Chapter 3
Skin Biopsy with Cutaneous Nerve Fiber Evaluation

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Introduction

The past two decades have seen the development and increasingly use of skin biopsy with intraepdiermal nerve fiber density (IENFD) evaluation. Skin biopsy has become the gold standard diagnostic test for small fiber neuropathy (SFN).

SFN is a common neuromuscular disorder which predominantly affects myelinated Aδ and unmyelinated C fibers. According to a Dutch study, the minimum prevalence of SFN is 52.95 per 100,000 population [1]. SFN can be associated with many medical conditions, including diabetes mellitus, connective tissue diseases, sarcoidosis, B12 deficiency, amyloidosis, monoclonal gammopathy, thyroid dysfunction, HIV infection, sodium channelopathy, and paraneoplastic syndrome, among others [2–5].

Small fibers consist of small somatic sensory fibers and autonomic C fibers, which mediate somatic sensory and autonomic functions. Small sensory fibers innervate skin to control the perception of pinprick and thermal stimuli. Autonomic C fibers innervate involuntary muscles, which include cardiac muscle and smooth muscle. Smooth muscle is present in the walls of blood vessel, gastrointestinal (GI) tract, and genitourinary (GU) tract, among others. Autonomic fibers also innervate some glands, including lacrimal gland, salivary gland, and sweat gland. They control cardiac muscle contractility, blood vessel constriction and dilatation, GI and GU motility, and gland functions. Patients with small somatic sensory fiber abnormalities commonly present with pain, burning, tingling, and numbness. Examination often shows allodynia, hyperalgesias, and reduced pinprick and thermal sensation in the affected areas. Motor strength, proprioception, and tendon reflexes are usually preserved, because these modalities are the functions of large nerve fibers. When autonomic fibers are affected, patients may experience dry eyes, dry mouth, ortho-

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static dizziness, palpitations, tachycardia, bowel constipation, urinary retention, sexual dysfunction, sweating abnormalities, and red or white skin discoloration. Examination may show orthostatic hypotension or skin changes [2, 6].

Patients with SFN may predominantly present with pain, and examination findings can be limited. Since pain is subjective, which can be caused by neurological conditions other than SFN, such as radiculopathy and central nervous system (CNS) disorders, or by a variety of non-neurological conditions, such as musculoskeletal disorders and arthritis, a specific diagnostic test is needed for SFN. Routine nerve conduction study (NCS) and electromyography (EMG), a valuable test for evaluating large fiber neuropathy, is typically normal in SFN, because the conduction velocities of small sensory nerve fibers are too slow for their conduction responses to be captured on the screen of routine NCS. EMG evaluates the function of motor nerve fibers which are large fibers. Small sensory nerve fibers were difficult to evaluate before the development of skin biopsy with intraepidermal nerve fiber density (IENFD) evaluation in 1990s [7–9]. This test allows direct visualization and evaluation of small cutaneous nerve fibers. The test is useful with a high diagnostic efficiency for evaluating distal SFN [10–13]. It is more sensitive and less invasive than sural nerve biopsy with electron microscopic evaluation of small myelinated and unmyelinated axons [14–17]. It is also useful for diagnosing non-length-dependent SFN [18–23] and focal SFN [24–27].

Skin biopsy is an office procedure. It is easy to perform and minimally invasive. The procedure takes about 10–15 minutes [28]. It has become more and more widely used by treating neurologists to diagnose patients with SFN. A growing number of diagnostic cutaneous nerve laboratories have been established in tertiary care centers and commercial settings. A task force of the European Federation of Neurological Societies (EFNS) published the first guideline paper regarding the use of skin biopsy in diagnosing SFN in 2005 [29], and a joint task force of EFNS and the Peripheral Nerve Society (PNS) published the second guideline paper in 2010 [30]. This chapter will give a brief review of skin biopsy procedure, skin biopsy specimen processing, and small cutaneous nerve fiber evaluation.

Skin Biopsy Procedure

Two methods are used to biopsy skin for evaluating cutaneous innervation, the 3-mm punch biopsy [8] and the blister technique [31]. The blister technique only removes epidermis by placing a suction capsule over the skin without damaging dermal capillaries. Although it is less invasive, painless, and does not cause bleeding, it is not commonly used because it is time-consuming, does not allow evaluation of dermal innervation, and no normative reference value of IENFD is established using this technique [30]. The 3-mm punch biopsy is the standard method for sampling skin. The current technique was initially developed at the Karolinska Institute [9], and later standardized by the groups at the University of Minnesota [7] and the Johns Hopkins University [8].

The 3-mm punch biopsy is routinely done in one lower limb. The biopsy is taken from the distal leg, which is 7–10 cm above the lateral malleolus. Additional biopsies may be taken from the lateral distal thigh (7–10 cm above the knee) and the lateral proximal thigh (7–10 cm below the greater trochanter) for evaluating the severity and the pattern of SFN, length-dependent *vs.* non-length-dependent [18–20, 22]. Biopsies taken from other sites may be indicated if focal or unilateral small fiber impairment is suspected, such as complex regional pain syndrome, meralgia paresthetica, and diabetic truncal neuropathy [24–27]. If no normative values are established at these sites, the contralateral unaffected sites should also be biopsied for comparison.

The 3-mm punch skin biopsy is minimally invasive. It can be done by a trained neurologist in an outpatient clinic. It takes about 10–15 minutes. It is done under local anesthesia, and the only time a patient may feel pain is when the anesthetic solution is injected to numb the biopsy site. After a biopsy site is identified, it is cleansed with alcohol swabs and injected with 1% lidocaine with 1:100,000 epinephrine. The vasoconstrictive effect of epinephrine can help reduce the bleeding. A 3-mm (diameter) disposable circular punch is then placed on the skin perpendicular to the skin surface and slowly twisted down until the punch is 3–4 mm (2/3 of the metal part) in. The biopsy is removed with the forceps and surgical blade technique. It is very important that the epidermis should not be pinched because intraepidermal nerve fibers will be evaluated (Fig. 3.1). Bleeding is usually minimum and easy to control by applying firm pressure to the biopsy site. It may not be necessary for patients to hold anticoagulants, antiplatelet agents, or non-steroidal anti-inflammatory agents for the procedure. But the biopsy site may need prolonged pressure and placement of an absorbable gelatin sponge (gelfoam) for hemostasis. No sutures are needed. The biopsy site is usually healed within 7–10 days by granulation, which leaves a small circular scar that gradually resolves. The biopsy sites

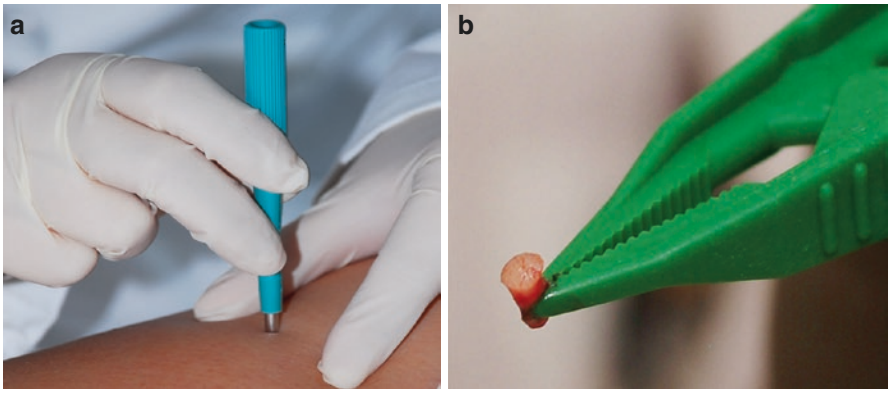

Fig. 3.1 3-mm punch skin biopsy for diagnosing small fiber neuropathy. (**a**) After cleaning the biopsy site, a 3-mm punch is placed on the site perpendicular to the skin surface and twisted down. (**b**) The skin biopsy should be picked up by a forceps to pinch the subcutaneous layer but not the top epidermis. (Reprinted by permission from Zhou [28])

should be covered with pressure gauzes after the biopsy is taken to prevent bleeding. The patient may start to take shower the day after the biopsy, remove the gauzes after the shower, and cove the biopsy sites with regular Band-Aids. The Band-Aids are then changed every day after shower for 7 days. To prevent infection, the patient may not take bath or go swimming during these 7 days. The 3-mm punch biopsy is safe. No serious side effects have been encountered by the author or reported in the literatures. The estimated frequency of non-serious side effects, including mild infection and excessive bleeding, is 1.9:1000 [30]. Mild infection at the biopsy site can be controlled by topical antibiotics, such as over-the-counter Neosporin, and bleeding can be controlled by prolonged pressure to the biopsy site without sutures.

Skin Biopsy Specimen Processing

The biopsy specimen should be placed into a tube filled with special fixative solution immediately after the biopsy is taken. The tube should be labeled with the patient's identification and the biopsy side and site. The normative values of small fiber densities at different sites are different [11, 12]. The normative values are also influenced by age and gender [32]. Therefore, these pieces of information should be clearly provided to pathologists. The specimens should be submitted to a cutaneous nerve laboratory, not a routine reference laboratory, as a special technique is used for processing. It is very important to contact a specialized cutaneous nerve laboratory regarding the fixative and specimen handling before planning a biopsy.

Immunohistochemical assays are used to detect an antigen expressed by nerve axons to visualize cutaneous nerve fibers for morphometric and morphological evaluation. Two methods of immunostaining have been used, the bright-field immunohistochemistry [8] and the immunofluorescence with [7] or without [9, 33] confocal microscopy. Since most diagnostic cutaneous nerve laboratories use the bright-field immunohistochemistry, this immunostaining method is briefly reviewed here.

After a skin biopsy is removed, it should be fixed immediately in fixative solution for approximately 24 hours. Two types of fixatives can be used, 2% paraformaldehyde-lysine-periodate (2% PLP) and Zamboni (2% paraformaldehyde and picric acid). Formalin, which is commonly used by routine histopathology laboratories, should be avoided because it may cause fragmented appearance of nerve fibers [11]. The skin specimen is then cryoprotected for at least 6 hours using 20% glycerol in 0.1 M Sorrensons phosphate buffer. After freezing, the specimen is sectioned at 50 μm. The wavy nerve fibers can be better viewed in thick 50-μm sections than in routine 5-μm sections. About 45–55 skin sections can be obtained from each specimen. Four non-adjacent sections from each specimen are chosen for immunostaining, and the rest can be stored in antifreeze solution (30% ethylene glycol) at −20°C for future use when needed.

Immunostaining is done manually under a dissecting microscope using free-floating skin sections and 96-well plates (Fig. 3.2). The primary antibody used in our lab for the immunostaining is a polyclonal antibody against protein gene product 9.5 (PGP9.5). PGP9.5 is an ubiquitin carboxyl-terminal hydrolase [34], which is a neuronal cytoplasmic marker. It is found in all types of efferent and afferent nerve

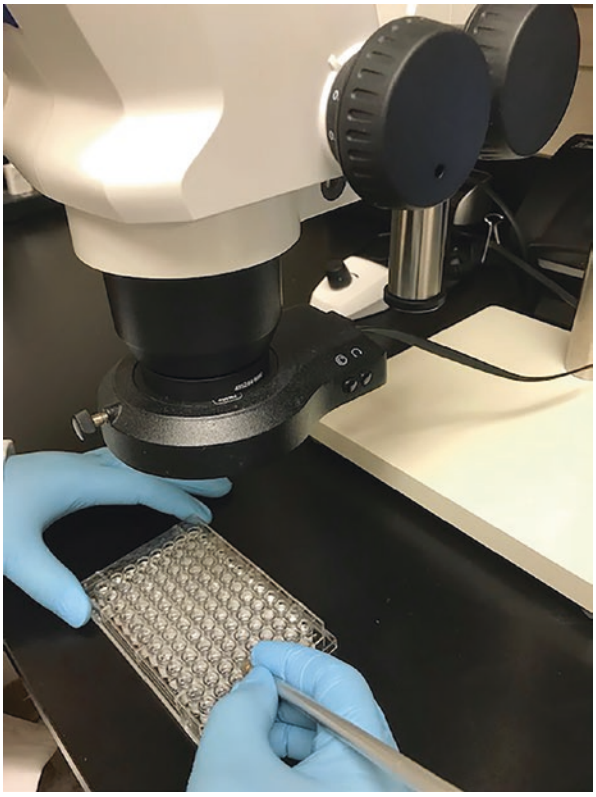

Fig. 3.2 Skin biopsy specimen processing. PGP9.5 immunostaining is done manually under a dissecting microscope using free-floating skin sections and 96-well plates

axons [35, 36], so it is a useful pan-axonal marker to highlight all the nerve fibers. After the primary antibody incubation, sections are incubated with a biotin-conjugated secondary antibody which binds to the primary antibody. This is followed by incubation with avidin-conjugated horseradish peroxidase, and avidin can bind to biotin. The immunostaining signal is then developed using an SG kit (blue chromogen/peroxidase substrate) which produces a blue-gray reaction product [8].

Small Cutaneous Nerve Fiber Evaluation

Intraepidermal Nerve Fiber Density Evaluation

Skin consists of three layers which are firmly attached to one another: the outer epidermis, the deeper dermis, and the subcutaneous layer. The cutaneous innervation was initially thought to mainly consist of a plexus of nerve fibers in the reticular dermis and a more superficial plexus of nerve fibers in the papillary dermis parallel to the skin surface. Rich innervation of epidermis was not demonstrated until late

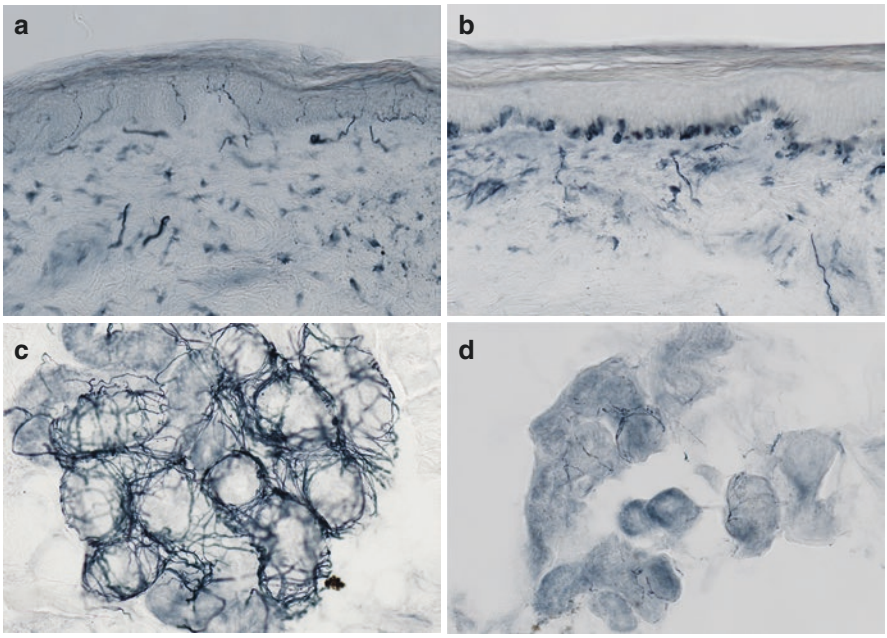

Fig. 3.3 Cutaneous innervation and denervation. (**a**) The epidermis is well-innervated by intraepidermal nerve fibers (arrows). (**b**) The epidermis is devoid of intraepidermal nerve fibers. (**c**) The sweat glands are well-innervated by sudomotor autonomic fibers. (**d**) The sweat glands are largely denervated

1980s and early 1990s by immunostaining using PGP9.5 antibodies [7, 9, 37]. The intraepidermal unmyelinated C fibers originate from sensory nerves as they express substance P and calcitonin gene-related peptide (CGRP) [38, 39]. In addition, these fibers arise entirely from dorsal root ganglions (DRG) as they disappear from skin after experimental dorsal root ganglionectomy, but not after dorsal rhizotomy, ventral rhizotomy, or sympathectomy [40]. Before reaching the epidermis, the unmyelinated C fibers are arranged in Remak bundles which also consist of non-myelinating Schwann cells. Axons exchange among Remak bundles as they pass from DRG to skin [41]. The Remak bundles lose their Schwann cells, and the S-100 staining signal of Schwann cells ends at the dermal-epidermal junction [8]. The unmyelinated C fibers ascend vertically through the epidermis between adjacent keratinocytes as free nerve endings [42] (Fig. 3.3).

Intraepidermal nerve fibers are quantified using a light microscope with 40x objective. A counting rule has been established [43] and recommended to use by EFNS/PNS [29, 30]. Briefly, the nerve fibers that cross the dermal-epidermal junction into the epidermis are counted. The nerve fibers that do not cross the dermal-epidermal junction are not counted. If a nerve fiber branches within epidermis, count as one fiber. If a nerve fiber branches below or within the dermal-

epidermal junction, count as two fibers. According to the EFNS/PNS guideline, the nerve fragments within epidermis that do not cross the dermal-epidermal junction are not counted due to the concern that these fragments may be the extension of adjacent fibers on the same skin section that are visualized to cross the dermal-epidermal junction and already counted. Counting these fragments may result in overcounting. However, the original fibers that cross the dermal-epidermal junction may not be shown on the same section due to the wavy nature of the nerve fibers, so excluding these fragments may result in undercounting. Some cutaneous nerve laboratories do count these individual fibers that are within epidermis but without crossing the dermal-epidermal junction [8, 12, 20, 44, 45].

The diagnosis of SFN is made based on the reduction of IENFD. To calculate the linear density of IENF, the length of the epidermal surface is measured [30]. The IENFD is expressed as the number of IENF per length of section (IENF/mm). An alternative "ocular" method has been described and used [46–48], in which special sections are chosen for immunostaining with the assumption that the length of the epidermal surface of these sections is close to 3 mm. So the IENFD is calculated simply by dividing the number of IENF by 3. It has been shown that the IENFD obtained by this "ocular" method significantly correlate with the IENFD obtained from the quantification by measuring the length of the epidermal surface [46]. Further studies are deemed warranted to establish the reliability of the "ocular" method [29].

IENFD measurement is highly reproducible. Reproducibility is the highest when four sections from each biopsy specimen are counted [44]. After reviewers are trained to use the same counting rule, the interobserver and intraoberserver reliabilities are high [8, 12, 44, 49, 50]. There is no significant difference in IENFD when skin sections are stained by different cutaneous nerve laboratories as long as an identical methodology is used by these laboratories to process skin specimens and measure IENFD [44].

The technique of 3-mm punch biopsy with IENFD evaluation using the PGP9.5 immunostaining was standardized and first utilized to evaluate patients with SFN by University of Minnesota [7] and Johns Hopkins University [8]. In 1995, the Johns Hopkins group published the method of the bright-field PGP9.5 immunostaining and IENFD quantification [8]. The majority of the diagnostic cutaneous nerve laboratories adopted this method. By using this method, the Johns Hopkins group showed that the IENFD at the distal leg was lower in patients with HIV-seropositive and HIV-seronegative sensory neuropathy than in normal controls. They subsequently developed normative reference ranges at the distal leg and proximal thigh in 98 healthy subjects with age ranging from 13–82 years [12]. They showed a significantly higher IENFD in the youngest age decile (10–19 years) [11, 12]. By using the cut-off derived from the fifth percentile of the normative range at the distal leg to evaluate 20 patients with sensory neuropathy, they showed that the technique had a diagnostic efficiency of 88%. The high diagnostic efficiency of this technique was

also demonstrated by other laboratories [10, 13]. By studying the cutaneous innervation at 5 sites, including distal leg, proximal calf, distal thigh, proximal thigh, and trunk in 10 healthy controls (ages 23–75 years), the Johns Hopkins group showed a normal rostral-to-caudal gradient of IENFD with a linear relationship to the distance from the spine [11]. IENFD at a proximal site was higher than that at a distal site. The IENFD at the proximal thigh was higher than that at the distal leg by about 60% [12].

Several laboratories studied normative reference values at the distal leg and found a decline of the IENFD with age [17, 46, 48–52]. A multicenter study developed the normative values of IENFD at the distal leg by evaluating 550 healthy subjects recruited from eight cutaneous nerve laboratories in Europe, USA, and Asia [32]. The study confirmed the age-related decline of IENFD. IENFD was also found to be influenced by gender but not height or weight. The study developed worldwide age- and sex-adjusted IENFD normative values for clinical use. However, the sensitivity, specificity, and diagnostic efficiency have not been fully determined. Our recent small-scale study suggested that the IENFD at the distal leg appeared influenced by the ethnicity, as the diagnostic sensitivity of using the worldwide age- and sex-adjusted normative reference values was lower in Chinses Americans than in non-Chinese Americans who had pure small fiber sensory neuropathy based on the clinical and electrodiagnostic evaluations [53]. Future large-scale studies are needed to fully address the ethnic differences in IENFD at the distal leg. The normative values may need to be adjusted in certain ethnic groups to improve the diagnostic sensitivity.

Intraepidermal Nerve Fiber Morphology Evaluation

IENFD can be normal at the early stage of SFN, which makes the disease difficult to diagnose because the skin biopsy diagnosis of SFN is based on the reduction of IENFD. However, in this setting, skin biopsy often shows prominent morphological changes of small fibers, including swellings, increased branching and fragmentation, and tortuous appearance (Fig. 3.4) [11, 14, 16, 47, 54–56]. Two studies investigated the diagnostic implication of IENF swellings in SFN [16, 47]. Both found a higher prevalence of IENF swellings at the distal leg in neuropathy patients than in healthy controls. Increased IENF swellings at the distal leg correlated with impaired heat-pain threshold, development of symptomatic neuropathy, and progression of neuropathy. In patients with small fiber sensory symptoms but normal IENFD, the presence of the large swellings of intraepidermal C fibers was found to be able to identify those who subsequently developed epidermal denervation [54]. Therefore, the abnormal morphological changes, especially the large swellings of intraepidermal nerve fibers, may represent small fiber degeneration. If these changes are prominent but IENFD are still normal, a repeat biopsy in 12 months may detect the reduction of IENFD and reach a final diagnosis of SFN.

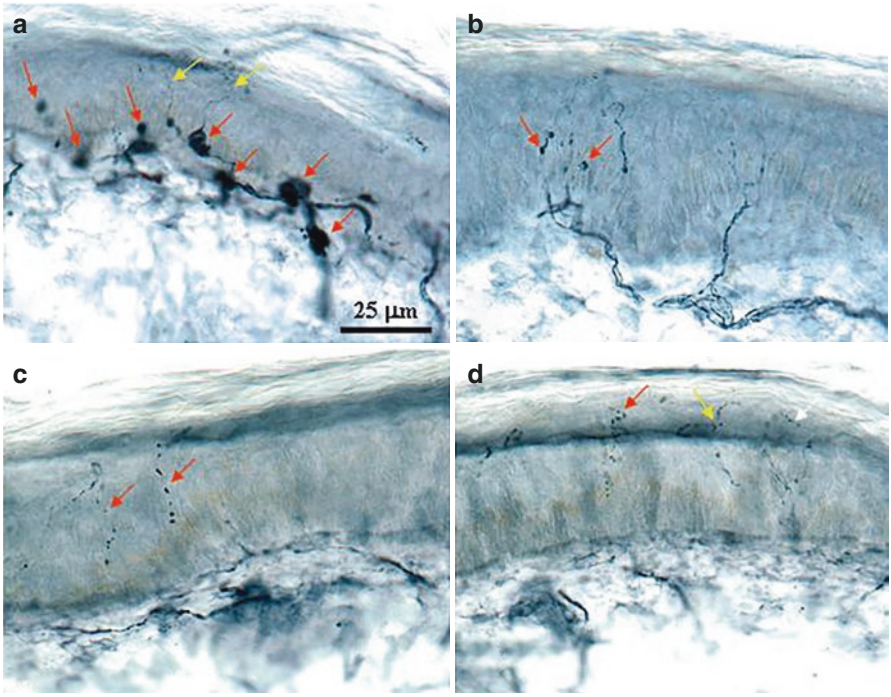

Fig. 3.4 Abnormal morphological changes of intraepidermal nerve fibers. (**a**) Abundant nerve fiber swellings of varying size (red arrows) are noted in epidermis, papillary dermis, and dermal-epidermal junction. (**b**) Many small IENF swellings are seen (red arrows). (**c**) Intraepidermal fibers are fragmented (red arrows) as compared to continuous fibers in **a** (yellow arrows). (**d**) Tortuous (red arrow), branched (yellow arrow), and horizontal (white arrow) fibers are present. (Reprinted with permission from Zhou et al. [45])

Cutaneous Autonomic Nerve Fiber Evaluation

There are several types of autonomic C fibers in the dermis that innervate blood vessel wall (vasomotor fibers), sweat gland (sudomotor fibers), and arrector pilorum smooth muscle (pilomotor fibers) (Fig. 3.5). A few reports have described the reduction of dermal autonomic fiber densities in patients with idiopathic SFN [57] or SFN and dysautonomia associated with diabetes [58], multiple system atrophy [59], and CADASIL [60]. Several studies have attempted to establish standard and reproducible methods to quantify dermal autonomic nerve fiber densities [58, 61–63] to facilitate clinical evaluation and research of autonomic dysfunction associated with SFN. Gibbons et al. have developed an automated method to quantify sudomotor fibers, and the sudomotor fiber density correlates well with the Neuropathy Impairment Score in the Lower Limb (NIS-LL) and the symptoms of reduced sweat production [62, 63]. Some cutaneous nerve laboratories include the measurements of sudomotor fiber densities in their skin biopsy reports. It remains to be determined

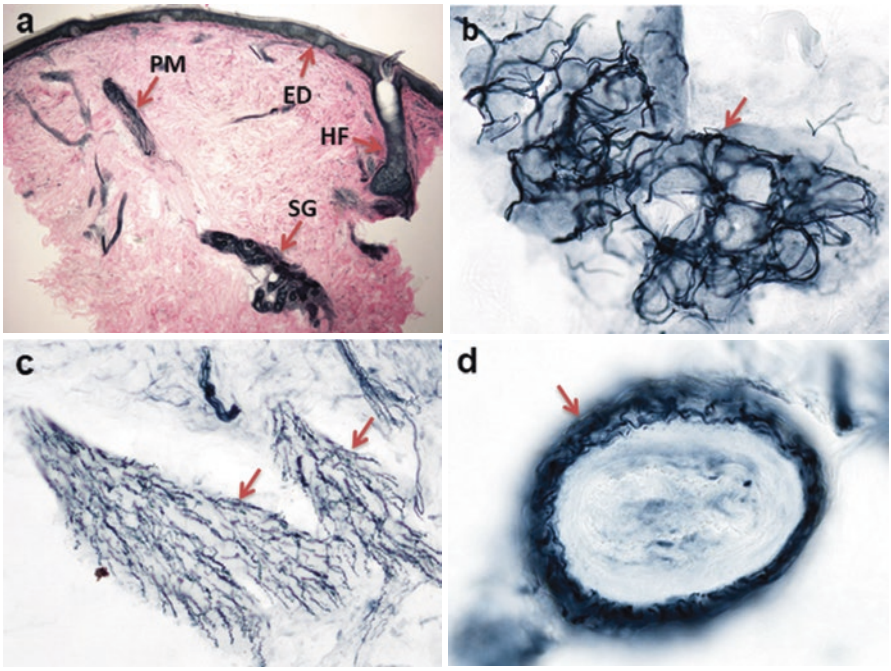

Fig. 3.5 Cutaneous autonomic nerve fibers. (**a**) A skin section immunostained with PGP9.5 shows the top layer of epidermis (ED), and hair follicle (HF), sweat glands (SG), and arrector pili smooth muscle (PM) in the dermis. (**b**) Dermal sudomotor autonomic fibers innervating sweat glands (arrow). (**c**) Dermal pilomotor autonomic fibers innervating arrectores pili muscle (arrows). (**d**) Dermal vasomotor autonomic fibers innervating a blood vessel (arrow)

whether the sudomotor fiber density correlates with the sudomotor function gauged by quantitative sudomotor axon reflex testing (QSART). Nolano et al. have developed a method to quantify pilomotor nerve fiber density (PNFD), and by using this method they have found that the PNFD is significantly reduced in diabetic patients as compared with normal controls. However, PNFD does not correlate with IENFD or total neuropathy score [58]. Future studies are needed to refine the measurements of dermal autonomic fibers and to fully determine the diagnostic utility of detecting dermal autonomic denervation [3, 30].

Treating neurologists who evaluate patients with SFN commonly ask whether they should order skin biopsy, QSART, cardiovascular autonomic testing or all of these tests, and which test has the highest diagnostic yield. The decision should be made based on the patient's symptoms. Skin biopsy, QSART, and cardiovascular autonomic testing evaluate different types of small fibers with different functions. Skin biopsy is mainly used to evaluate the number and morphology of somatic intraepidermal sensory fibers. QSART is to evaluate the functions of sudomotor autonomic fibers. Cardiovascular autonomic testing is to evaluate the functions of cardiovascular autonomic fibers. If a patient mainly presents with sensory symptoms, such as pain, burning, tingling, and numbness, skin biopsy should be ordered

for evaluation. If a patient also has sweating abnormalities, QSART should be added. If a patient presents with orthostatic dizziness, palpitations, tachycardia, near-syncope, or syncope, cardiovascular autonomic testing should be ordered for evaluation. SFN that is associated with diabetes, sarcoidosis, Sjogren syndrome, or amyloidosis often manifests both somatic sensory symptoms and autonomic dysfunction. In these settings, skin biopsy, QSART, and cardiovascular autonomic testing can be complementary, and the diagnostic sensitivity can be improved if used together [64, 65].

Limited Usefulness of Skin Biopsy in Evaluation of SFN Etiologies

In addition to the PGP9.5 immunostaining, hematoxylin and eosin staining (HE) and Congo red staining are routinely done by most cutaneous nerve laboratories to evaluate for possible vasculitis and amyloidosis. Since skin biopsy for neuropathy is not a lesion biopsy, the likelihood of finding these abnormalities is extremely low, although amyloid deposition has been reported on skin biopsies taken for evaluation of SFN from patients with amyloidosis [66, 67]. Overall, the usefulness of skin biopsy in evaluation of SFN etiologies is very limited. The test is mainly used to diagnose SFN.

References

1. Peters MJ, Bakkers M, Merkies IS, Hoeijmakers JG, van Raak EP, Faber CG. Incidence and prevalence of small-fiber neuropathy: a survey in the Netherlands. Neurology. 2013;81(15):1356–60.
2. Tavee J, Zhou L. Small fiber neuropathy: a burning problem. Cleve Clin J Med. 2009;76(5):297–305.
3. Cazzato D, Lauria G. Small fibre neuropathy. Curr Opin Neurol. 2017;30(5):490–9.
4. Chan AC, Wilder-Smith EP. Small fiber neuropathy: getting bigger! Muscle Nerve. 2016;53(5):671–82.
5. Gibbons CH. Small fiber neuropathies. Continuum (Minneap Minn). 2014;20(5 Peripheral Nervous System Disorders):1398–412.
6. Lacomis D. Small-fiber neuropathy. Muscle Nerve. 2002;26(2):173–88.
7. Kennedy WR, Wendelschafer-Crabb G. The innervation of human epidermis. J Neurol Sci. 1993;115(2):184–90.
8. McCarthy BG, Hsieh ST, Stocks A, Hauer P, Macko C, Cornblath DR, et al. Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. Neurology. 1995;45(10):1848–55.
9. Wang L, Hilliges M, Jernberg T, Wiegleb-Edstrom D, Johansson O. Protein gene product 9.5-immunoreactive nerve fibres and cells in human skin. Cell Tissue Res. 1990;261(1):25–33.
10. Devigili G, Tugnoli V, Penza P, Camozzi F, Lombardi R, Melli G, et al. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. Brain. 2008;131.(Pt 7:1912–25.
11. Lauria G, Holland N, Hauer P, Cornblath DR, Griffin JW, McArthur JC. Epidermal innervation: changes with aging, topographic location, and in sensory neuropathy. J Neurol Sci. 1999;164(2):172–8.

12. McArthur JC, Stocks EA, Hauer P, Cornblath DR, Griffin JW. Epidermal nerve fiber density: normative reference range and diagnostic efficiency. Arch Neurol. 1998;55(12):1513–20.
13. Vlckova-Moravcova E, Bednarik J, Dusek L, Toyka KV, Sommer C. Diagnostic validity of epidermal nerve fiber densities in painful sensory neuropathies. Muscle Nerve. 2008;37(1):50–60.
14. Herrmann DN, Griffin JW, Hauer P, Cornblath DR, McArthur JC. Epidermal nerve fiber density and sural nerve morphometry in peripheral neuropathies. Neurology. 1999;53(8):1634–40.
15. Holland NR, Crawford TO, Hauer P, Cornblath DR, Griffin JW, McArthur JC. Small-fiber sensory neuropathies: clinical course and neuropathology of idiopathic cases. Ann Neurol. 1998;44(1):47–59.
16. Lauria G, Morbin M, Lombardi R, Borgna M, Mazzoleni G, Sghirlanzoni A, et al. Axonal swellings predict the degeneration of epidermal nerve fibers in painful neuropathies. Neurology. 2003;61(5):631–6.
17. Periquet MI, Novak V, Collins MP, Nagaraja HN, Erdem S, Nash SM, et al. Painful sensory neuropathy: prospective evaluation using skin biopsy. Neurology. 1999;53(8):1641–7.
18. Gemignani F, Giovanelli M, Vitetta F, Santilli D, Bellanova MF, Brindani F, et al. Non-length dependent small fiber neuropathy. A prospective case series. J Peripher Nerv Syst. 2010;15(1):57–62.
19. Gorson KC, Herrmann DN, Thiagarajan R, Brannagan TH, Chin RL, Kinsella LJ, et al. Non-length dependent small fibre neuropathy/ganglionopathy. J Neurol Neurosurg Psychiatry. 2008;79(2):163–9.
20. Khan S, Zhou L. Characterization of non-length-dependent small-fiber sensory neuropathy. Muscle Nerve. 2012;45(1):86–91.
21. Lauria G, Sghirlanzoni A, Lombardi R, Pareyson D. Epidermal nerve fiber density in sensory ganglionopathies: clinical and neurophysiologic correlations. Muscle Nerve. 2001;24(8):1034–9.
22. Provitera V, Gibbons CH, Wendelschafer-Crabb G, Donadio V, Vitale DF, Loavenbruck A, et al. The role of skin biopsy in differentiating small-fiber neuropathy from ganglionopathy. Eur J Neurol. 2018;25(6):848–53.
23. Chai J, Herrmann DN, Stanton M, Barbano RL, Logigian EL. Painful small-fiber neuropathy in Sjogren syndrome. Neurology. 2005;65(6):925–7.
24. Chemali KR, Zhou L. Small fiber degeneration in post-stroke complex regional pain syndrome I. Neurology. 2007;69(3):316–7.
25. Wongmek A, Shin S, Zhou L. Skin biopsy in assessing meralgia paresthetica. Muscle Nerve. 2016;53(4):641–3.
26. Lauria G, McArthur JC, Hauer PE, Griffin JW, Cornblath DR. Neuropathological alterations in diabetic truncal neuropathy: evaluation by skin biopsy. J Neurol Neurosurg Psychiatry. 1998;65(5):762–6.
27. Oaklander AL, Rissmiller JG, Gelman LB, Zheng L, Chang Y, Gott R. Evidence of focal small-fiber axonal degeneration in complex regional pain syndrome-I (reflex sympathetic dystrophy). Pain. 2006;120(3):235–43.
28. Zhou L. Skin Biopsy. In: Katirji B, Kaminski H, Ruff R, editors. Neuromuscular disorders in clinical practice. New York: Springer; 2014.
29. Lauria G, Cornblath DR, Johansson O, McArthur JC, Mellgren SI, Nolano M, et al. EFNS guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy. Eur J Neurol. 2005;12(10):747–58.
30. Lauria G, Hsieh ST, Johansson O, Kennedy WR, Leger JM, Mellgren SI, et al. European Federation of Neurological Societies/Peripheral Nerve Society guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society. Eur J Neurol. 2010;17(7):903–12, e44–9.
31. Kennedy WR, Nolano M, Wendelschafer-Crabb G, Johnson TL, Tamura E. A skin blister method to study epidermal nerves in peripheral nerve disease. Muscle Nerve. 1999;22(3):360–71.

32. Lauria G, Bakkers M, Schmitz C, Lombardi R, Penza P, Devigili G, et al. Intraepidermal nerve fiber density at the distal leg: a worldwide normative reference study. J Peripher Nerv Syst. 2010;15(3):202–7.
33. Provitera V, Gibbons CH, Wendelschafer-Crabb G, Donadio V, Vitale DF, Stancanelli A, et al. A multi-center, multinational age- and gender-adjusted normative dataset for immunofluorescent intraepidermal nerve fiber density at the distal leg. Eur J Neurol. 2016;23(2):333–8.
34. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, Pohl J. The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. Science. 1989;246(4930):670–3.
35. Gulbenkian S, Wharton J, Polak JM. The visualisation of cardiovascular innervation in the guinea pig using an antiserum to protein gene product 9.5 (PGP 9.5). J Auton Nerv Syst. 1987;18(3):235–47.
36. Lundberg LM, Alm P, Wharton J, Polak JM. Protein gene product 9.5 (PGP 9.5). A new neuronal marker visualizing the whole uterine innervation and pregnancy-induced and developmental changes in the guinea pig. Histochemistry. 1988;90(1):9–17.
37. Dalsgaard CJ, Rydh M, Haegerstrand A. Cutaneous innervation in man visualized with protein gene product 9.5 (PGP 9.5) antibodies. Histochemistry. 1989;92(5):385–90.
38. Bloom SR, Polak JM. The Prosser-White Oration 1981. Regulatory peptides and the skin. Clin Exp Dermatol. 1983;8(1):3–18.
39. Dalsgaard CJ, Jonsson CE, Hokfelt T, Cuello AC. Localization of substance P-immunoreactive nerve fibers in the human digital skin. Experientia. 1983;39(9):1018–20.
40. Li Y, Hsieh ST, Chien HF, Zhang X, McArthur JC, Griffin JW. Sensory and motor denervation influence epidermal thickness in rat foot glabrous skin. Exp Neurol. 1997;147(2):452–62.
41. Griffin JW, McArthur JC, Polydefkis M. Assessment of cutaneous innervation by skin biopsies. Curr Opin Neurol. 2001;14(5):655–9.
42. Hsieh ST, Choi S, Lin WM, Chang YC, McArthur JC, Griffin JW. Epidermal denervation and its effects on keratinocytes and Langerhans cells. J Neurocytol. 1996;25(9):513–24.
43. Kennedy WRMJ, Polydefkis MJ, Wendelschafer G, editors. Pathology and quantification of cutaneous innervation. Philadelphia: Elsevier Saunders; 2005.
44. Smith AG, Howard JR, Kroll R, Ramachandran P, Hauer P, Singleton JR, et al. The reliability of skin biopsy with measurement of intraepidermal nerve fiber density. J Neurol Sci. 2005;228(1):65–9.
45. Zhou L, Kitch DW, Evans SR, Hauer P, Raman S, Ebenezer GJ, et al. Correlates of epidermal nerve fiber densities in HIV-associated distal sensory polyneuropathy. Neurology. 2007;68(24):2113–9.
46. Chien HF, Tseng TJ, Lin WM, Yang CC, Chang YC, Chen RC, et al. Quantitative pathology of cutaneous nerve terminal degeneration in the human skin. Acta Neuropathol. 2001;102(5):455–61.
47. Herrmann DN, McDermott MP, Henderson D, Chen L, Akowuah K, Schifitto G. Epidermal nerve fiber density, axonal swellings and QST as predictors of HIV distal sensory neuropathy. Muscle Nerve. 2004;29(3):420–7.
48. Pan CL, Lin YH, Lin WM, Tai TY, Hsieh ST. Degeneration of nociceptive nerve terminals in human peripheral neuropathy. Neuroreport. 2001;12(4):787–92.
49. Bakkers M, Merkies IS, Lauria G, Devigili G, Penza P, Lombardi R, et al. Intraepidermal nerve fiber density and its application in sarcoidosis. Neurology. 2009;73(14):1142–8.
50. Goransson LG, Mellgren SI, Lindal S, Omdal R. The effect of age and gender on epidermal nerve fiber density. Neurology. 2004;62(5):774–7.
51. Chang YC, Lin WM, Hsieh ST. Effects of aging on human skin innervation. Neuroreport. 2004;15(1):149–53.
52. Umapathi T, Tan WL, Tan NC, Chan YH. Determinants of epidermal nerve fiber density in normal individuals. Muscle Nerve. 2006;33(6):742–6.
53. Jin P, Cheng L, Chen M, Zhou L. Low sensitivity of skin biopsy in diagnosing small fiber neuropathy in Chinese Americans. J Clin Neuromuscul Dis. 2018;20(1):1–6.

54. Gibbons CH, Griffin JW, Polydefkis M, Bonyhay I, Brown A, Hauer PE, et al. The utility of skin biopsy for prediction of progression in suspected small fiber neuropathy. Neurology. 2006;66(2):256–8.
55. Scott LJ, Griffin JW, Luciano C, Barton NW, Banerjee T, Crawford T, et al. Quantitative analysis of epidermal innervation in Fabry disease. Neurology. 1999;52(6):1249–54.
56. Smith AG, Ramachandran P, Tripp S, Singleton JR. Epidermal nerve innervation in impaired glucose tolerance and diabetes-associated neuropathy. Neurology. 2001;57(9):1701–4.
57. Dabby R, Vaknine H, Gilad R, Djaldetti R, Sadeh M. Evaluation of cutaneous autonomic innervation in idiopathic sensory small-fiber neuropathy. J Peripher Nerv Syst. 2007;12(2):98–101.
58. Nolano M, Provitera V, Caporaso G, Stancanelli A, Vitale DF, Santoro L. Quantification of pilomotor nerves: a new tool to evaluate autonomic involvement in diabetes. Neurology. 2010;75(12):1089–97.
59. Provitera V, Nolano M, Caporaso G, Stancanelli A, Manganelli F, Iodice R, et al. Postganglionic sudomotor denervation in patients with multiple system atrophy. Neurology. 2014;82(24):2223–9.
60. Nolano M, Provitera V, Donadio V, Caporaso G, Stancanelli A, Califano F, et al. Cutaneous sensory and autonomic denervation in CADASIL. Neurology. 2016;86(11):1039–44.
61. Donadio V, Nolano M, Provitera V, Stancanelli A, Lullo F, Liguori R, et al. Skin sympathetic adrenergic innervation: an immunofluorescence confocal study. Ann Neurol. 2006;59(2):376–81.
62. Gibbons CH, Illigens BM, Wang N, Freeman R. Quantification of sweat gland innervation: a clinical-pathologic correlation. Neurology. 2009;72(17):1479–86.
63. Gibbons CH, Illigens BM, Wang N, Freeman R. Quantification of sudomotor innervation: a comparison of three methods. Muscle Nerve. 2010;42(1):112–9.
64. Tavee JO, Karwa K, Ahmed Z, Thompson N, Parambil J, Culver DA. Sarcoidosis-associated small fiber neuropathy in a large cohort: clinical aspects and response to IVIG and anti-TNF alpha treatment. Respir Med. 2017;126:135–8.
65. Thaisetthawatkul P, Fernandes Filho JA, Herrmann DN. Contribution of QSART to the diagnosis of small fiber neuropathy. Muscle Nerve. 2013;48(6):883–8.
66. Ebenezer GJ, Liu Y, Judge DP, Cunningham K, Truelove S, Carter ND, et al. Cutaneous nerve biomarkers in transthyretin familial amyloid polyneuropathy. Ann Neurol. 2017;82(1):44–56.
67. Visser AC, Klein CJ. Wild-type TTR neuropathy with cardiomyopathy presenting with burning feet. Neurology. 2017;88(11):1101–2.