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Skin biopsy as a diagnostic tool in peripheral neuropathy

Giuseppe Lauria* and Grazia Devigili

SUMMARY

Skin biopsy is a safe, minimally invasive, painless and cheap tool for providing diagnostic information on small nerve fibers, which are invisible to routine neurophysiological tests. Biopsy can be performed in hairy skin to investigate unmyelinated and thinly myelinated fibers and in glabrous skin to examine large myelinated fibers. Morphometric analysis of skin nerves is readily accomplished through the use of immunohistochemical techniques, and has proved to be reliable, reproducible and unaffected by the severity of neuropathy. One further advantage of skin biopsy over conventional nerve biopsy is that it allows somatic nerve fibers to be distinguished from autonomic nerve fibers. Morphological changes, axonal degeneration and abnormal regeneration occur in cutaneous nerves very early in the course of peripheral neuropathies, making skin biopsy a promising tool for investigating the progression of neuropathy and the effect of neuroprotective treatments in clinical practice and trials. This article reviews the techniques that are used to investigate the innervation of human skin, the possible uses of skin biopsy in diagnosing and monitoring peripheral neuropathies, and correlations between skin biopsy findings and those of other diagnostic methods.

KEYWORDS autonomic neuropathy, neuropathic pain, peripheral neuropathy, skin biopsy, small-fiber neuropathy

REVIEW CRITERIA

PubMed was searched using Entrez for articles published up to 4 May 2007, including electronic early release publications. Search terms included "skin biopsy", "neuropathic pain", "small-fiber neuropathy", "autonomic neuropathy", "sensory neuropathy" and "peripheral neuropathy". Full articles were obtained and references were checked for additional material where appropriate.

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Learning objectives

Upon completion of this activity, participants should be able to:

1 List advantages of skin biopsy over nerve biopsy.
2 Identify the disadvantage of the blister method compared with the punch method of skin biopsy for neuropathy.
3 List conditions for which immunohistochemical methods have been used to demonstrate small-fiber neuropathy using punch biopsy.
4 Identify systemic diseases in which skin biopsy plays a role in the diagnosis of small-fiber neuropathy and nerve degeneration.
5 Describe conditions under which spontaneous nerve regrowth can be followed using skin biopsy.

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INTRODUCTION

Skin biopsy is proving to be a reliable diagnostic tool in patients complaining of symptoms consistent with small-fiber neuropathy, a condition that has been underdiagnosed in the past. Skin biopsy samples can demonstrate the selective degeneration of somatic unmyelinated fibers that convey pain and thermal sensations. These fibers cannot be observed in routine neurophysiological tests. Skin biopsy can also provide diagnostic information when there is little or no clinical evidence of neuropathy. The minimal invasiveness of skin biopsy makes it a useful tool not only in clinical practice, but also for monitoring the progression of neuropathy in trials of neuroprotective treatments. The range of applications of skin biopsy has recently been expanded to include autonomic neuropathies and immune-mediated and inherited

demyelinating neuropathies. In addition, correlation of skin biopsy findings with the overall clinical picture and with the results of neurophysiological examinations has provided important insights into the pathogenesis and features of neuropathic pain in peripheral neuropathies. Finally, an extremely interesting field of investigation is that of the functional properties of skin nerves. The emerging view is that the transduction of temperature and pain sensation might be regulated by a network involving the epidermal cells, which are also thought to have a distinct role in the pathogenesis of neuropathic pain.

THE SKIN AND ITS INNERVATION

Structure of the superficial skin

The topmost living layer of the skin is the epidermis, which is composed of four layers of keratinocytes. These keratinocytes undergo gradual differentiation as they progress from the basal layer to the stratum corneum. Epidermal cells have a turnover time of about 30 days. Other cells that reside in the epidermis include Langerhans cells, melanocytes and Merkel cells. The basement membrane (dermal–epidermal junction) separates the epidermis from the subpapillary dermis, which is organized as papillae that contain vascular plexus and capillary loops. In the glabrous skin, the apexes of the papillae contain Meissner's corpuscles. The mean (± SD) density of these mechanoreceptors in the fingertip is 33.0 ± 13.2/mm^2.[1] Pacini's and Ruffini's corpuscles reside in the deeper layers of the dermis and are commonly excluded from routine skin biopsy examination. The matrix of the superficial dermis also includes fibroblasts, hair follicles, arrectores pilorum muscles, blood vessels, sebaceous glands and sweat glands.

Immunohistochemical examination of skin nerve fibers

The epidermis is innervated by somatic naked axons arising from nerve bundles that run through the subpapillary dermis. Intraepidermal nerve fibers (IENFs) lose Schwann cell ensheathment as they cross the dermal–epidermal junction and run towards the skin surface between the keratinocytes, sometimes with horizontal branches. In healthy individuals, IENFs have a simple morphology, with small bead-like varicosities.[2] IENFs can be labeled with antibodies against various structures, including cytoplasmic epitopes (e.g. protein gene product 9.5 [PGP9.5]), components of the cytoskeleton (e.g. microtubules, phosphorylated neurofilaments or axonal membrane), and the capsaicin receptor (transient receptor potential vanilloid receptor 1 [TRPV1]).[3] The density of IENFs is highest in the paravertebral region of the trunk, and shows a decreasing proximal-to-distal gradient in the limbs, being about 40% lower in the supramalleolar area than in the thigh (Figure 1).

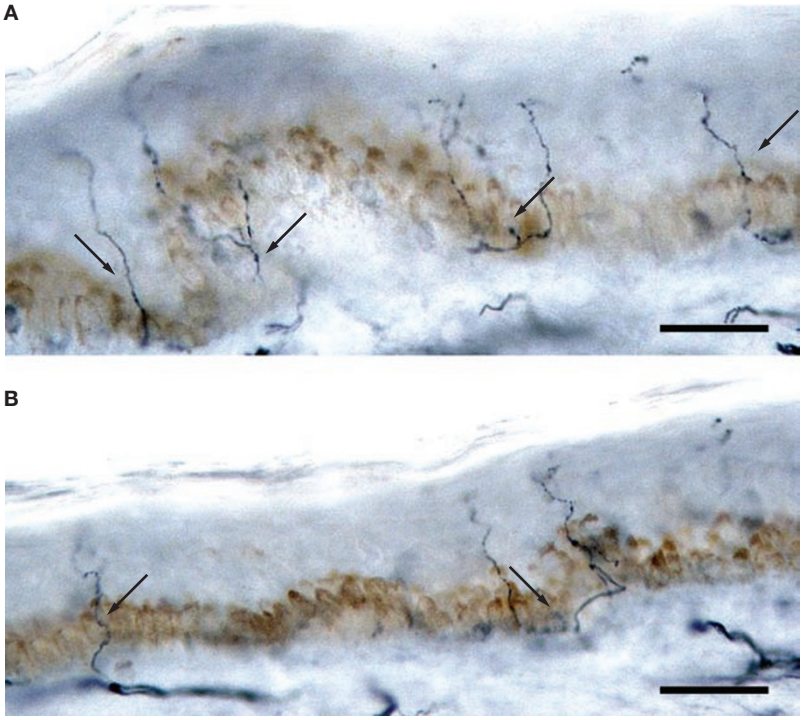

Figure 1 Normal innervation of the epidermis. (**A**) Proximal thigh. (**B**) Distal leg. Note the normal morphology of intraepidermal nerve fibers, indicated by the arrows. The fibers have a straight course and slight varicosities. Bright-field immunohistochemistry in 50 µm sections stained with polyclonal rabbit anti-protein-gene-product 9.5 antibody (Ultraclone, Wellow, Isle of Wight, UK). Bar = 60 µm.

The subpapillary dermis of both hairy and glabrous skin is innervated by bundles of unmyelinated and thinly myelinated fibers. Glabrous skin also contains several large myelinated fibers, which can be immunostained with antibodies against myelin proteins.[1,4] Recent studies have demonstrated that myelin ultrastructure and protein expression do not differ between skin and sural nerves.[5,6]

The innervation of dermal autonomic structures, including sweat glands, blood vessels, arrectores pilorum muscles and hair follicles, can be investigated using antibodies against molecules displayed on adrenergic sympathetic fibers (e.g. tyrosine hydroxylase and dopamine beta-hydroxylase), noradrenergic sympathetic

fibers (e.g. neuropeptide Y), cholinergic sympathetic fibers (e.g. vasointestinal peptide), and vasodilatory peptidergic fibers (e.g. calcitonin-gene-related peptide [CGRP] and substance P [SP]).[7] Skin biopsy, therefore, allows the investigation, separately and specifically, of somatic and autonomic nerve fibers, as well as small-caliber and large-caliber nerve fibers.

Functional properties of skin nerve fibers

Traditionally, skin nerves have been considered to be transducers of certain sensory stimuli (e.g. temperature, pain and touch) and to have a primary role in maintaining tissue integrity. Recent studies have demonstrated, however, that these nerves are part of a more complex network involving epidermal cells. The emerging view is that keratinocytes and other cells residing in the epidermis communicate with neighboring nerve fibers and might themselves participate in the transduction of certain physical and chemical stimuli. For instance, *in vitro* studies have shown that mechanical stimulation of keratinocytes can influence the activation of sensory neurons via an intercellular signaling mechanism involving ATP and purinergic receptors. Moreover, keratinocytes secrete chemical substances (e.g. neurotrophins, ATP, β-endorphin and interleukins) that can influence sensory neurons.[8] Peptidergic nerves (i.e. those expressing CGRP and SP) influence the maturation of keratinocytes and the ability of Langerhans cells to present antigens to lymphocytes.[9,10] Neuropeptides released from skin nerves exert various actions on resident cells and blood vessels, resulting in responses such as erythema, edema, hyperthermia and itch. Two groups of CGRP-positive IENFs, projecting to different layers of the dorsal horns, have been described: a large group co-expressing somatostatin, and a smaller group co-expressing SP. Activation of CGRP–somatostatin-positive axons inhibits the activation of CGRP–SP-positive axons during inflammatory responses.[11] Opioid and N-methyl-D-aspartate (NMDA) receptors have been also identified on skin nerves, suggesting a role for these nerves in neuropathic pain.[12,13]

IENFs are the endings of dorsal root ganglion nociceptors, as demonstrated by their expression of the capsaicin receptor TRPV1,[4,14] a protein that is essential for thermal hyperalgesia induced by tissue inflammation.[15] Keratinocytes also express receptors of the TRP family, such as TRPV3 and TRPV4,[16,17] which participate in temperature homeostasis and have a role in the pathogenesis of mechanical hyperalgesia.[18] Consequently, the whole epidermis can be considered to be a huge polymodal receptor, in which transmission of somatosensory sensation occurs by means of functional contacts between resident cells and nerves. No synaptic contact between keratinocytes and axons has been described, but the loss of Schwann cell ensheathment when IENFs cross the dermal–epidermal junction, similar to that seen when large myelinated fibers reach the inner core of mechanoreceptors,[19] probably favors communication through paracrine pathways.

The active participation of skin cells in the transduction of temperature and pain sensations could explain why IENF density alone does not correlate with the severity of neuropathic pain (see below). In fact, patients with chronic painful neuropathy can show a complete loss of IENFs, suggesting that IENFs are not the generators of pain in these individuals. On the other hand, complete skin denervation is found in patients with insensitivity to pain resulting from hereditary sensory and autonomic neuropathy type IV.[20] Skin cells could have a role in sensitizing undamaged unmyelinated fibers, in keeping with the concept of 'irritable nociceptors' proposed for postherpetic neuralgia.[21] Tissue damage could, therefore, lead to widespread functional and degenerative neuropathological changes, as demonstrated in complex regional pain syndrome type I, a condition characterized by a broad spectrum of sensory, autonomic and motor symptoms, but with no apparent evidence of nerve fiber degeneration.[22,23]

SKIN BIOPSY TECHNIQUES

Skin biopsy is usually performed under topical anesthesia with lidocaine using a sterile disposable 3 mm punch. The incision does not require suturing, the risk of bleeding and local infection is very low, and no side effects have been reported, even in patients with diabetes. Healing is usually complete within 1 week, and a barely visible scar can remain. For diagnostic purposes in patients with polyneuropathy one skin biopsy sample is taken at the distal end of the leg—10 cm above the lateral malleolus—within the territory of the sural nerve. A further biopsy sample can be taken at the proximal thigh—20 cm below the iliac spine—to demonstrate either the length-dependent process typical of polyneuropathy or the absence

of a length-dependent pattern, as observed in sensory neuronopathies. One advantage of the skin biopsy method is that its location can be chosen on the basis of the patient's signs and symptoms, and it can be conducted in regions where nerve conduction tests cannot be performed, such as the trunk and fingers.

The 'blister method' is another, even less invasive, method for obtaining samples of epidermis. This technique is performed by applying to the skin surface a suction capsule with single or multiple holes of 2–3 mm diameter,[24] which exerts a negative pressure and separates the epidermis from the dermis. This method does not cause bleeding and does not require local anesthesia, and it allows a large area of epidermis to be investigated. The blister method does not, however, provide information on the morphology of IENFs and the innervation of the dermis, so its usefulness in clinical practice is limited.

Quantification of somatic and autonomic skin nerve fibers

An important advantage of skin biopsy over nerve biopsy is that it allows morphometric analysis of small nerve fibers using bright-field immunohistochemistry or indirect immunofluorescence, rather than relying on more-complex electron microscope examination. Most laboratories use bright-field microscopy for routine diagnostic purposes (Figure 2). The biopsied tissue is cut into 50 μm sections, which are then immunostained with PGP9.5, a neuronal form of ubiquitin carboxyl-terminal hydrolase widely distributed in the PNS. PGP9.5-positive fibers crossing the dermal–epidermal junction are counted in at least three sections. The number of positive fibers is divided by the length of the epidermal surface, calculated using software for biological measurements, to calculate the linear density of IENFs (IENF/mm). Quantification of linear IENF density is a reliable tool, with high interobserver, intraobserver and interlaboratory agreement.[25] Linear density measurements correlate significantly with data obtained using stereological techniques of skin nerve morphometry,[26,27] and with quantification of nerve fibers per epidermal area.[28] No significant variation has been seen in IENF densities calculated in adjacent sections from the same biopsy or in adjacent biopsies from the same site.[29]

Indirect immunofluorescence provides an opportunity to investigate multiple epitopes in the same neural structure or in different structures. The use of confocal laser microscopy allows three-dimensional reconstruction of skin sections, which is particularly useful in the study of cutaneous receptors, sweat glands and blood vessels. Quantification of IENF density is performed using computerized image analysis.

Evaluation of dermal innervation is not currently included in routine morphometric analysis of skin biopsy tissue because of the lack of a validated protocol, although this parameter has recently been measured using the density of PGP9.5-immunoreactive structures and expressed as a percentage of the whole subepidermal area analyzed.[30] Morphometric data on normal sweat gland innervation are limited, and have been obtained using various methods, including calculation of nerve fiber length[31] and innervation per unit area.[32] Other authors have used semiquantitative[33] or qualitative approaches (Figure 3).[34,35] Sweat gland denervation and altered expression of neuropeptides were demonstrated in Ross syndrome and familial dysautonomia.[33,35] In idiopathic pure sudomotor failure,[36] IENF density and sweat gland innervation are preserved, suggesting that anhidrosis is caused by a functional impairment of cholinergic transmission (V Tugnoli, personal communication). Reduced skin innervation, inversely correlated with the size of blood vessels, has been found in port-wine stains, supporting the hypothesis of a causative defect in autonomic vessel innervation.[37] No study has systematically quantified the innervation of arrectores pilorum muscles, hair follicles and blood vessels.

Using immunohistochemical methods, punch biopsy has also demonstrated small-fiber neuropathy in burning mouth syndrome,[38] gastric denervation in diabetic patients,[39] and altered mucosal innervation in rectal hypersensitivity[40] and vulvodynia.[41]

Normative values of intraepidermal nerve fiber density

Reference ranges of IENF density in the leg are available for both bright-field immunohistochemistry and indirect immunofluorescence techniques. Four studies that investigated IENF density in the distal region of the leg using bright-field immunohistochemistry reported similar results. The mean (±SD) linear densities measured in these studies were 12.4 ± 4.6 IENF/mm (n = 98),[29] 13.8 ± 6.7 IENF/mm (n = 106),[42] 12.9 ± 5.3 IENF/mm

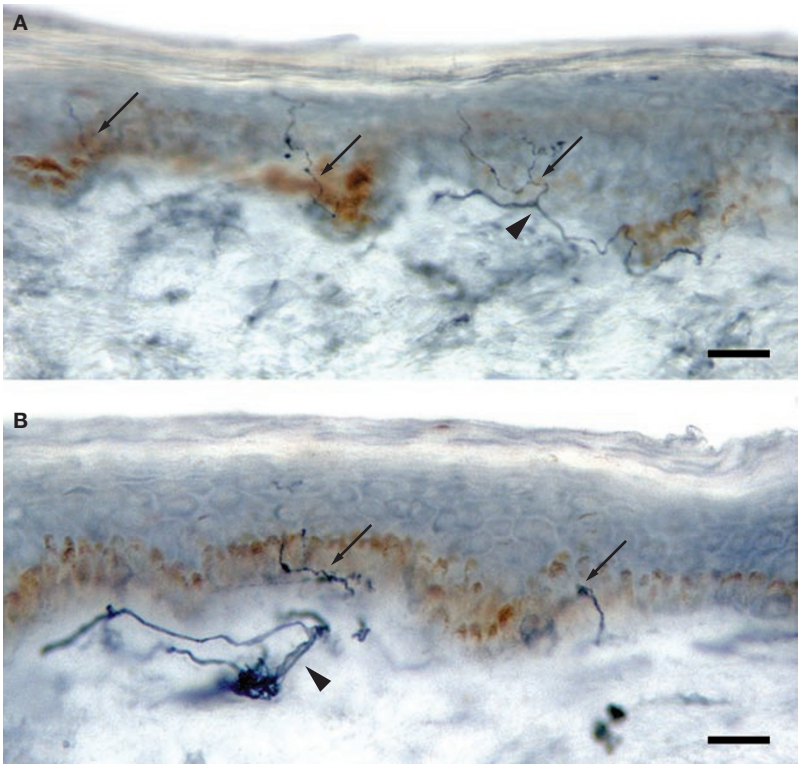

Figure 2 Skin biopsy samples from a patient with diabetic small-fiber neuropathy. (**A**) Proximal thigh. (**B**) Distal leg. Arrows indicate intraepidermal nerve fibers, arrowheads indicate dermal nerve bundles. The density of intraepidermal nerve fibers is reduced, particularly in the distal part of the leg, reflecting the length-dependent denervation of the skin. Bright-field immunohistochemistry in 50 µm sections stained with polyclonal rabbit anti-protein-gene-product 9.5 antibody (Ultraclone, Wellow, Isle of Wight, UK). Bar = 80 µm.

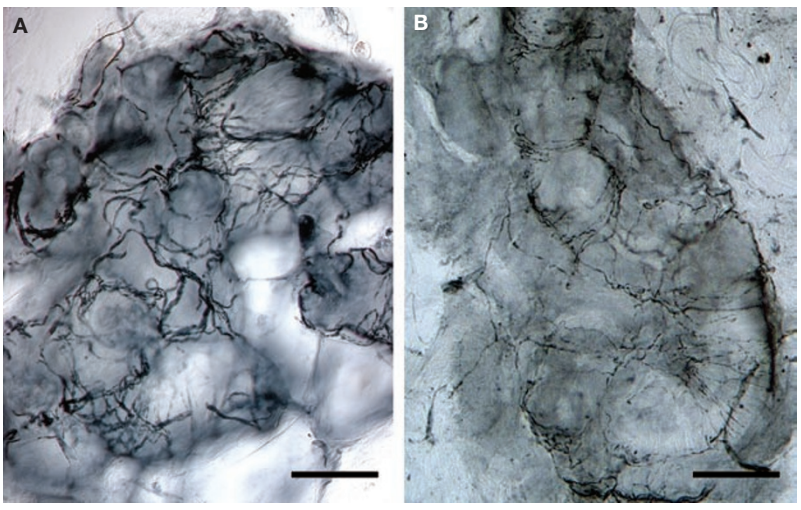

Figure 3 Innervation of the sweat gland. (**A**) Normal innervation in a healthy subject. (**B**) Reduced density of nerve fibers in a patient with peripheral neuropathy. Bright-field immunohistochemistry in 50 µm sections stained with polyclonal rabbit anti-protein-gene-product 9.5 antibody (Ultraclone, Wellow, Isle of Wight, UK). Bar = 80 µm.

($n = 55$),[43] and 15.0 ± 5.0 IENF/mm ($n = 84$).[44] Two of these studies also reported the IENF density in the proximal thigh, as 21.1 ± 10.4 IENF/mm[29] and 22.8 ± 6.9 IENF/mm.[44] Several smaller studies[45–49] have reported similar values. Indirect immunofluorescence (with or without confocal microscopy) has been used in fewer studies, none of which was specifically designed to assess the normative range of IENF density. Values reported for the distal region of the leg were higher than those found using bright-field immunohistochemistry, ranging between 17.4 ± 7.4/mm and 33.0 ± 7.9/mm.[50–53]

The normal IENF density in the arm was investigated in two studies that used different techniques. Pan and colleagues[43] found a density of 17.3 ± 6.2 IENF/mm in the distal forearm using bright-field immunohistochemistry, whereas Nolano and colleagues[1] estimated a density of 11.3 ± 2.9 IENF/mm in the glabrous skin of the fingertip using confocal microscopy.

The effect of aging on skin innervation remains uncertain. In the first normative study performed at the Johns Hopkins University, Baltimore, MD,[29] sex and age did not affect IENF density in the distal part of the leg. Similarly, two studies showed that IENF density in the fingertip[1] and abdominal skin[54] did not change with age. Aging did, however, correlate with decreasing IENF density in the distal part of the leg in studies performed in Norway and Singapore.[42,44] Researchers in Taiwan found a difference in density in the distal part of the leg between subjects aged below 60 years (11.1 ± 3.7 IENF/mm) and those over 60 years of age (7.6 ± 3.0 IENF/mm).[43,55,56] No other anthropometric variable influences IENF density in the distal part of the leg,[44] although male gender was associated with lower values in one study.[42]

DIAGNOSTIC YIELD OF SKIN BIOPSY IN SMALL-FIBER NEUROPATHY

In 2005, a task force of the European Federation of Neurological Societies published guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathies, in which the usefulness of the technique was established.[57] IENF density in the distal part of the leg has been found to be useful for confirming a diagnosis of peripheral neuropathy of various etiologies with specificity ranging from 95%[55] to 97%,[29] sensitivity ranging from 45%[29] to 80%,[55] a positive predictive value of 92%, and a negative predictive value of 90%.[29] Since 2005, other

papers, focused on pure small-fiber neuropathy, have provided additional support for the important role of skin biopsy in diagnosis.[58–61] Small-fiber neuropathy is frequently encountered in clinical practice, but can be misdiagnosed because of the absence of neurophysiological tests that can investigate small nerve fibers. In most studies, patients' complaints—mainly burning feet—are taken as the gold standard against which the performance of skin biopsy is measured. Studies in patients with clinically suspected pure small-fiber neuropathy reported that skin biopsy had a sensitivity of 90% and a specificity of 95%.[25,55] In idiopathic and secondary (diabetic, cytotoxic or amyloid) small-fiber neuropathy, skin biopsy analysis showed a positive predictive value of 95% and a negative predictive value of 91%.[28] Most recently, a study compared 99 patients who had painful neuropathy with 37 healthy subjects and assessed sensitivity and specificity of both IENF and dermal nerve fiber density quantification using receiver operating characteristic analysis.[30] In patients with pure small-fiber neuropathy, using a cut-off of 8.8 IENF/mm in the distal region of the leg, sensitivity of IENF density was 77% and specificity was 79%. Quantification of dermal nerve fiber density in addition to IENF density increased the sensitivity to 86%. IENF quantification is highly reproducible and its reliability as a diagnostic tool is not affected by the severity of the neuropathy.[29] Several smaller studies[45–53,62–69] involving a total of more than 500 patients have confirmed these data, demonstrating an important diagnostic role for skin biopsy in patients who have little clinical and neurophysiological evidence of neuropathy.

MORPHOLOGICAL CHANGES IN SKIN NERVES IN NEUROPATHIES

The presence of diffuse swellings on IENFs (Figure 4) has been shown to predict the progression to overt neuropathy in patients with HIV, diabetes or other causes of small-fiber neuropathy,[47,60,69] and to correlate with paresthesia.[60] Swelling was defined either quantitatively (e.g. enlargement above 1.5 µm)[47] or semi-quantitatively (e.g. enlargements to more than twice the diameter of the parent fiber).[60,69] Although swellings are commonly considered to be predegenerative changes, they can also reflect axonal regeneration, as has been observed after capsaicin denervation[70] or steroid treatment.[71]

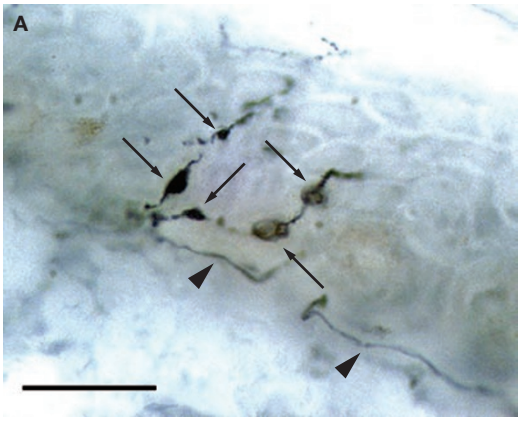

Figure 4 Skin biopsy tissue from the distal part of the leg in a patient with peripheral neuropathy. Arrows indicate the multiple swellings, some of them partly vacuolated, of an intraepidermal nerve fiber branching above the dermal–epidermal junction. Arrowheads indicate the nerves in the subpapillary dermis. Bright-field immunohistochemistry in 50 µm sections stained with polyclonal rabbit anti-protein-gene-product 9.5 antibody (Ultraclone, Wellow, Isle of Wight, UK). Bar = 50 µm.

Increased branching and sprouting of IENFs was found more frequently in patients with neuropathy than in healthy controls, and might represent an early marker of nerve fiber dysfunction.[2,72] We do not know, however, whether collateral sprouting represents attempted axonal regeneration with the goal of achieving functional recovery of nerve fibers.[73,74]

CORRELATES OF INTRAEPIDERMAL NERVE FIBER DENSITY

Clinical picture, etiology and neuropathic pain

The clinical picture of small-fiber neuropathy is dominated by spontaneous and stimulus-evoked positive sensory symptoms—namely thermal and pinprick hypoesthesia—that can mask the signs of small-fiber loss. Only a few studies have attempted to correlate IENF density with validated clinical scales. In patients with diabetic neuropathy, a negative correlation between IENF density and neuropathy symptom score was reported.[53,56] These studies also showed that the extent of epidermal denervation correlated with the duration of diabetes but not with hemoglobin A_{1C} levels, suggesting that IENF density might be useful as a marker of neuropathy progression. A recent study found a

high concordance between reduced IENF density and loss of pinprick sensation in the foot.[61]

Skin biopsy has allowed small-fiber neuropathy to be demonstrated in restless legs syndrome[75] and erythromelalgia.[76] In systemic diseases, such as systemic lupus erythematosus, sarcoidosis, Sjögren's syndrome, celiac disease and hypothyroidism, skin biopsy has enabled correlations to be found between neuropathic symptoms and small-fiber degeneration.[52,65,77–79] Although IENF density is a general marker of axonal integrity in peripheral neuropathies, it cannot be used to directly address the question of etiology. Skin biopsy findings can, however, indirectly contribute to the assessment of etiology. For example, in 40% of patients with small-fiber neuropathy diagnosed only after skin biopsy, oral glucose tolerance testing revealed a previously undetected impaired glucose tolerance.[49] Similarly, the distribution of IENF loss can help to differentiate between a non-length-dependent sensory neuronopathy and a length-dependent axonal neuropathy,[78,80] thereby leading to focused screening for associated diseases.

The relationship between IENF density and neuropathic pain remains uncertain. In HIV neuropathy, IENF density correlated inversely with pain severity when assessed by the patient, but not when the Gracely Pain Scale was used.[66] Another study found only a trend towards an inverse correlation between IENF density and pain intensity in this setting.[81] In diabetic neuropathy, patients with pain had lower IENF densities than did asymptomatic patients, but IENF density did not correlate with pain intensity within the group of symptomatic patients.[82] In patients with impaired glucose tolerance, diet and exercise induced a slight recovery of IENF density that was associated with a reduction in pain symptoms.[83] Similarly, epidermal reinnervation coincided with pain reduction after steroid treatment.[71] In length-dependent neuropathies, therefore, more-severe IENF loss seems to increase the risk of developing pain, the intensity of which might decrease in parallel with recovery of IENF density.

In postherpetic neuralgia, on the basis of evidence of relatively preserved skin innervation in the area of severe allodynia, normal thermal sensory function, pain relief in response to topical lidocaine, and worsening of pain with application of capsaicin, surgical removal of painful skin has been attempted.[84] After initial relief, pain increased, became intractable, and spread to previously unaffected dermatomes, suggesting the involvement of central mechanisms in the pathogenesis of neuropathic pain.

Sensory nerve conduction studies

Sural sensory nerve action potential (SNAP) amplitude, which reflects the integrity of large-diameter fibers, showed concordance with IENF density in the distal part of the leg in patients with large-fiber or mixed small-fiber and large-fiber neuropathy. Not surprisingly, skin biopsy analysis seemed to be more sensitive than sural nerve conduction studies for diagnosing small-fiber neuropathy.[62] One study,[85] however, showed that in patients with symptoms of small-fiber neuropathy and normal sural nerve conduction, reduced IENF density correlated with a decrease in SNAP amplitude in the medial plantar nerve. This finding suggests subclinical involvement of the most-distal large fibers in small-fiber neuropathy.

Psychophysical tests

The detection of thermal and pain thresholds using quantitative sensory testing has been widely used to assess the function of small nerve fibers. Although this approach is useful in population studies, it is an unreliable tool for diagnosing small-fiber neuropathy in clinical practice.[86] Moreover, the size of the probe used for the test can affect the results.[87]

In view of the fact that unmyelinated fibers and thinly myelinated fibers convey warm and cold sensation, respectively, thermal thresholds would be expected to correlate with IENF density. In diabetic neuropathy, IENF density was found to be inversely correlated with thermal and pain thresholds, showing the highest correlation with warm threshold.[53,56,82] Similarly, in Guillain–Barré syndrome lower IENF density was associated with increased warm threshold.[67] One study reported a significant correlation between cold pain threshold and signs of large-fiber impairment.[59] By contrast, others studies did not find any correlation between quantitative sensory testing results and IENF density.[45,51,88]

Autonomic tests

As IENFs are somatic unmyelinated fibers, their density would not be expected to correlate with autonomic fiber function. Intriguingly, however,

in patients with Guillain–Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy, lower IENF density was associated with a higher risk of developing dysautonomia.[64,67] These findings suggest that the integrity of IENFs might reflect the integrity of the whole class of small nerve fibers, including autonomic fibers. A few studies have investigated the correlation between IENF density and the results of a quantitative sudomotor axonal reflex test in patients with painful neuropathy and autonomic symptoms in order to test the hypothesis that IENF density and sweat output might decrease concomitantly. IENF density correlated with test results in one study,[63] but not in another.[51] In leprosy neuropathy, reduced nicotine-induced axon-reflex sweating correlated with decreased innervation of sweat glands.[88]

Nonconventional neurophysiological tests

Laser-evoked potentials (LEPs) have been used to investigate peripheral and central nociceptive pathways in trigeminal neuralgia and peripheral neuropathies. Late LEPs, reflecting Aδ-fiber activation, are delayed in patients with neuropathic pain, but can be enhanced when the pain has a psychogenic origin.[89] Recording of ultralate LEPs, reflecting activation of unmyelinated C-fibers, is less reliable than recording of late LEPs, thereby limiting the overall usefulness of LEPs in clinical practice. LEPs and skin biopsy findings have been examined in single case reports.[90] In two patients with Ross syndrome, abnormal LEPs correlated with decreased IENF density and increased thermal thresholds.[91] No study has yet looked for a correlation between results of skin biopsy analysis and recording of contact heat-evoked potentials, a technique that was recently proposed for investigating small-fiber function, but which cannot be used to assess C-fiber-related responses.[92]

Microneurography allows single-fiber recordings from nerves in awake patients. This technique demonstrated loss of nociceptive and skin sympathetic C-fiber activity that correlated with IENF and sweat gland denervation in a patient with hereditary sensory and autonomic neuropathy type IV.[20] In two patients with generalized anhidrosis, C-fiber recording and sweat gland innervation analysis distinguished postganglionic autonomic nerve fiber impairment from eccrine gland dysfunction.[34]

Sural nerve biopsy

The diagnosis of small-fiber neuropathy is better assessed by skin biopsy than by sural nerve biopsy.[57] IENF density can be reduced despite normal morphometry of unmyelinated and thinly myelinated fibers in sural nerve biopsy.[58] In a large comparative study,[62] skin and sural nerve biopsy findings were concordant in 73% of patients, but in 23% of patients IENF density was the only indicator of small-fiber neuropathy. Skin biopsy offers the opportunity to differentiate small nerve fibers with somatic function from those with autonomic function, thereby giving it a further advantage over nerve biopsy. In Charcot–Marie–Tooth disease and related hereditary neuropathies, a biopsy sample of the glabrous skin demonstrated the typical neuropathological abnormalities known from sural nerve studies.[5,6]

Immunohistochemical studies demonstrated IgM deposited specifically in the myelinated fibers of hairy and glabrous skin in patients with anti-myelin-associated-glycoprotein neuropathy.[93] Although skin biopsy can be contemplated in genetic and immune-mediated neuropathies, sural nerve biopsy should always be considered to confirm the diagnosis in inflammatory polyradiculoneuropathy with atypical presentation, or when vasculitic or amyloid neuropathy is suspected.

MONITORING THE PROGRESSION OF NEUROPATHY WITH SKIN BIOPSY

Skin biopsy is minimally invasive and can be repeated close to the site of a previous biopsy, within the territory of the same sensory nerve, to investigate the progression of neuropathy and the effect of neuroprotective treatments. IENFs can spontaneously regenerate, in parallel with sensory recovery, after nerve injury; for example, in diabetic truncal neuropathy,[94] following chemical denervation with capsaicin,[70] or after intracutaneous axotomy.[73] The ability of skin nerves to regenerate after topical capsaicin treatment prompted study of the rate of fiber regrowth in patients at risk of developing neuropathy. In diabetic or HIV-infected patients with no clinical or neurophysiological signs of neuropathy, the IENF regeneration rate is slower than in healthy subjects.[73,95] This finding provided insights into the pathogenesis of these common neuropathies and suggested the need for early neuroprotective interventions. In patients with impaired glucose tolerance, drastic

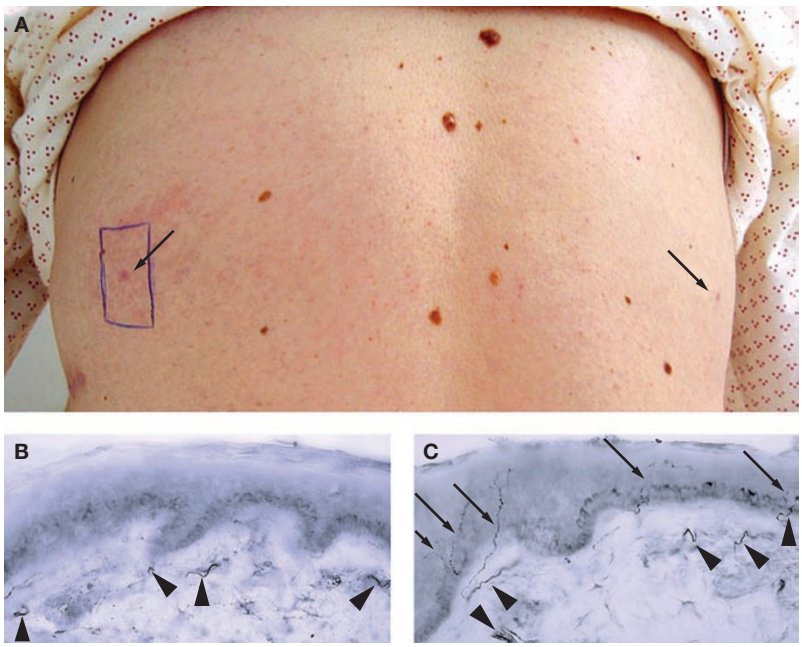

Figure 5 Skin biopsy in notalgia paresthetica. (**A**) Biopsies were performed within an area of notalgia paresthetica, indicated by the blue square on the left side of the trunk, and on the healthy contralateral dermatome. Arrows indicate the scars 2 weeks after biopsy. (**B**) Note the complete loss of intraepidermal nerve fibers and the denervation of the dermis in the pathological area. (**C**) Normally innervated skin in the healthy area for comparison. Arrows indicate intraepidermal nerve fibers, arrowheads indicate dermal nerve bundles. Bright-field immunohistochemistry in 50 µm sections stained with polyclonal rabbit anti-protein-gene-product 9.5 antibody (Ultraclone, Wellow, Isle of Wight, UK). Bar = 60 µm.

lifestyle change resulted in partial recovery of IENF density and sural SNAP amplitude, and reduction in pain.[83] Similarly, skin nerves were shown to regenerate in steroid-responsive neuropathy.[72]

Follow-up skin biopsy demonstrated that IENF swellings are early markers of axonal degeneration,[47,69] and that a reduction in IENF density predicts the transition to symptomatic neuropathy.[81] Evidence in experimental neuropathies[96–98] led to the use of skin biopsy results as outcome measures in trials of neuroprotective treatments for HIV neuropathy,[99] diabetic neuropathy,[100] Fabry's disease,[101] and leprosy.[102]

Given the accessibility of large myelinated fibers in glabrous skin by skin biopsy,[5] an ongoing trial with ascorbic acid in Charcot–Marie–Tooth disease type 1A has included the detection of myelin protein expression in skin biopsy tissue among the outcome measures.[103]

CONCLUSIONS: USEFULNESS AND LIMITATIONS OF SKIN BIOPSY

Skin biopsy should be considered in patients with symptoms of small-fiber neuropathy when nerve conduction studies do not reveal abnormalities. Innervation density should be assessed using a standardized quantitative method, the protocol for which has been recently published.[104] Qualitative examination of epidermal innervation density could be misleading, and should not be used alone to diagnose small-fiber neuropathy. Once small-fiber neuropathy has been diagnosed, focused screening (e.g. glucose tolerance test) and treatment of neuropathic pain can begin. Skin biopsy provides an opportunity to identify subclinical involvement of autonomic nerve fibers and degeneration of somatic nerves in neuropathies otherwise considered exclusively autonomic, leading to a better comprehension of symptoms and awareness of potential complications.

Skin biopsy allows preganglionic injury (e.g. radiculopathy), in which nerve fiber density is preserved, to be differentiated from postganglionic damage (e.g. plexopathy and neuropathy), in which nerve fibers degenerate. The technique can be useful when painful symptoms are localized in the trunk (e.g. notalgia paresthetica; Figure 5) or proximal regions of the limbs (e.g. meralgia paresthetica), where sensory nerve conduction cannot be recorded. Despite high specificity, sensitivity and positive and negative predictive values, some patients with neuropathy can show normal IENF density. In patients with typical length-dependent distribution of symptoms, however, predegenerative changes of skin nerves, including axonal swellings and weaker PGP9.5 immunoreactivity, are commonly found, and a follow-up biopsy should be performed to evaluate the progression of the neuropathy. IENF density within the normal range of values can be found in patients with demyelinating neuropathy with predominant large-fiber impairment.

One limitation of skin biopsy is that it cannot help in assessment of the etiology of neuropathy. The technique cannot replace nerve biopsy when neuropathological examination of mixed or large-fiber neuropathy is needed and when a vasculitic pathogenesis is suspected. Finally, despite high positive and negative predictive values in small-fiber neuropathy, normal IENF density cannot exclude a functional impairment of unmyelinated fibers.

KEY POINTS

- Skin biopsy analysis is a reliable tool for diagnosing small-fiber neuropathy
- Skin biopsy is a safe and painless procedure that allows somatic fibers carrying temperature and pain sensation to be differentiated from autonomic fibers
- Biopsy of glabrous skin can be performed to examine large sensory fibers in immune-mediated and inherited demyelinating neuropathies
- Loss of intraepidermal nerve fibers correlates with increased severity of neuropathy and a higher risk of developing neuropathic pain
- Skin nerve fibers are able to regenerate, and their regrowth rate could be a marker of early axonal damage in patients at risk of developing peripheral neuropathy
- Skin biopsy can be repeated in close proximity to a previous biopsy in order to assess the progression of neuropathy and the response to neuroprotective treatments, and can be used as a measure of outcome in clinical trials

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