SMALL FIBER NEUROPATHY: PATHOPHYSIOLOGICAL MECHANISMS AND DIAGNOSTIC METHODS

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Abstract

Peripheral neuropathy is a prevalent disorder dominated by both sensory and motor fiber loss, leading to a significant reduction in quality of life. Nevertheless, only a minority of patients with peripheral or central lesions suffers from neuropathic pain. Here, we summarize diagnostic tests that assess the severity of structural and functional impairment such as quantitative sensory testing, axon reflex flare responses, evoked potentials, corneal confocal microscopy and intra-epidermal nerve fiber density evaluated with skin biopsies. We focus on processes and conditions that may lead from peripheral neuropathy to neuropathic pain, and on the identification of pain mechanisms also including results from direct nerve recordings (microneurography). Recent studies analyzing specific neuronal markers such as CGRP, sodium or calcium channels, inflammatory mediators and growth factors may help to characterize the mechanisms that induce neuropathic pain.

Keywords: Neuropathic pain, painful neuropathy, peripheral neuropathy, nerve fibers, pathogenesis of painlatelet, anticoagulation

ΠΕΡΙΦΕΡΙΚΗ ΝΕΥΡΟΠΑΘΕΙΑ: ΠΑΘΟΦΥΣΙΟΛΟΓΙΚΟΙ ΜΗΧΑΝΙΣΜΟΙ ΚΑΙ ΜΕΘΟΔΟΙ ΔΙΑΓΝΩΣΗΣ

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Περίληψη

Η περιφερική νευροπάθεια αποτελεί μία επικρατούσα διαταραχή στην οποία επικρατούν τόσο αισθητικές όσο και κινητικές διαταραχές, οδηγώντας σε εκσεσημασμένη μείωση της ποιότητας ζωής του ασθενούς. Παρόλα αυτά, μόνο μία μειοψηφία ασθενών με περιφερικές είτε κεντρικές εντοπίσεις νευροπάθειας παρουσιάζουν νευροπαθητικό πόνο. Στη παρούσα ανασκόπιση, συνιψίζονται οι διαγνωστικοί μέθοδοι οι οποίες αξιολογούν τη σοβαρότητα της δομικής και λειτουργικής βλάβης που προκαλεί η νευροπάθεια, τον ποσοτικό έλεγχο της αισθητικότητας με διαδερμική ηλεκτική διέγερση, τα προκλητά δυναμικά και την πυκνότητα ενδοεπιδερμικών νευρικών ινών αξιολογούμενη με διενέργεια βιοψιών δέρματος. Εστιάζουμε στις καταστάσεις που μπορεί να οδηγήσουν από την περιφερική νευροπάθεια στον νευροπαθητικό πόνο και στον εντοπισμό των μηχανισμών που το προκαλούν. Πρόσφατες μελέτες που αναλύουν συγκεκριμένους νευρωνικούς δείκτες όπως το CGRP, τα κανάλια νατρίου ή ασβεστίου, φλεγμονώδεις μεσολαβητές και αυξητικούς παράγοντες οι οποίοι, μπορούν να βοηθήσουν στην διασαφήνιση των μηχανισμών που οδηγούν από την περιφερική νευροπάθεια στον νευροπαθητικό πόνο.

Λέξεις-κλειδιά: νευροπαθητικός πόνος, χρόνιος πόνος, περιφερικές νευροπάθειες, επώδυνες νευροπάθειες, παθογένεση του πόνου

Introduction

The basic characteristics of peripheral neuropathy include both motor and sensory loss including all types of sensory nerve fibers—large myelinated and small unmyelinated. Thus, primarily, one might expect an impaired pain sensation. However, a minority of patients with either peripheral or central lesions develop neuropathic pain despite the reduced responsiveness to acute noxious stimuli. Neuropathic pain is defined by the International Association for the Study of Pain (IASP) as, "pain caused by a lesion or disease of the somatosensory nervous system." Management of patients who present with chronic pain is a common problem in medical care (Jensen and Finnerup, 2007) and therapeutic options are often insufficiant. Well-established functional assessment is enabling the identification of different sensory phenotypes in neuropathy, but it is still unclear to determine whether these functional phenotypes are linked to pain mechanisms (Baron et al., 2010) in particular, as these sensory phenotypes do not differentiate between neuropathy patients with and without pain (Held et al., 2019;Forstenpointner et al., 2020;Matesanz et al., 2020;Schmelz, 2020). Patients with predominant features of small fiber neuropathy coupled with clinical and nerve conduction findings from large sensory fibers dysfunction should be considered as having a mixed (small and large fiber) sensory neuropathy, also referred to as predominant small fiber neuropathy (SFN) (Cazzato and Lauria, 2017; Lauria, 2010).

Mechanisms that determine whether a periphery lesion leads to a complete loss of sensory or is accompanied with chronic pain have not been described yet. In the attempt to clarify the structural and functional patterns of small fibers to patients with neuropathy and pain, research has been focusing on quantitative sensory testing (QST) (Aasvang et al., 2008; Dyck et al., 2000; Freynhagen et al., 2007; Jaaskelainen et al., 2005; Maier et al., 2010; Rolke et al., 2006), the spatial extent of the axon reflex flare (Bickel et al., 2002; Kramer et al., 2004b; Novak et al., 2001), and intraepidermal nerve fiber density in skin biopsies(Devigili et al., 2008; Ho et al., 2008; Oaklander, 2001; Oaklander, 2008; Polydefkis et al., 2002; Cazzato and Lauria, 2017; Kokotis et al, 2016).

Although earlier studies (Oaklander, 2001; Polydefkis et al., 2002) had shown that the more pronounced loss of nerve fibers in the epidermis is able to predict the intensity of neuropathic pain, recent studies no longer find this correlation and epidermal nerve fiber density (histologically evaluated) as an objective indicator of small fiber neuropathy (Cruccu et al., 2010; Devigili et al., 2008; Ho et al., 2008; Oaklander, 2008). However, no obvious correlation between reduced fiber density and pain was detected (Devigili et al., 2008; Landerholm et al., 2010; Karlsson et al., 2019). As a result, the mechanisms determining the development of neuropathic pain remain unclear.

In an attempt to clarify this question, studies have been conducted that combined the functional and structural examinations of small nerve fibers in chronic patients with and without pain in order to reveal these mechanisms that lead some of the patients with neuropathy to feel pain, either localized, or generalized and diffused. More specifically, quantitative sensory testing, axon reflex flare, microneurography, and skin biopsy are used, as well as additional tests, such as measuring small fiber-related evoked potentials and corneal confocal microscopy, that might contribute to a better understanding of these neuropathies. Biochemical markers might also serve a tool in screening patients for the presence of small fiber neuropathy and in assessing disease progression.

DIAGNOSTIC METHODS

Skin biopsy

Punch biopsy is used for the diagnosis procedure. The removed skin tissue, after being properly treated with immunohistochemistry and immunofluorescence to be readable, is analyzed with an optical fluorescence microscope (e.g. Zeiss Axiophot) with conventional filters that allow different lengths of radiation to pass through the tissue depending on the fluorescent antibody that is conjugated within the target structure. Fluorescent images are collected with a high-definition camera (e.g. Sony, dkc-ST5) and processed with appropriate software (eg imageJ, Photoshop) to measure cutaneous, subcutaneous, and dermal nerve fibers. Specific fiber subpopulations such as the sympathetic postganglionic fibers for the sweat glands, or depending on the expression of peptides, such as peptide P or CGRP, can also be evaluated. Finally, the number of macrophages or T lymphocytes in the skin can be assessed. The reliability of skin biopsy has been strengthened by the evidence that the value of Intra epidermal nerve fibers (IENF) density is consistent when comparing biopsies taken from the right and left ankle of both healthy individuals and neuropathic pain patients, and that it is stable at 3-week follow-up, through a follow-up biopsy in the same sensory territory (Lauria and Devigli, 2007; Lauria, 2010; McCarthy et al., 1995; Cazzato and Lauria, 2017; Sopacua et al., 2018).

Corneal confocal microscopy

Corneal confocal microscopy (CCM) was introduced very recently for assessing abnormalities of the small corneal fibers in patients with diabetes.

Recent studies have strengthened the use of this non-invasive and repeatable technique that has been already applied to neuropathies of several causes. Automated analysis of corneal nerve images and the development of normative values have made CCM more reliable for diagnosing small fiber neuropathy. The diagnostic sensitivity and specificity of this technique in diabetic polyneuropathy are 91% and 93% respectively, and the quantification of small fibers in the cornea by use of corneal confocal microscopy has been shown to be associated with the severity of diabetic polyneuropathy. Despite the promising findings of corneal confocal microscopy to date, additional studies are needed to determine its specificity and sensitivity for neuropathic pain conditions before the method can be used as a tool for diagnosing SFN in clinical practice and research settings. While intra-epidermal fiber density quantifies single unmyelinated axons, CCM quantifies the density of small subcorneal nerve fascicles. Decreased density or alterations in corneal nerve fibers have been demonstrated in patients with Sjögren's syndrome through CCM, and more recently, in those with HIV infection, amyloidosis, Fabry's disease, or sarcoidosis (Cazzato and Lauria, 2017; Terkelsen et al, 2017; Sène, 2017).

Axon reflex erythema

Laser Doppler scans are performed to assess the increase of the axon reflex erythema that develops upon increasing the stimulation intensity. For the electrically induced axonal reflex erythema, transcutaneous electrical stimulation (1 Hz, 0.5 ms duration) is applied through bipolar electrodes. In parallel, the severity of electrically induced pain is monitored.

The center of the stimulus is usually identical to the site of the subsequent skin biopsy and should display the center of the painful area—it is the most painful part for localized neuropathies and the dorsal surface of the foot for generalized neuropathies. The scan area must be at least 50 cm^2.

The area and extent of increased blood flow is assessed with a Laser Doppler Flowmeter, either Moor Laser Doppler Imaging (LDI) or Perimed Laser Doppler (Periscan PIM).

The area and amplitude of the electrically generated stimulus are defined by the increase in blood flow which should be 2 times greater than the initial one. Each point above the threshold is included in the axon reflex erythema surface (given in cm^2). This test evaluates the function of peptidergic C-nociceptors, presumably, mainly mechano-insensitive chemonociceptors (Schmelz et al., 2000).

Evoked potentials

Nociceptive evoked potentials can be generated by either radiant heat (laser-evoked potentials, LEPs) or contact heat (contact heat-evoked potentials, CHEPs). Both LEPs and CHEPs are based on selective activation of Aδ- and C-fibers, whereas induction of pain-related evoked potentials (PREPs) involves the preferential stimulation of Aδ-fibers. Skin denervation induced by topical capsaicin tends to decrease the LEP amplitude. Laser-generated radiant heat pulses selectively excite free nerve endings in superficial skin layers, synchronously activating Aδ and C nociceptors and resulting in heat that evokes potentials. Research has shown that LEP amplitudes are grossly in accordance with the reported intensity of perceived pain, but do adapt upon repetition unlike the pain (Liberati et al., 2018). Recent studies on different peripheral and central nervous system diseases suggest a highly significant difference in controls, with high specificity but low sensitivity. As mentioned above, CHEPs are recorded after the thermal stimulation of the skin through a contact thermode using heat pulses with different peak temperatures to stimulate Aδ- and C-fibers. The main advantage of CHEPs when compared to LEPs is that there is no evident lesion of the skin. A strong correlation was found in a cohort between CHEP amplitudes and the degree of skin innervation. Patients with sensory neuropathy and an Intra epidermal nerve fiber (IENF) loss seem to have lower-amplitude CHEPs. Age and gender adjusted standardized values have been reported for the clinical use of CHEPs. It should be noted that due to the slower rise time of the contact heat stimulus compared to the laser pulse, the latencies of CHEPs are longer than that of the LEPs. Even though no microneurographic studies are available to establish the selective activation of nociceptors after contact heat cutaneous stimulation, a dipole modeling study demonstrated that the same cerebral areas activated by laser pulses delivered on the skin are also involved in CHEP topography building. Further studies about CHEPs and their correlation with neuropathic pain need to be conducted. Intraepidermal electrical stimulation (IES) has also been described as a potential additional tool in detecting functional changes in Aδ-fibers and C-fibers in patients with neuropathic pain (Sopacua et al, 2018; Lauria et al, 2010; Pazzaglia and Valeriani, 2009; Lacesa et al., 2015).

Although laser stimuli activate both Aδ- and C-fibers, 'ultra-late' potentials (750-1200 ms), are related to C-fiber activation (C-LEPs). These potentials can be obtained only with dedicated techniques that have not yet been standardized for clinical application. To obtain evoked potentials specifically related to C-fibers, the Aδ component of the evoked potential's afferent current must be suppressed by

pressure-block of A-fibers, or by various techniques such as stimulation of tiny skin areas, spectral analysis of expected time windows, selection of single trials devoid of Aδ-LEPs or stimulation of large areas at low intensities. LEPs are more sensitive than any other neurophysiological test, and the finding of LEP suppression helps to diagnose neuropathic pain (Cruccu et al, 2010).

Quantitative sensory testing

For the quantitative sensory testing, a bilateral functional assessment is conducted on the painful site and the corresponding healthy one in unilateral diseases (e.g. postherpetic neuralgia, radiculitis), while in systemic neuropathies, the test is conducted in the peripheral and proximal parts of the limbs, using standardized stimulators (e.g. Somedic® Thermo Test, Medoc®). Measurements are taken for thermal thresholds, i.e. warm/cold and painfully hot/cold. The average of at least 3 measurements is used to determine the sensory threshold.

In some studies, thermal threshold deterioration was associated with the intensity of pain in peripheral neuropathy (Ng Wing Tin, et al., 2014), but more recent studies did not find such correlation (Held et al., 2019;Forstenpointner et al., 2020). Interestingly, increasing pain intensity may also have some central inhibitory effects on pain detection as observed in patients with carpal tunnel syndrome that also had impaired sensory function in the non-injured radial nerve (Matesanz et al., 2020). While confirming the utility of QST for the diagnosis of sensory neuropathies, particularly diabetic and small fiber neuropathy (SFN), QST is not being suggested as a stand-alone examination for diagnosing neuropathic pain. Therefore, a standard methodology, validated algorithms and reference values adjusted for anatomical site, age, and sex should be used. The German Research Network on Neuropathic Pain has developed a standardized QST protocol consisting of seven different tests measuring 13 parameters. Standardizing reference values are available for both sexes, all age groups, and several body regions (including the face, hand, and foot). QST parameters have proven to be region specific and age dependent, and less sensitive in older patients than in young individuals. Such a comprehensive test protocol has been shown to be sensitive in detecting small fiber neuropathy even in children (Blankenburg et al., 2012). However, QST mirrors sensory function rather than ongoing pain experience (Gierthmuhlen et al., 2019), and cannot be used for the evaluation of clinical pain levels (Forstenpointner et al., 2020).

Patients' mood and cognitive settings that could make the results unreliable should also be taken into consideration when attempting the interpretation in the clinical context. Nevertheless, using appropriate standards of equipment and examination protocols, and the certification of examiners should reduce variability, thereby contributing to a more reliable classification of patients (Cazzato and Lauria, 2017; Bakkers et al, 2013; Terkelsen et al, 2017). Thus, phenotypes based on various aspects of clinical and ongoing pain appear to have profound implications for the preferred therapeutic approach (Bouhassira et al., 2020) and may also include somatization, depression, and anxiety to sensory function (Gaynor et al., 2020).

Microneurography

Microneurography is used to record the activity of C-nociceptors and sympathetic fibers and to test the efficacy of different compounds in blocking abnormal on-going activity in both animal models and in patients. The use of microneurography is increasing in disorders affecting the peripheral nervous system. Signs of hyperexcitability have been observed in peripheral nerve fibers in connection with neuropathies and peripheral neuropathic pain conditions, and the affected fibers have been shown to be greater in neuropathic patients with chronic pain than in neuropathic patients without pain (Sopacua et al, 2018; Mainka et al, 2015). Most importantly, higher levels of spontaneously active nociceptors were found in patients with painful neuropathy as compared to patients with painless neuropathy (Kleggetveit et al., 2012).

Co-evaluation of the tests

Perceptions of heat, cold, and pain from heat were reduced on patients' skin, as was the area of axonal reflex erythema and the epidermal density of nerve fibers. Therefore, the functional and structural parameters of the afferent sensory nerve fibers confirmed the neuropathy in patients' skin. The impairment of cold and warm thresholds was associated with lower density of the epidermal fibers while the pain caused by the hot, cold or electrical stimulus, as well as the area of the axonal reflex erythema was correlated with the deeper inner layer of dermal innervation. Pain intensity was found to be higher in those patients with a smaller reduction in epidermal fiber density and a better threshold for cold sensation. However, no reliable indicator of the onset or absence of pain was determined in patients (Cazzato and Lauria, 2017; Karlsson et al., 2019; Matesanz et al., 2020).

Patients with localized pain had the highest clinical pain ratings (spontaneous and induced) and were more likely to experience cold or tactile allodynia,

as reported by other researchers in traumatic injury (Landerholm et al., 2010; Kleggetveit and Jorum, 2010). In contrast, these patients had a better retained sense of heat, with no apparent relationship between the heat threshold and neuropathic pain. Thus, to interpret this finding we must either speculate about a connection of the warm-sensitive channels (TRPV3) to those of the neuropathic pain (Facer et al., 2007), or it is much more likely that the less affected heat threshold reflect the slightly higher density of epidermal fibers in patients with pain.

In patients with generalized neuropathy, a greater reduction of the heat threshold and the corresponding pain was found, which was associated with a greater loss of skin fibers. This means that skin denervation can be protective against allodynia. A weak correlation between epidermal fiber density and clinical pain assessments confirms recent observations by other researchers in postherpetic neuralgia (Petersen et al., 2010) where a greater reduction in the number of epidermal fibers does not mean a greater reduction in pain sensation. Cutaneous innervation obviously reflects the degree of small fiber neuropathy (Attal et al., 2008; Devigili et al., 2008; Ho et al., 2008; Oaklander, 2008) but does not appear to be a determining factor of pain sensation in these patients.

In another research for diabetes without sensory large nerve involvement, a significantly lower (IENFD) Intra epidermal nerve fiber density and higher cold perception threshold were found in comparison with controls, with no correlation to whether they had symptoms of polyneuropathy or not. Nevertheless, a reduction of IENFD was the most common abnormal finding in the subgroup of patients with neuropathic symptom, and consequently seemed more sensitive as a diagnostic tool (Loseth, et al., 2008; Sopacua et al., 2019).

Although skin biopsy selectively assesses endodermal nerve fiber density and accurately diagnoses small fiber neuropathy, no studies have reported a direct relationship between neuropathic pain and skin biopsy data. In other words, whereas the examination of skin biopsy invariably points to reduced dermal nerve fiver density in patients with painful neuropathy, it also does so in patients with painless neuropathy (Cruccu et al, 2010).

MEDICAL CONDITIONS THAT ARE ASSOCIATED WITH NEUROPATHY

I. Alcohol induced painful neuropathy

Regarding alcohol induced neuropathy, recent reviews have indicated that it involves sensation disorders with or without neuropathic pain, also, distal paresis and vegetative dysfunction may also appear. Axonal sensorimotor neuropathy has been confirmed through nerve conduction studies. Neuropathological tests reveal that the small nerve fibers are particularly affected, explaining the associated pain. Treatment includes alcohol abstinence and modified dietary habits to correct malnutrition. If abstinence is maintained, neuropathy can resolve within months to years (Sommer et al., 2018).

II. HAART treatment and IEFND

It has been observed that the duration of highly active antiretroviral therapy (HAART) treatment, expressed as the sum of the months of administration, is clearly associated with decreased cutaneous nerve fiber density (IENFD) in the lower leg and an increment in the latency of sympathetic skin response, SSR, (foot-arm) indicative lesion, both afferent and efferent. This result is consistent with previous observations that indicate a significant reduction in IENFD accompanied by other structural abnormalities. These changes can occur in the early stages of HIV treatment in patients, even during the asymptomatic stage of neuropathy. The above findings were initially identified in the lower leg as a result, of a dying back process (Pardo et al., 2001; McCarthy et al., 1995; Herrmann et al., 2004; Zhou et al., 2007; Skopelitis et al., 2007).

However, in addition to the change in the number of epidermal fibers, increased branching and sprouting were considered as early signs of nerve fiber dysfunction in many neuropathies (Lauria and Devigili, 2007). Therefore, we evaluated the epidermal fibers in the skin biopsy of our patients and observed that their branching, as estimated by counting the epidermal fibers not passing through the basal membrane of the epidermis, decreased slightly only during treatment. In contrast, the number of nerves passing through it (IENFD) decreased significantly. Therefore, the sprouting / IENFD ratio increases with the duration of HAART therapy, indicating an intact compensatory mechanism that promotes the germination of the remaining number of fibers. One study investigated the global spatial sampling in order to determine the epidermal nerve fiber length density (ENFLD), taking into account its biologic complexity (Karlsson, et al., 2013). Results showed that ENFLD is comparable to IENFD in differentiating between SFN and healthy individuals (Karlsson, et al., 2013). It should be noted that there is no consensus about the benefits of certain molecular mechanisms linked to HAART treatment, leading to neuropathic pain. Even worse, there is even no consensus if it's induced from peripheral or central pathways. We therefore summarized the most common candidates.

III. Fabry Disease(FD)

Fabry disease (FD) is X-linked, lysosomal storage disorder caused by a mutation in the GLA gene on chromosome Xq22 resulting in alpha-galactosidase A enzyme (a-Gal A) deficiency. In Fabry's Disease, increased amounts of glycosphingolipids (Gb3) can lead to signs of neuropathy. Ion channels such a Nav1.7 characterize acute pain sensation and as a result, loss of Nav1.7 function leads to congenital insensitivity to pain. Meanwhile mutations in the SCN9A gene, that encodes Nav1.7 are associated with painful neuropathies. Also, NaV1.8 channels in nociceptors has been associated with neuropathy, and ablation of NaV1.8 channels are linked with the development of mechanical allodynia and thermal hyperalgesia. In Fabry disease NaV1.7 and NaV1.8 channels were analyzed in relation to conduction velocity and this resulted to heat hyposensitivity in FD compared to controls Furthermore, the over-expression of hyperpolarizing activated cyclic nucleotide-gated (HCN) channels is been observed in animal models of chronic, neuropathic pain and was suggested to be associated with the development of FD neuropathy and possibly neuropathic pain. Other ion channels associated with FD are KCa channels because they speed the repolarization of the action potential as well as they generate the after-hyperpolarization of the plasma membrane. These channels were found altered in FD patients inducing nociceptive neuron hyperexcitability, ectopic firing, and spontaneous pain (Weissman et al, 2021).

IV. Amyloid neuropathy

Amyloid and toxic oligomers may cause cell damage through various mechanisms involving cellular damage, tissue injury, and organ failure. This damage caused by amyloid is involved in the development of peripheral neuropathy. To begin with, amyloid proteins disrupt the integrity of the phospholipid bilayer, but oligomers form small pores in the plasma membrane that can act as non-specific ion channels. These are called "amyloid pores" In neurons, causing ectopic discharges and neuronal damage by membrane depolarization. Thus, the disruption of membrane integrity due to amyloid depolarization increases the exposure of polyunsaturated fatty acids to cytosolic reactive oxygen species (ROS) leading to composition of reactive aldehydes. Amyloidogenic proteins tend to misfold and aggregate, which puts pressure on the endothelial reticulum and this stress is implicated in cytotoxicity in amyloid neuropathies. Also, peripheral neuropathy is associated with mitochondrial dysfunction, such as reduced oxidative phosphorylation, reduced ATP production and increased production of reactive oxygen species (ROS) and altered mitochondrial transportation, suggesting a mitochondrial involvement. Then amyloid strongly activates macrophages to clear protein aggregates, but also causes inflammatory responses and cell death due to the inability of macrophages to process amyloid effectively. As a result, these inflammatory macrophage processes can cause neurotoxicity and neuron apoptosis. In familial amyloid neuropathy is observed a phenomenon of defective myelination and impairment in Schwann cell function leading in a defective structural and functional integrity of peripheral nerves (Asiri et al, 2020).

V. Chemotherapy-induced peripheral neuropathy (CIPN)

It must be noted that nervous system complications have been noted for as many as 21 different chemotherapy agents, including cisplatin, carboplatin and oxaliplatin, either autonomic, or motor and sensory complications. The complications associated with chemotherapy are specific to each chemotherapy agent. Chemotherapeutic agents deregulate activity of cell membrane proteins such as receptors and ion channels and affect mitochondrial morphology, disbalancing, intracellular homeostasis, signaling and neurotransmission. The final outcomes include production of reactive oxygen species (ROS), neuroinflammation, DNA damage, demyelination, cytoskeleton damage and apoptosis. For example, platina chemotherapy agent induces unregulated proteolysis through an array of intracellular signaling pathways and intracellular Ca2+ plays a very important role in cellular homeostasis. The disturbance of Ca2+ is involved in CIPN, since Ca2+ is stored in mitochondria and endoplasmic reticulum. Alternations in Ca2+ concentration may interfere with membrane excitability, neurotransmitter release and gene expression. Development of CIPN has also been associated with malfunction of ion channels such as sodium and potassium channels and transient receptor potential (TRP) channels. Voltage-gated channels initiate action potentials in neural cells, and alternations in their function changes peripheral nerve excitability leading to neuropathy (Stevanović et al.2020).

Pathogenesis of neuropathic pain

Microvascular Blood Flow

Structural and functional microvascular abnormalities of the vasa-nervorum have been identified to be involved in the pathogenesis of distal symmetrical neuropathy. Studies have shown that regulation of peripheral blood flow is altered in

patients with painful- compared with painless-painful neuropathy. Moreover, other studies that estimate serum markers of angiogenesis (vascular endothelial growth factor, VEGF) and endothelial dysfunction (soluble intercellular adhesion molecule-1, sICAM-1) both been found to be increased in painful neuropathy.

Furthermore, punch skin biopsy studies have identified that skin microcirculation is maybe a pathogenic factor of painful neuropathy. Also, hypoxia, inflammation, overstimulation, and chemical damage can induce fiber degeneration and alterations in channel expression and composition, resulting in ectopic firing and faulty signal transmission which are recognized as factors of painful neuropathy too. Thus, in response to axonal damage, satellite glia and autonomic neurons can induce pain-promoting states though alterations in their overall numbers, distribution, sprouting patterns, and channel expression. Recently, the group of Shillo et al. has also found cutaneous von Willebrand factor (vWF) immunoreactivity as a blood vessel marker, to be significantly elevated in subjects with painful diabetic peripheral neuropathy, in comparison to subjects with painless diabetic peripheral neuropathy, patients with diabetes mellitus but without diabetic peripheral neuropathy, and healthy volunteers (Shillo, 2007; Meacham et al., 2017; Vinik et al., 2013; Grotle, 2019).

Nerve growth factor (NGF)

The axons sprouting process in their final stages depends on the neurotrophic factor, NGF (nerve growth factor), a critical protein for the survival and maintenance of sympathetic and sensory neurons. NGF secretion increases during the acute phase of denervation but may also decrease in the long term in denervated skin (Madduri et al., 2009). As a result of the reduced density of nerve fibers, relatively more NGF may be available for the remaining fibers and thus trigger the sprouting. Contrary to the results of the treatment, the increase in the severity of the disease did not reduce the number of IENFDs (intraepidermal nerve fiber density) as much, but especially the number of sprouting fibers. Thus, our results suggest a different mechanism of HIV damage than highly active antiretroviral therapy HAART therapy on small fibers. The reduction in IENFD density from neurotoxic antiviral therapy has already been described in models in rodents, which was done with neurotoxic antiviral therapy (Wallace et al., 2007). However, gp120 may not be the only mechanism of HIV neurotoxicity and, therefore, the link between the clinical stages of HIV infection and the preferred reduction of sprouting fibers may be interpreted. HIV is known to interact with Langerhans skin cells and the keratin layer. However, the mechanisms that control the growth of nerve fibers in the epidermis are unclear.

It is important to note that neuropathic and other types of pains are often present in the same patient (e.g., degenerative spine disease). Even in cases of definite neuropathic pain, a coexisting inflammatory pain may be clinically more crucial. Identification of the presence of neuropathic pain requires evidence for a disease process or lesion affecting a neuroanatomically identifiable part of the peripheral or central somatosensory system, which is concordant with the distribution of the pain (Treede et al., 2007).

The CGRP peptide

The peptide CGRP (calcitonin gene related peptide) is an indicator for a class of afferent fibers and has been shown to be involved in the production of mechanical and thermal hyperalgesia (Khodorova et al., 2009). In addition, the CGRP peptide has been associated with the expression of nerve growth factor (NGF) (Dallos et al., 2006), a neurotrophin associated with nociceptor sensitization (Rukwied et al., 2010) (Obreja et al., 2018) and chronic pain (Lane et al., 2010)(Schmelz et al., 2019). Indeed, it was found in this study that the threshold of heat-induced pain was associated with staining of CGRP in the subcutaneous layer of nerves. This confirms that the CGRP-positive subset of the C-fiber population is highly associated with the heat pain thresholds and therefore might contribute to peripheral sensitization (Dussor et al., 2009).

Transduction proteins

Similarly, a wide variety of sensory proteins such as Transient Receptor Potential (TRP) channels and sodium channels involved in painful or non-sensory mechanisms expressed in cutaneous fiber subpopulations, as well as in the epidermis (Petersen et al., 2010; Albrecht et al., 2006; Zhao et al., 2008), may be possible biomarkers of neuropathic pain. In a large cohort of 921 patients, 75% of them had no known comorbidities before the diagnostic workup. Immunological conditions were found in 175 patients. Other associated conditions found were sodium channel gene variants, diabetes mellitus, vitamin B12 deficiency, alcohol abuse, chemotherapy, monoclonal gammopathy of undetermined significance (MGUS), and haemochromatosis (de Greef BT, 2017). Another study demonstrated that the prevalence of Fabry's disease is not correlated with adult small fiber neuropathy (SFN) patients, a finding that allows the exclusion of this genetic screening in patients with

confirmed diagnosis of SFN (de Greef, et al., 2016).

However, in addition to the above methods, which are quite specialized and not available in most laboratories, there are possible simpler tests that combine the functional and structural assessment of small nerve fibers to draw useful conclusions regarding their subpopulations in specific neuropathies. In such an effort by our team, part of which is still unpublished, we examined small fiber neuropathy in HIV patients known to cause potentially painful peripheral neuropathy (Skopelitis et al., 2006). We used a combination of sympathetic dermal reflex (SSR) to functionally assess sympathetic C-fibers and morphological features of epidermal nerve fibers in skin biopsy to structurally assess small fibers.

Voltage-gated sodium and calcium channelopathies in small fiber neuropathy

Sodium channel-related small fiber neuropathy can be induced during childhood, adolescence, or adulthood, and both sporadic and familial cases have been identified. The clinical picture is mainly characterized by burning feet, but single mutations can cause different phenotypes and cell electrophysiological changes. The mechanism leading to the preferential degeneration of small nerve fibers in sodium channel gene mutations is supposed to involve the altered function. Recent research has identified various neuropathic disorders as well as Chemotherapy induced peripheral neuropathy (CIPN) due to mitochondrial impairment, relevant impairment of Ca2+ signal pathway, and reactive oxygen species (ROS) that are related to pain behavior. Various neuropathic disorders as well as CIPN are due to mitochondrial impairment, serious impairment of Ca2+ signaling pathways and reactive oxygen species (ROS) that ultimately lead to apoptosis (Waseem et al., 2018).

It is remarkable that mutations in COL6A5 have been first described in familial and sporadic patients with neurogenic itching and small fiber neuropathy. Sensitive skin and itch have recently been defined as thermo-occurrence of unpleasant sensations (stinging, burning, pain, pruritus, and tingling sensations) in response to stimuli that normally should not provoke such sensations. These unpleasant sensations cannot be explained by lesions attributable to any skin disease. The skin can appear normal or be accompanied by erythema." Degeneration of small fibers is a common finding, before the development of any symptoms in patients carrying TTR (Transient Receptor Potential) mutations, whereas in the symptomatic stage of familial amyloid neuropathy, patients more likely present a mixed neuropathy. It is proposed that in a patient with neuropathic pain, characterized by constant pain with exacerbations that are provoked by body temperature changes, and a decreased cold sensation as measured with QST and an abnormal intraepidermal nerve fiber density (Cazzato and Lauria, 2017; El-Abassi, 2014; Schmelz, M. ,2019;).

The sodium channels Nav1.7, Nav1.8, and Nav1.9, coded by SCN9A, SCN10A, and SCN11A respectively, are all preferentially expressed in peripheral nerves. Although the exact mechanism for axonal degeneration is not completely clear, it is plausible that DRG (dorsal root ganglion) neuron hyperexcitability results in neuropathic pain (De Greef, B. T. A et al., 2017; Ossipov et al., 2005). Drugs blocking calcium channels play an important role in neuropathic pain, but with synaptic mechanism, they in fact reduce neurotransmitter release. Nav 1.8 and 1.9 sodium channels are expressed mainly in the medium and small diameter size of DRG neuron and the selective block of these channel subtypes should reduce hyperexcitability without serious effects on central nervous system and on the heart. It's not just the ionic channel that determines membrane excitability but also the current passing through it. Consequently, an increase of the current flux through a fixed number of channels could have the same effect as increasing the number of present channels. This could happen through an increase of channel conductivity. As a result, the change in the channel kinetics further represents a process explaining neuronal hyperexcitability. This change could be related to a cAMP-phosphorylation and/or dephosphorylation of some sodium channels. If it is possible to reduce sodium channel density, the threshold of repeated discharge will increase, distancing itself from the threshold for a single discharge, so that the single discharge can be evoked and spread without the opposite stimulus to the repeated discharge (Ossipov et al, 2005; Aurillio et al., 2008).

Moreover, a recent review comparing painful and painless diabetic neuropathies with advances in gene sequencing technology have led to several studies examining genetic variants associated with distal symmetrical polyneuropathy (DPN) and disabling neuropathic pain (painful-DPN). In genome-wide association studies, Chr8p21.3 and Chr8p21.3 polymorphisms were associated with neuropathic pain. Recently also, there has been great interest in voltage-gated sodium channels and their role in neuropathic pain. The Nav1.7 sodium channel is well recognized to be involved in pain signaling, and the "gain of function" mutations of its encoding gene, SCN9A, cause rare pain disorders. Finally, studies have identified Nav1.7 mutations in idiopathic small fiber neuropathy and painful-DPN and found that none of the participants with painless-DPN were

found to have a genetic variant. However, patients with painful-DPN and the genetic variants were found to have a shorter duration of diabetes, yet more severe burning pain (Shillo et Al., 2007; Waseem et al., 2018).

Conclusion

In conclusion, there are numerous well-established techniques for determining the severity of nerve damage in the periphery, even for specific subpopulations of neurons requiring subjective and objective approaches. However, neither severity nor sensory phenotype appears to provide crucial information about pain mechanisms or treatment options. Concerning molecular mechanism of neuropathic pain, analysis of neuronal markers as seen in CGRP or sodium channels seems to be a more promising approach to characterizing the mechanisms that characterize how neuropathic pain is induced. However, the clinical expression of pain is the result of a complex neuronal interaction between primary peripheral sensory neurons and processing of the transmitted signal in the spinal cord and brain. Thus, numerous interdependent circuits and functional markers remain to be studied in their specific role and contribution to the clinical manifestation of neuropathic pain.

Refercences

[1] Aasvang EK, Brandsborg B, Christensen B, Jensen TS, Kehlet H. Neurophysiological characterization of postherniotomy pain. Pain. 2008;137:173-181.

[2] Albrecht, P.J., Hines, S., Eisenberg, E., Pud, D., Finlay, D.R., Connolly, M.K., Pare, M., Davar, G., and Rice, F.L. Pathologic alterations of cutaneous innervation and vasculature in affected limbs from patients with complex regional pain syndrome. Pain. 2008;120, 244-266.

[3] Asiri, M. M. H., Engelsman, S., Eijkelkamp, N., & Höppener, J. W. M. Amyloid Proteins and Peripheral Neuropathy. Cells, 2020;9(6), 1553. doi:10.3390/cells9061553

[4] 10.3390/cells9061553

[5] Attal N, Fermanian C, Fermanian J, Lanteri-Minet M, Alchaar H, Bouhassira D. Neuropathic pain: are there distinct subtypes depending on the aetiology or anatomical lesion? Pain. 2008;138:343-353.

[6] Aurilio, C., Pota, V., Pace, M. C., Passavanti, M. B., & Barbarisi, M. Ionic channels and neuropathic pain: Phisiopatology and applications. Journal of Cellular Physiology, 2008; 215(1), 8–14.

[7] Bakkers, M., Faber, C. G., Peters, M. J. H., Reulen, J. P. H., Franssen, H., Fischer, T. Z., & Merkies, I. S. J. Temperature threshold testing: a systematic review. Journal of the Peripheral Nervous System, 2013,18(1), 7–18.

[8] Baron, R., Binder, A., and Wasner, G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. 2010; 9, 807-819.

[9] Bickel A, Kramer HH, Hilz MJ, Birklein F, Neundorfer B, Schmelz M. Assessment of the neurogenic flare reaction in small-fiber neuropathies. Neurology. 2002;59:917-919.

[10] Blankenburg, M., Kraemer, N., Hirschfeld, G., Krumova, E.K., Maier, C., Hechler, T., Aksu, F., Magerl, W., Reinehr, T., Wiesel, T., and Zernikow, B. Childhood diabetic neuropathy: functional impairment and non-invasive screening assessment. Diabet Med 29, 2008; 1425-1432.

[11] Bouhassira, D., Branders, S., Attal, N., Fernandes, A.M., Demolle, D., Barbour, J., Ciampi De Andrade, D., and Pereira, A. Stratification of patients based on the Neuropathic Pain Symptom Inventory: development and validation of a new algorithm. Pain.2020.

[12] Cazzato, D., & Lauria, G. Small fibre neuropathy. Current Opinion in Neurology, 2017; 30(5), 490–499.

[13] Cruccu G, Sommer C, Anand P, Attal N, Baron R, Garcia-Larrea L, Haanpaa M, Jensen TS, Serra J, Treede RD. EFNS guidelines on neuropathic pain assessment: revised 2009. Eur.J Neurol. 2010.

[14] Cruccu G, Anand P, Attal N, et al. EFNS guidelines on neuropathic pain assessment. European Journal of Neurology. 11(3):153-162. 2004; doi:10.1111/j.1468-1331.2004.00791.x

[15] Dallos A, Kiss M, Polyanka H, Dobozy A, Kemeny L, Husz S. Effects of the neuropeptides substance P, calcitonin gene-related peptide, vasoactive intestinal polypeptide and galanin on the production of nerve growth factor and inflammatory cytokines in cultured human keratinocytes. Neuropeptides. 2006;40:251-263.

[16] Devigili G, Tugnoli V, Penza P, Camozzi F, Lombardi R, Melli G, Broglio L, Granieri E, Lauria G. The diagnostic criteria for small fibre neuropathy: from symptoms to neuropathology. Brain. 2008;131:1912-1925.

[17] Dussor G, Koerber HR, Oaklander AL, Rice FL, Molliver DC. Nucleotide signaling and cutaneous mechanisms of pain transduction. Brain Res.Rev. 2009;60:24-35.

[18] Dyck PJ, Larson TS, O'Brien PC, Velosa JA. Patterns of quantitative sensation testing of hypoesthesia and hyperalgesia are predictive of diabetic polyneuropathy: a study of three cohorts. Nerve growth factor study group. Dia-

betes Care. 2000;23:510-517.
[19] El-Abassi, R., Singhal, D., & England, J. D. Fabry's disease. Journal of the Neurological Sciences, 2014; 344(1-2), 5–19.
[20] Facer, P., Casula, M.A., Smith, G.D., Benham, C.D., Chessell, I.P., Bountra, C., Sinisi, M., Birch, R., and Anand, P.. Differential expression of the capsaicin receptor TRPV1 and related novel receptors TRPV3, TRPV4 and TRPM8 in normal human tissues and changes in traumatic and diabetic neuropathy. BMC.Neurol. 2007; 7:11., 11.
[21] Forstenpointner, J., Ruscheweyh, R., Attal, N., Baron, R., Bouhassira, D., Enax-Krumova, E.K., et al. No pain, still gain (of function): the relation between sensory profiles and the presence or absence of self-reported pain in a large multicenter cohort of patients with neuropathy. Pain.2020; doi: 10.1097/j.pain.0000000000002058.
[22] Freynhagen R, Rolke R, Baron R, Tolle TR, Rutjes AK, Schu S, Treede RD. Pseudoradicular and radicular low-back pain - A disease continuum rather than different entities? Answers from quantitative sensory testing. Pain. 2007;.:
[23] Gaynor, S.M., Bortsov, A., Bair, E., Fillingim, R.B., Greenspan, J.D., Ohrbach, R., Diatchenko, L., Nackley, A., Tchivileva, I.E., Whitehead, W., Alonso, A.A., Buchheit, T.E., Boortz-Marx, R.L., Liedtke, W., Park, J.J., Maixner, W., and Smith, S.B. Phenotypic profile clustering pragmatically identifies diagnostically and mechanistically informative subgroups of chronic pain patients. Pain.2020.
[24] Gierthmuhlen, J., Schneider, U., Seemann, M., Freitag-Wolf, S., Maihofner, C., Enax-Krumova, E.K., Azad, S.C., Uceyler, N., Birklein, F., Maier, C., Tolle, T., Treede, R.D., and Baron, R. Can self-reported pain characteristics and bedside test be used for the assessment of pain mechanisms? An analysis of results of neuropathic pain questionnaires and quantitative sensory testing. Pain 2020; 160, 2093-2104.
[25] Grotle, A.-K., & Stone, A. J. Exaggerated exercise pressor reflex in type 2 diabetes: Potential role of oxidative stress. Autonomic Neuroscience, 2019; 222, 102591.
[26] Held, M., Karl, F., Vlckova, E., Rajdova, A., Escolano-Lozano, F., Stetter, C., et al. Sensory profiles and immune related expression patterns of patients with and without neuropathic pain after peripheral nerve lesion. Pain. 2019; doi: 10.1097/j.pain.0000000000001623.
[27] Ho TW, Backonja M, Ma J, Leibensperger H, Froman S, Polydefkis M. Efficient assessment of neuropathic pain drugs in patients with small fiber sensory neuropathies. Pain. 2008.
[28] Jaaskelainen SK, Teerijoki-Oksa T, Forssell H. Neurophysiologic and quantitative sensory testing in the diagnosis of trigeminal neuropathy and neuropathic pain. Pain 2005;117:349-357.
[29] Jensen, T.S. and Finnerup, N.B. Management of neuropathic pain. Curr.Opin.Support.Palliat. Care. 2007; 1, 126-131.
[30] Karlsson, P., Hincker, A.M., Jensen, T.S., Freeman, R., and Haroutounian, S. Structural, functional, and symptom relations in painful distal symmetric polyneuropathies: a systematic review. Pain 2019; 160(2), 286-297. doi: 10.1097/j.pain.0000000000001381.
[31] Keswani SC, Jack C, Zhou C, Höke A. Establishment of a rodent model of HIV-associated sensory neuropathy. J Neurosci 2006; 26:10299-10304
[32] Khodorova A, Richter J, Vasko MR, Strichartz G. Early and late contributions of glutamate and CGRP to mechanical sensitization by endothelin-1. J Pain. 2009;10:740-749.
[33] Kokotis, P., Schmelz, M., Kostouros, E., Karandreas, N., & Dimopoulos, M.-A. Oxaliplatin-Induced Neuropathy: A Long-Term Clinical and Neurophysiologic Follow-Up Study. Clinical Colorectal Cancer, 2016; 15(3), e133–e140.
[34] Kramer HH, Schmelz M, Birklein F, Bickel A. Electrically stimulated axon reflexes are diminished in diabetic small fiber neuropathies. Diabetes. 2004b;53:769-774.
[35] Kleggetveit, I.P., Namer, B., Schmidt, R., Helas, T., Ruckel, M., Orstavik, K., et al. High spontaneous activity of C-nociceptors in painful polyneuropathy. Pain 2012; 153(10), 2040-2047.
[36] Kleggetveit, I.P. and Jorum, E. Large and Small Fiber Dysfunction in Peripheral Nerve Injuries With or Without Spontaneous Pain. J Pain.2010;
[37] Landerholm SH, Ekblom AG, Hansson PT. Somatosensory function in patients with and without pain after traumatic peripheral nerve injury. Eur.J Pain. 2010;14:847-853.
[38] Lane NE, Schnitzer TJ, Birbara CA, Mokhtarani M, Shelton DL, Smith MD, Brown MT. Tanezumab for the treatment of pain from osteoarthritis of the knee. N.Engl.J Med. 2010;363:1521-1531.
[39] Lauria G, Devigili G. Skin biopsy as a diagnostic tool in peripheral neuropathy. Nat Clin Pract Neurol 2007; 3:546-557.
[40] Lauria, G., Hsieh, S. T., Johansson, O., Kennedy, W. R., Leger, J. M., Mellgren, S. I., … Valls-Solé, J. (2010). 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 Fe-deration of Neurological Societies and the Peripheral Ne. European Journal

of Neurology, 17(7), 903–e49.
[41] La Cesa, S., Tamburin, S., Tugnoli, V., Sandrini, G., Paolucci, S., Lacerenza, M., … Truini, A. . How to diagnose neuropathic pain? The contribution from clinical examination, pain questionnaires and diagnostic tests. Neurological Sciences, Sciences, 2015; 36(12), 2169–2175.
[42] Liberati, G., Algoet, M., Klocker, A., Ferrao Santos, S., Ribeiro-Vaz, J.G., Raftopoulos, C., and Mouraux, A. Habituation of phase-locked local field potentials and gamma-band oscillations recorded from the human insula. Sci Rep 2018; 8, 8265.
[43] Madduri S, Papaloïzos M, Gander B. Synergistic effect of GDNF and NGF on axonal branching and elongation in vitro. Neurosci Res 2009; 65:88–97.
[44] Maier C, Baron R, Tolle TR, Binder A, Birbaumer N, Birklein F, Gierthmuhlen J, Flor H, Geber C, Huge V, Krumova EK, Landwehrmeyer GB, Magerl W, Maihofner C, Richter H, Rolke R, Scherens A, Schwarz A, Sommer C, Tronnier V, Uceyler N, Valet M, Wasner G, Treede RD. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes. Pain. 2010;150:439-450.
[45] Mainka, T., Maier, C., & Enax-Krumova, E. K. Neuropathic pain assessment. Current Opinion in Anaesthesiology, 2015;28(5), 537–545.
[46] Matesanz, L., Hausheer, A.C., Baskozos, G., Bennett, D.L., and Schmid, A.B. Somatosensory and psychological phenotypes associated with neuropathic pain in entrapment neuropathy. Pain.2020.
[47] Meacham, K., Shepherd, A., Mohapatra, D. P., & Haroutounian, S. Neuropathic Pain: Central vs. Peripheral Mechanisms. Current Pain and Headache Reports, 2017;21(6).
[48] McCarthy BG, Hsieh ST, Stocks A, Hauer P, Macko C, Cornblath DR, Griffin JW, McArthur JC .Cutaneous innervation in sensory neuropathies: evaluation by skin biopsy. Neurology 1995; 45:1848-1855
[49] Novak V, Freimer ML, Kissel JT, Sahenk Z, Periquet IM, Nash SM, Collins MP, Mendell JR. Autonomic impairment in painful neuropathy. Neurology. 2001;56:861-868.
[50] Oaklander AL. The density of remaining nerve endings in human skin with and without postherpetic neuralgia after shingles. Pain. 2001;92:139-145.
[51] Oaklander AL. Mechanisms of pain and itch caused by herpes zoster (shingles). J.Pain. 2008;9:S10-S18
[52] Obreja, O., Rukwied, R., Nagler, L., Schmidt, M., Schmelz, M., and Namer, B. Nerve growth factor locally sensitizes nociceptors in human skin. Pain. 2018;159(3), 416-426. doi: 10.1097/j.pain.0000000000001108.
[53] Ossipov, M. H., & Porreca, F. Challenges in the development of novel treatment strategies for neuropathic pain. NeuroRX,2005; 2(4), 650–661.
[54] Pazzaglia, C., & Valeriani, M. (2009). Brain-evoked potentials as a tool for diagnosing neuropathic pain. Expert Review of Neurotherapeutics, 9(5), 759–771.
[55] Pardo CA, McArthur JC, Griffin JW (2001). HIV neuropathy: insights in the pathology of HIV peripheral nerve disease. J Peripher Nerv Syst 6:21-27.
[56] Petersen KL, Rice FL, Farhadi M, Reda H, Rowbotham MC. Natural history of cutaneous innervation following herpes zoster. Pain. 2010.
[57] Polydefkis M, Yiannoutsos CT, Cohen BA, Hollander H, Schifitto G, Clifford DB, Simpson DM, Katzenstein D, Shriver S, Hauer P, Brown A, Haidich AB, Moo L, McArthur JC. Reduced intraepidermal nerve fiber density in HIV-associated sensory neuropathy. Neurology. 2002;58:115-119.
[58] Rolke R, Baron R, Maier C, Tolle TR, Treede RD, Beyer A, Binder A, Birbaumer N, Birklein F, Botefur IC, Braune S, Flor H, Huge V, Klug R, Landwehrmeyer GB, Magerl W, Maihofner C, Rolko C, Schaub C, Scherens A, Sprenger T, Valet M, Wasserka B. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. Pain. 2006;123:231-243.
[59] Rukwied R, Mayer A, Kluschina O, Obreja O, Schley M, Schmelz M. NGF induces non-inflammatory localized and lasting mechanical and thermal hypersensitivity in human skin. Pain. 2010;148:407-413.
[60] Schmelz, M. Itch Processing in the Skin. Frontiers in Medicine, 2019; 6.
[61] Skopelitis E, Kokotis P, Kontos AN, Panayiotakoloulos GD, Konstantinou K, Kordossis T, Karandreas N. Distal sensory polyneuropathy in HIV positive patients in the HAART era: an entity underestimated by clinical examination. Int J STD AIDS 2019;17:467-472.
[62] Skopelitis E, Aroni K, Kontos AN, Konstantinou K, Kokotis P, Karandreas N, Kordossis T Early detection of subclinical HIV sensory polyneuropathy using intraepidermal nerve fiber density quantification: association with HIV stage and surrogate markers. Int J STD AIDS 2018; 18:856-860.
[63] Sène D., Small fiber neuropathy: Diagnosis, causes, and treatment. Joint Bone Spine.2017.

[64] Shillo, P., Sloan, G., Greig, M., Hunt, L., Selvarajah, D., Elliott, J., … Tesfaye, S. Painful and Painless Diabetic Neuropathies: What Is the Difference? Current Diabetes Reports, 2019; 19(6).
[65] Schmelz, M., Mantyh, P., Malfait, A.M., Farrar, J., Yaksh, T., Tive, L., et al. Nerve growth factor antibody for the treatment of osteoarthritis pain and chronic low-back pain: mechanism of action in the context of efficacy and safety. Pain 2019; 160(10), 2210-2220. doi: 10.1097/j.pain.0000000000001625.
[66] Schmelz, M., Michael, K., Weidner, C., Schmidt, R., Torebjörk, H.E., and Handwerker, H.O. Which nerve fibers mediate the axon reflex flare in human skin? Neuroreport 2000;11(3), 645-648.
[67] Schmelz, M. What can we learn from the failure of quantitative sensory testing? Pain.2020;
[68] Schmelz, M., Mantyh, P., Malfait, A.M., Farrar, J., Yaksh, T., Tive, L., and Viktrup, L. Nerve growth factor antibody for the treatment of osteoarthritis pain and chronic low-back pain: mechanism of action in the context of efficacy and safety. Pain 2019; 160, 2210-2220.
[69] Sommer C, Geber C, Young P, Forst R, Birklein F, Schoser B Polyneuropathies, Dtsch Arztebl Int. 2018; 9;115(6):83-90.
[70] Sopacua, M., Hoeijmakers, J. G. J., Merkies, I. S. J., Lauria, G., Waxman, S. G., & Faber, C. G. Small fibre neuropathy: expanding the clinical pain universe. Journal of the Peripheral Nervous System.2018.
[71] Stevanović M., Lazic, A., Popović J., Paunesku, T., & Woloschak, G. E. Insights into platinum-induced peripheral neuropathy–current perspective. Neural Regeneration Research, 2020;15(9), 1623. https://doi.org/10.4103/1673-5374.276321
[72] Terkelsen, A. J., Karlsson, P., Lauria, G., Freeman, R., Finnerup, N. B., & Jensen, T. S. The diagnostic challenge of small fibre neuropathy: clinical presentations, evaluations, and causes. The Lancet Neurology, 2017; 16(11), 934–944.
[73] Treede, R.-D., Jensen, T. S., Campbell, J. N., Cruccu, G., Dostrovsky, J. O., Griffin, J. W., … Serra, J. Neuropathic pain: Redefinition and a grading system for clinical and research purposes. Neurology, 2007;70(18), 1630–1635.
[74] Vinik, A. I., Nevoret, M.-L., Casellini, C., & Parson, H. Diabetic Neuropathy. Endocrinology and Metabolism Clinics of North America, 2013;42(4), 747–787.
[75] Wallace VC, Blackbeard J, Segerdahl AR, Hasnie F, Pheby T, McMahon SB, Rice AS . Characterization of rodent models of HIV-gp120 and antiretroviral-associated neuropathic pain. Brain 2007;130:2688-2702.
[76] Waseem, M., Kaushik, P., Tabassum, H., & Parvez, S. Role of Mitochondrial Mechanism in Chemotherapy-Induced Peripheral Neuropathy. Current Drug Metabolism, 2018;19(1), 47–54.
[77] Weissmann, C., Albanese, A. A., Contreras, N. E., Gobetto, M. N., Castellanos, L. C. S., & Uchitel, O. D. Ion channels and pain in Fabry disease. Molecular Pain, 17, 174480692110331.2021; doi:10.1177/17448069211033172
[78] Zhao, P., Barr, T.P., Hou, Q., Dib-Hajj, S.D., Black, J.A., Albrecht, P.J., Petersen, K., Eisenberg, E., Wymer, J.P., Rice, F.L., and Waxman, S.G. Voltage-gated sodium channel expression in rat and human epidermal keratinocytes: evidence for a role in pain. Pain 2008; 139, 90-105.
[79] Zhou L, Kitch DW, Evans SR, Hauer P, Raman S, Ebenezer GJ, Gerschenson, M, Marra CM, Valcour V, Diaz-Arrastia R, Goodkin K, Millar L, Shriver S, Asmuth DM, Clifford DB, Simpson DM, McArthur JC. NARC and ACTG A5117 Study Group. Neurology 2007; 68:2113-2119.