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The Cutaneous Biopsy for the Diagnosis of Peripheral Neuropathies: Meissner's Corpuscles and Merkel's Cells

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Abstract

Cutaneous biopsy is a complementary method, alternative to peripheral nerve biopsy, for the analysis of nerve involvement in peripheral neuropathies, systemic diseases, and several pathologies of the central nervous system. Most of these neuropathological studies were focused on the intraepithelial nerve fibers (thin-myelinated A δ fibers and unmyelinated C fibers), and few studies investigated the variations in dermal innervation, that is, large myelinated fibers, Merkel's cell-neurite complexes, and Meissner's corpuscles. Here, we updated and summarized the current data about the quantitative and qualitative changes that undergo MCs and MkCs in peripheral neuropathies. Moreover, we provide a comprehensive rationale to include MCs in the study of cutaneous biopsies when analyzing the peripheral neuropathies and aim to provide a protocol to study them.

Keywords: skin biopsy, peripheral neuropathy, Meissner's corpuscles, Merkel's cells

1. Introduction

Since the last half of the past century, the analysis in the cutaneous biopsy of nerves, Merkel's cells (MkCs), and sensory corpuscles, especially Meissner's corpuscles (MCs), become a complementary method to diagnose peripheral neuropathies [1] and a reliable alternative to peripheral nerve biopsy. Nevertheless, it has been during the last decade that numerous studies have provided consistent evidence to support this technique as a valuable tool to understand the etiologies of some neurological diseases and to follow up clinical trials [2–4] (**Figures 1 and 2**).

Most of the neuropathological studies on cutaneous biopsies were focused on intraepithelial nerve fibers, which are thin-myelinated A δ fibers or unmyelinated C fibers [2, 3, 5–9]. Conversely, few studies have investigated the large myelinated fibers (although it can offer notable advantages over the unmyelinated ones [10]). Also, the quantitative and qualitatively changes in MCs and MkCs associated to peripheral neuropathies are poorly known although the study of MCs has gained interest [11–13].

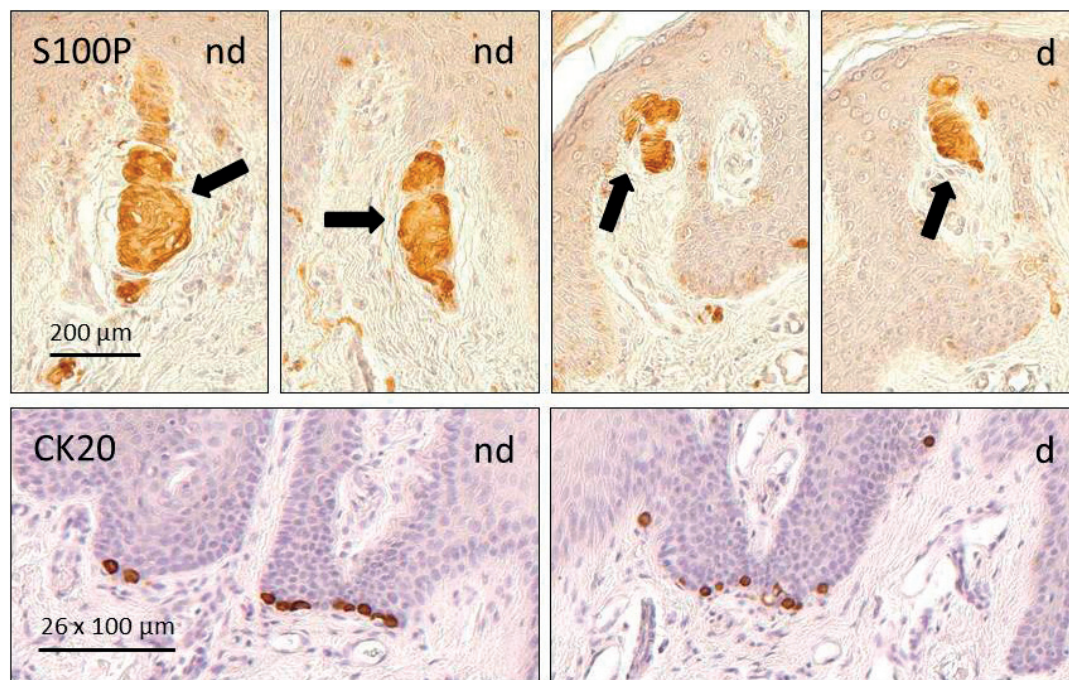


Figure 1.

Meissner's corpuscles (arrows) and Merkel's cells in the first toe skin of nondiabetic (nd) and diabetic (d) subjects as observed using immunohistochemistry for S100 protein (S100P) and cytokeratin-20 (CK20), specific markers for lamellar cells and Merkel's cells, respectively.

The evaluation of the dermal innervation, including large fibers, MCs, and MkCs, is not currently included within the routine analysis of skin biopsies because of the lack of a validated protocol. Changes in the density and size of MC and MkCs (i.e., variations in number/unit of surface, atrophy and/or hypertrophy, protein expression, etc.), can reflect quantitative or qualitative variations in the number of sensory neurons or nerve fibers innervating them or in the cells forming MCs themselves. Even more, they might also reflect pathologies of the central nervous system, and in these cases, the cutaneous biopsy becomes a method to study diseases difficult to be analyzed without invasive surgery.

This chapter is aimed to update the current data about the quantitative and qualitative changes in MCs and MkCs in peripheral neuropathies, as well as to provide a comprehensive rationale to include them in the study of cutaneous biopsies when analyzing the peripheral neuropathies. Furthermore, our purpose is to provide a technical protocol for analyzing MCs and MkCs in cutaneous biopsies. We have excluded from this review the intraepidermic nerve fibers because they have been extensively studied in peripheral neuropathies, and standardized method has been proposed and accepted [4, 9].

2. State of the art: a review and update of the literature

2.1 Why do we study Meissner's corpuscles and Merkel's cells for clinical purposes

The cutaneous MCs are sensory structures placed just beneath the epidermis within the dermal papillae in areas especially sensitive to light touch, like the fingertips, palms, soles, lips, and male and female genital skin [14–16]. They show an ellipsoid morphology with the main axis perpendicular to the skin surface and a size largely variable (length of 80–150 μm and diameter of 20–40 μm). Structurally, they consist of an axon that runs between the stacked nonmyelinating Schwann-like cells (the so-called lamellar cells) and habitually lacks a differentiated capsule [14, 16, 17].

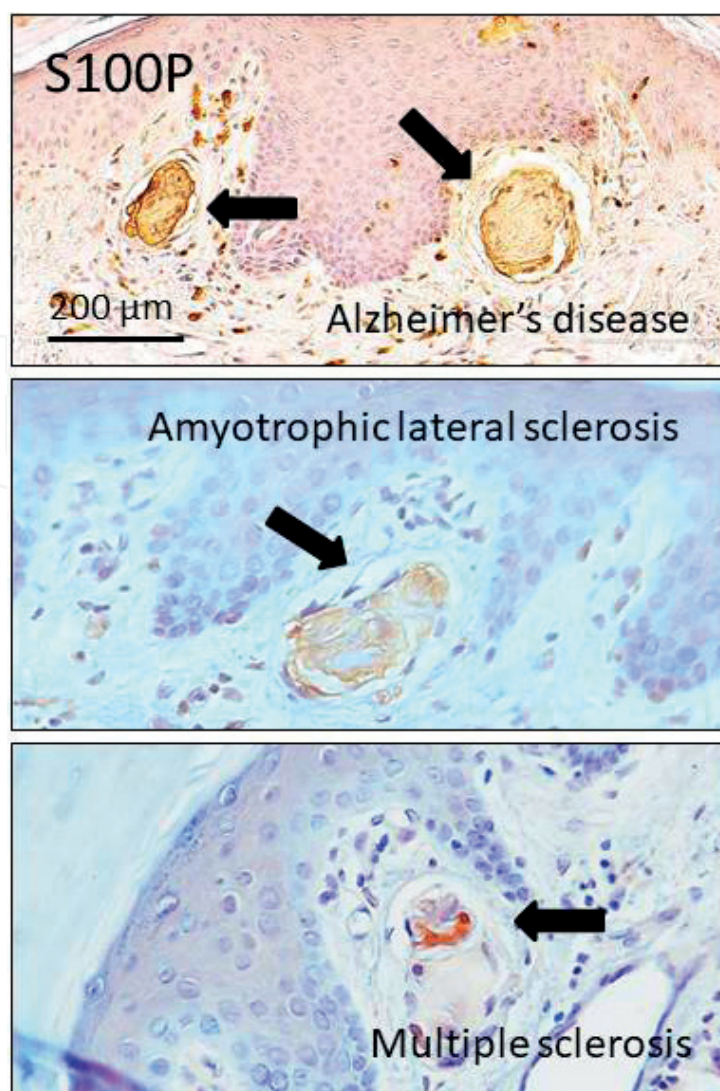


Figure 2.
 Meissner's corpuscles (arrows) of the palmar aspect of the fingers of patients diagnosed of Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis, as observed using immunohistochemistry for S100 protein (S100P). The samples were obtained during necropsy and in compliance with Spanish law.

MCs are particularly abundant in the fingers and palm hand, which are two zones easily accessible for biopsy. Nevertheless, the analysis of MCs from these zones has many problems. First of all, the normal density (MCs/mm²) at this localization should be determined to compare normal and pathological conditions. The most ancient studies established that the density of MCs in the human hand is ~10–24 MCs/mm² [18–20], it is higher in the fingertip (2.7/mm² ± 0.68) than in the palm (1.33/mm² ± 0.6), and it does not change significantly with age [21]. Nolano et al. [22] found 33.02/mm² ± 13.2 in the fingertip of digit III and 45/mm² in the digit V; Herrmann et al. [12] determined that the density of MCs on the palmar side of digit V is 12/mm² ± 5.3, whereas in the skin of the thenar eminence, it is 5.1/mm² ± 2.2.

The second trouble for the use of MCs in the diagnosis of neuropathies is whether or not MCs change in density and characteristic with aging. A reduction in number and size of MCs in elderly is generally assumed [18, 23–25], but detailed studies are not available. Preliminary data from our laboratory demonstrate that aging is accomplished of a reduction in the number and size of digital MCs, as well as changes in their architecture and immunohistochemical properties (García-Piqueras et al., unpublished). However, the variations in the corpuscular size and morphology of MCs are difficult to evaluate because of their large variability within the same skin sample. Therefore, in the absence of evident atrophy, hypertrophy, or

corpuscular disruption, the evaluation of these parameters must be cautiously considered when evaluating cutaneous biopsies.

The main constituents of MCs, that is, the axon and lamellar cells, contain specific proteins as widely demonstrated using immunohistochemistry [17, 26, 27]. These studies reported a large volume of information, but they are purely descriptive and do not consent to quantify those proteins and their possible variations in neuropathies. The central axon displays immunoreactivity for general neuronal markers (neuron-specific enolase, protein gene product 9.5, neurofilament subunit proteins). They also express Ca^{2+} -binding proteins such as calbindin D28k, parvalbumin, calretinin, and neurocalcin, which presumably regulate the axonic Ca^{2+} homeostasis and therefore participate in the mechanoelectric transduction. Recently, our research's group detected axonic TRPC6, TRPV4, ASIC2, and Piezo2 ion channels that work as putative mechanoproteins [28–30]. Regarding lamellar cells, the vimentin is the intermediate filament filling their cytoplasm, while the glial fibrillary acidic protein is always absent. They strongly express S100 protein collocated with parvalbumin or calbindin D-28 kDa. The lamellar cells also display immunoreactivity for TrkB, the signaling receptor for the neurotrophins BDNF/NT-4 [31]. Apart from axon- or lamellar cell-specific proteins, there are some others shared by both corpuscular constituents. They include p75NTR and TrkA (low-affinity pan-neurotrophin receptor and the high-affinity receptor for nerve growth factor, respectively; [32, 33]), the epidermal growth factor receptor [34], or cell death protein Bcl-2 [35]. The presence of some ion channels in the lamellar cells has been also reported [28–30]. It is possible that some of these proteins undergo changes during peripheral neuropathies, but limited information is so far available in this topic (see [17]). The proteins present in human MCs are summarized in **Table 1**.

The cutaneous MkCs are special epidermal cells placed in the basal layer of the epidermis, isolated or forming clusters, in both the glabrous and hairy skin. They are innervated by A β sensory axons connected through synapse-like contacts forming the so-called MkCs-neurite complexes. MkCs are involved in fine touch working as a part of slowly adapting type I low-threshold mechanoreceptors and express specific mechanoproteins [16, 30, 36–39]. MkCs have an epithelial origin and do not originate from the neural crest, as classically accepted [40–42].

Using immunohistochemistry, diverse proteins have been detected in the MkC-neurite complexes. They include low-molecular-weight cytokeratins and a repertoire of synaptic vesicles-related proteins (chromogranin A, synaptophysin), different neuropeptides as well as neurotransmitter receptors, neurotrophin receptors, ion channels (ASIC2 and Piezo2), and neuron-specific enolase [28, 43–46]. The axon of the MkC-neurite complexes displays immunoreactivity for general neuronal markers (**Table 1**).

The density of MCs varies from an anatomical region to another, and it is directly related to the sensibility of those zones [47]. In terms of density as far as we know, no age-dependent changes have been communicated. Recently, we have found significant reduction in of digital MkCs with aging (García-Piqueras et al., unpublished). On the other hand, whether or not MkCs, or the nerve fibers innervating them, are involved in peripheral neuropathies has been poorly studied, but this possibility should be explored because the easily accessibility to MkCs-neurite complexes.

2.2 Variations in MCs and MKCs in peripheral neuropathies

Data reporting changes in MCs in peripheral neuropathies are scarce and are restricted to diabetes and other rare inheretary neuropathies, HIV infection, mechanical or traumatic nerve entrapment, and a miscellaneous group of systemic diseases with neurological symptoms.

Protein	Meissner’s corpuscles		Merkel’s cell-neurite complex	
	Ax	LC	Ax	MC
<i>Axonal proteins</i>				
Neuron-specific enolase				
Protein gene product 9.5				
β-Arrestin 1				
GAP-43				
<i>Ca2+-binding proteins</i>				
S100 protein				
Calbindin D28K				
Calretinin				
Neurocalcin				
<i>Cytoskeletal proteins</i>				
Neurofilament proteins				
Vimentin				
<i>Growth factor receptors</i>				
p75NTR (pan-neurotrophin receptor)				
TrkA (NGF receptor)				
TrkB (BDNF/NT4 receptor)				
EGF receptor				
<i>Putative mechanoproteins (ion channels)</i>				
ASIC2				
Piezo2				
TRPC6				
TRPV4				
TRPM8				
<i>Cell death-live proteins</i>				
Bcl-2				
<i>Neuropeptides and bioactive amines</i>				
Serotonin				
Bombesin				
Vasoactive intestinal polypeptide				
Substance P				
CCK8				
Calcitonin gene-related peptide				
<i>Neuropeptide receptors</i>				
NMDA				
<i>Synaptic vesicle-associated proteins</i>				
Chromogranin A				
Synaptophysin				

Table 1.
Proteins detected in human Meissner’s corpuscles and Merkel’s cell neurite complexes using immunohistochemistry. Red: positivity for a protein in the axon of Meissner's corpuscles; Blue: positivty for a protein in the lamellar cells (LC) of Meissner's corpuscles.

2.2.1 Diabetic neuropathy

Distal symmetric peripheral neuropathy is one of the most common complications of diabetes [48] and involves motor, autonomic, and sensory nerve fibers. The histopathological studies have provided evidence that both the thin unmyelinated C fibers and the large myelinated ones are affected in on diabetic neuropathy. Consistently, the two most prominent complaints are peripheral pain and changes in touch [13, 49–52]. The intraepidermic nerve fibers as well as the nerve apparatus of the dermis are reduced in the diabetic neuropathy, and the reduction of the dermic nerves involves MCs. Importantly, although some authors have argued their interest in studying MCs and MkCs to better understand the diabetic neuropathy [53], only few studies have approached this topic.

In cutaneous biopsies, it was shown that the density of MCs is significantly reduced in diabetic patients with respect to the controls (10.2 ± 8.4 vs. $16.2 \pm 9.4/\text{mm}^2$, more evidently in type I than in type II diabetes), and this correlated with a reduction in median and ulnar nerves sensory amplitude; moreover, some MCs were hypertrophic or showed anomalies in their architecture (disorganization of the lamellar cells and increase in the irregularity of the axons) [54]. Similar findings as those obtained from cutaneous biopsy were observed using *in vivo* reflectance confocal microscopy at the thenar eminence and digit V [55]. We have recently communicated that long-term diabetic neuropathy courses with a reduction in the number and size of MCs and changes in their immunohistochemical profile [56] (**Figure 1**).

Nevertheless, the number and size of MCs are probably related with the time of evolution of the neuropathy. In fact, in an animal model of diabetes that develop neuropathy, MCs were found more abundant and hypertrophic during the first few years of hyperglycemia, whereas after a long time, the hypertrophy declines but the number of corpuscles remained higher than in age-matched nondiabetic subjects; furthermore, the MCs from the diabetic animals found had abnormal structure and immunochemistry properties [57].

On the other hand, as far as we know, the only study reporting a reduction in the number of immunohistochemically demonstrable MkCs in diabetic neuropathy was from our laboratory [56].

2.2.2 Charcot-Marie-Tooth disease

Charcot-Marie-Tooth (CMT) disease is a common inherited neuromuscular disorder characterized by neuropathies without known metabolic alterations. In the skin of patients with common and rare forms of CMT caused by different mutations, the density of MCs is reduced compared with normal controls [58–60]. Similar findings were reported by Almodovar et al. [61] using *in vivo* reflectance confocal microscopy.

2.2.3 Human immunodeficiency virus (HIV) neuropathy

HIV-sensory neuropathy is a common complication of HIV infection and may be associated with significant morbidity due to neuropathic pain [62]. Several approaches exist for quantitative assessment of human HIV-associated distal sensory polyneuropathy, and some of them have analyzed both unmyelinated and myelinated nerve fibers, as well as MCs. Using *in vivo* reflectance confocal microscopy, it was found a marked reduction in MCs [12, 63] in HIV+ subjects with and without distal sensory neuropathy [64].

2.2.4 Entrapment neuropathies

Surprisingly, little is known about the impact of entrapment neuropathy on target innervation. More than 20 years ago, we reported that human digital MCs survive to entrapment or section of peripheral nerves for more than 10 years, and although its number remains relatively stable, denervated MCs lack some antigens or change the pattern of expression of some others [65–67]. These data were confirmed recently in subjects undergoing carpal tunnel syndrome [68].

2.2.5 Miscellaneous

A reduction in density or loss of MCs has also been reported in the skin of patients suffering from Ross syndrome (a rare disorder of sweating associated with areflexia and tonic pupil) [69], POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) [70], systemic sclerosis [71], pachyonychia congenita (in contrast, MkC densities are higher) [72], chronic inflammatory demyelinating polyradiculoneuropathy [73], and systemic lupus erythematosus [12].

2.3 MCs are also altered in central nervous system disorders

In addition to the abovementioned peripheral neuropathies, changes in MCs have been reported in Parkinson's disease associated or not with dementia [74–76], spinobulbar muscular atrophy [77], Friedreich's ataxia [78], amyotrophic lateral sclerosis [79], or Guillain-Barré syndrome [73]. Furthermore, altered cutaneous innervation also has been observed in some psychiatric disorders [80] and mental deficiencies [81] (**Figure 2**).

3. Proposal of a method to systematically study MCs and MkCs in cutaneous biopsies

MCs are only present in glabrous skin, and therefore fingers or toes are appropriate regions to take cutaneous biopsies focused to evaluate them; in spite of the discrepancies regarding their density in these places, they are abundant enough.

In our opinion, the palmar aspect of fingertip IV would be an ideal region to be biopsied, because it is not involved in handling; the lateral borders should be excluded to avoid damaging the digital nerves and the formation of neuromas. On the other hand, toe pad biopsies can be also useful, but they contain a lower density of MCs than fingers [82].

The Joint Task Force EFNS/PNS [9] recommends to perform a 3 mm punch skin biopsy (including epidermis and the subpapillary and reticular dermis), using a sterile technique and under local anesthesia. A sample of these dimensions does not need sutures, heal completely within 1 week, and this normally guarantees no side effects or complaints. Informed consent is required, and information on the possible risks must be always provided. The fixation of the skin samples is recommended in 2% PLP (2% paraformaldehyde, 0.075 M lysine, 0.037 M sodium phosphate, 0.01 M periodate) or Zamboni's solution. We have also obtained excellent structural results and good antigen preservation using Bouin's fixative and buffered 10% formaldehyde. Conversely, 4% paraformaldehyde masked most of the antigens present in MCs. The thickness of the sections is also important. The Joint Task Force EFNS/

PNS especially recommends 50- μm thick sections to perform 3D reconstructions of MCs. Nevertheless, our experience demonstrates that to demonstrate the occurrence of most antigens present in the axon or in the lamellar cells of Meissner's corpuscles, 8 or 10 μm sections are appropriate.

There are different techniques for identification and assessment of MCs (silver impregnation techniques, electron microscopy, immunohistochemistry, and immunofluorescence), but the ideal one should allow to the quantification and specific immunostaining, distinguishing the different MCs constituents. In routine studies, at least one marker for the axon and one for the lamellar cells should be used. Indirect immunofluorescence, especially when associated with confocal microscopy, provides an opportunity to investigate multiple neuronal and nonneuronal proteins within the same MC and also to perform its 3D reconstruction using appropriate computerized image analysis systems. Ideally, double immunostaining for both axon and lamellar cells, associated or not with labeling of the nuclei, provides a global image of the morphology and size of the corpuscle, as well as of the arrangement of corpuscular constituents (**Figure 3**).

To quantify MCs, we use the method proposed by Verendelev et al. [83] to establish the density of MCs in the fingertips of primates. Briefly, 10- μm -thick sections, 200 μm apart, processed for S100 protein immunohistochemistry, are used. The sections are scanned by SCN400F scanner (Leica, Leica Biosystems™) and computerized using SlidePath Gateway LAN software (Leica, Leica Biosystems™). Then, in each section, MCs are identified and counted by two independent observers. The average numerical values were corrected applying the Abercrombie's formula: $N = n \cdot T / (T + H)$, where N is the corrected average number of MCs, n is the counted average number of MCs in all sections of a fingertip, T is the average section's thickness, and H is the average diameter of the counted MCs. Through a specific tool of the abovementioned software, the average MCs diameter was determinate measuring the horizontal axis by drawing a straight line approximately in the central region of each corpuscle. The longitudinal epidermis of each section (mm) is measured with the same tool, and the average length was multiplied by the section's thickness (mm) to give the measured surface area (mm^2). Finally, the average number of Meissner corpuscles (N) was divided by the surface area (mm^2) that is the density of MCs by squared millimeter of skin (number of MCs/ mm^2) (**Figure 4**). To establish the density of digital Merkel's cells, we used the same method immunostaining Merkel's cells for cytokeratin.

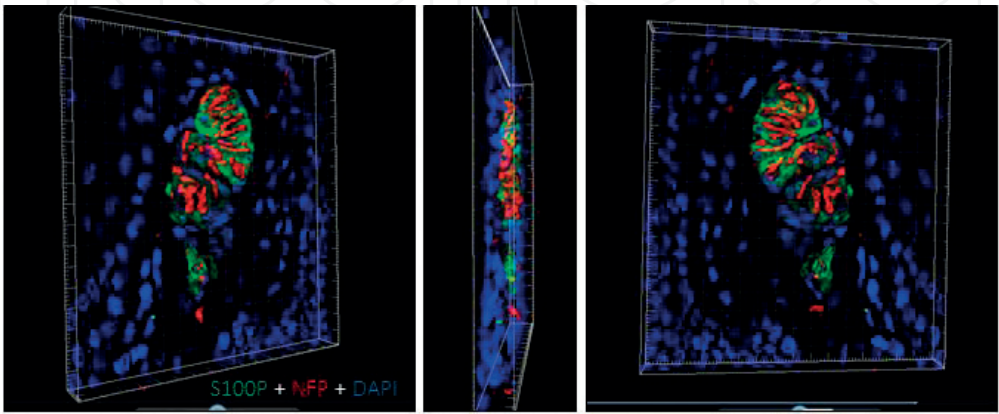


Figure 3. 3D reconstruction of a Meissner's corpuscle in a finger of a 25-year-old male. The axon is labeled in red, and the lamellar cells in green. The cell nuclei were labeled with DAPI. Scale bar = 20 μm .

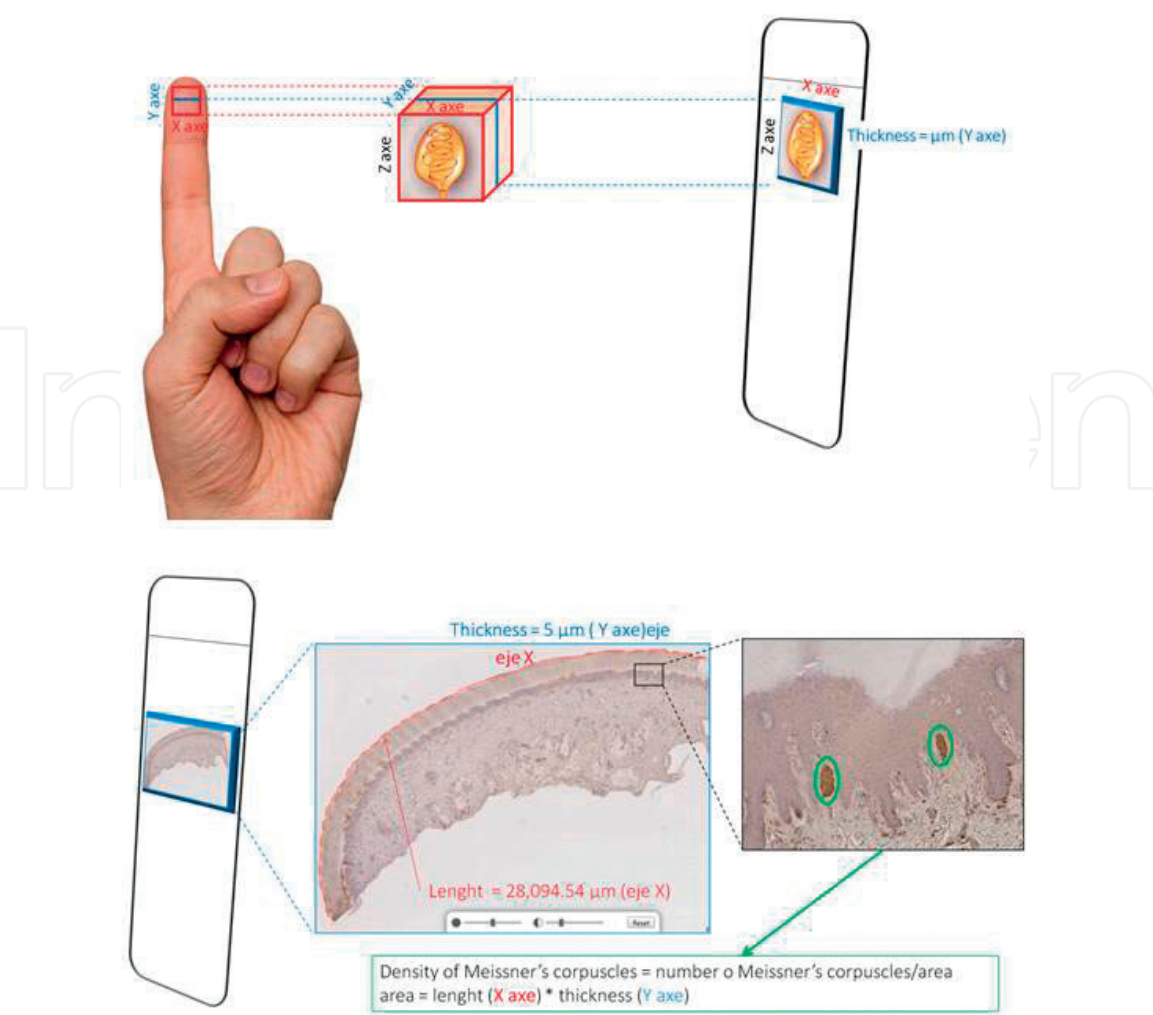


Figure 4.
Schematic representation of the technical procedure to quantify Meissner's corpuscles in sections of human digital skin immunostained for the detection of S100 protein.

4. Final remarks and future prospectives

Peripheral neuropathies are diverse and require a multidimensional approach for detection and monitoring clinical and research setting. The minimal invasiveness of skin biopsy makes it a useful tool not only for diagnostics but also for following the progression or the effects of a treatment in neuropathies.

Pathophysiological studies in patients with large nerve fiber polyneuropathies are limited because the difficulty in obtaining nerve samples due to the invasive nature of the procedure. For this reason, some authors utilized skin biopsies to obtain morphological and molecular information from large dermal myelinated nerve fibers. The development of new methods to evaluate skin innervation, including MCs, through noninvasive techniques, that is, in vivo reflectance confocal microscopy, may contribute to better understand the changes in sensory corpuscles in neuropathies [12, 55, 61, 84–86].

Nevertheless, to use MCs as a complementary method in the diagnosis of neurological diseases, more studies are still necessary. Firstly, the density of MCs must be mapped in the specific areas where they are abundant and easily accessible to cutaneous biopsy, especially the hand glabrous skin. Secondly, the physiological age-related changes in the number and protein composition of MCs of these selected areas must be established. Quantitative data, apart from qualitative, on

changes in protein composition of MCs with aging are necessary as a baseline for possible pathological changes. In addition to immunohistochemical studies, skin biopsy is amenable to the extraction of mRNA, RT-PCR, or microarrays for genes involved in neuropathies, and these methods should be used and standardized to study MCs. Finally, future studies should include not only neuropathies such as neurofibromatosis [85], or other rare metabolic neuropathies such as Gaucher type 1 disease [86], but also central nervous system diseases such as Alzheimer's disease.

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