Original Article

Neuropathic Pain and Nerve Growth Factor in Chemotherapy-Induced Peripheral Neuropathy: Prospective Clinical-Pathological Study

Roser Velasco, PhD, MD, Xavier Navarro, PhD, Miguel Gil-Gil, MD, Mireia Herrando-Grabulosa, PhD, Aina Calls, PhD student, and Jordi Bruna, PhD, MD

Neuro-Oncology Unit-IDIBELL (R.V., M.G.-G., J.B.), Hospital Universitari de Bellvitge-Institut Català d'Oncologia L'Hospitalet, Barcelona; and Department of Cell Biology, Physiology and Immunology (R.V., X.N., M.H.-G., A.C., J.B.), Institute of Neurosciences, Universitat Autònoma de Barcelona, and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Bellaterra, Spain

Abstract

Context. Neuropathic pain can be present in patients developing chemotherapy-induced peripheral neuropathy (CIPN). Nerve growth factor (NGF) is trophic to small sensory fibers and regulates nociception.

Objectives. We investigated the changes in serum NGF and intraepidermal nerve fiber density in skin biopsies of cancer patients receiving neurotoxic chemotherapy in a single-center prospective observational study.

Methods. Patients were evaluated before and after chemotherapy administration. CIPN was graded with Total Neuropathy Score©, nerve conduction studies, and National Common Institute-Common Toxicity Criteria for Adverse Events scale. Neuropathic pain was defined according to the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-CIPN20 questionnaire.

Results. Neuropathic pain was present in 13 of 60 patients (21%), who reported shooting or burning pain in the hands ($n = 9$) and the feet ($n = 12$). Patients displaying painful CIPN presented higher NGF after treatment compared with patients with painless or absent CIPN (8.7 ± 11.9 vs. 2.5 ± 1.4 pg/mL, $P = 0.016$). The change of NGF significantly correlated with neuropathic pain. Patients with painful CIPN did not show significant loss of IEFND compared with patients with painless or absent CIPN (6.16 ± 3.86 vs. 8.37 ± 4.82, $P = 0.12$). No correlation between IEFND and NGF was observed.

Conclusion. Serum NGF increases in cancer patients receiving taxane or platinum with painful CIPN, suggesting that it might be a potential biomarker of the presence and severity of neuropathic pain in this population. Long-term comprehensive studies to better define the course of NGF in relation with neurological outcomes would be helpful in the further design of therapies for CIPN-related neuropathic pain. J Pain Symptom Manage 2017;54:815–825.

Key Words

Chemotherapy-induced peripheral neuropathy, chemotherapy-induced neuropathy, neuropathic pain, nerve growth factor, skin biopsy

Introduction

Peripheral neuropathy is the most frequent neurological side effect in cancer patients receiving chemotherapy. Among these compounds, taxane (TX), platinum (PT), and bortezomib (BTZ) are the most neurotoxic agents, causing most observed neuropathies in oncological practice.[1] Typically, patients complain of paresthesia and/or numbness in hands and feet.[2] Neuropathic pain, usually described as shooting and/or burning pain, is reported by

Address correspondence to: Jordi Bruna, PhD, MD, Neuro-Oncology Unit, Hospital Universitari de Bellvitge and ICO Duran i Reynals, C/Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain. E-mail: 35078jbe@comb.cat

Accepted for publication: April 12, 2017.

0885-3924/$ - see front matter
http://dx.doi.org/10.1016/j.jpainsymman.2017.04.021

approximately 40% of patients with chemotherapy-induced peripheral neuropathy (CIPN).[3]

Innervation of the skin is provided by large myelinated (Aβ), small myelinated (Aδ), and unmyelinated (C) fibers, which are the endings of trigeminal nucleus (face) and dorsal root ganglia and of sympathetic ganglia neurons. The endings of Aβ fibers are in the dermis close to hair follicles or vascular structures and supply specialized sensory corpuscles. The unmyelinated fibers located in the epidermis are the terminal nerve endings of either C or Aδ fibers, which are indistinguishable from C fibers because of the loss of their myelin sheath before entering the epidermis. The immunohistochemical study of skin biopsy allows evaluating the degree of innervation of the skin by these terminals by measuring the intraepidermal nerve fiber density (IENFD). Skin biopsy is considered the reference standard technique for diagnosing small-fiber neuropathies.[4] However, contradictory results on the relationship between skin biopsy data and neuropathic pain exist in the literature.[5]

Nerve growth factor (NGF) belongs to the neurotrophin family of growth factors, which regulate the development and survival of neurons in the central and peripheral nervous systems.[6] NGF is produced and released from target tissues, including basal keratinocytes, and binds to receptors on the Aδ and C nerve fibers.[6] NGF binds to tropomyosin-related kinase A receptor expressed on the terminals of sensory neurons, activating several intracellular signaling cascades affecting the sensitivity of nociceptors. The constitutive synthesis of NGF in adult tissues correlates with peripheral nervous system neuron phenotypic features, such as innervation density, cell body size, and axonal terminal sprouting, among others.[6] NGF has been involved in neuropathic pain, and clinical trials to evaluate the usefulness of monoclonal antibodies against NGF to treat pain conditions are currently ongoing.[7]

The involvement of NGF in the pathogenesis of the CIPN has been reported in several experimental studies, demonstrating NGF neuroprotective properties *in vitro* and *in vivo*.[8,9] Changes in NGF serum level during chemotherapy treatment have been observed in preclinical models[9–11] and in two small clinical studies.[12,13] Most of them pointed out to a correlation between a decrease in NGF levels and the severity of the CIPN. Noteworthy, these clinical studies did not take into account the neuropathic painful status as a potential confounding factor. The aim of the present study was to investigate the changes in serum NGF and IENFD in cancer patients receiving neurotoxic chemotherapy schedules with regard to the presence or not of neuropathic pain.

Methods

From 2006 to 2011, adult cancer patients scheduled to receive PT, TX, or BTZ without previous symptomatic neuropathy were recruited from our center and included in three prospective studies previously reported.[14–16] Chemotherapy schedules were all administered according to standard doses and institution protocols. Only patients in whom NGF and/or skin biopsy were performed before and/or after finishing chemotherapy are included in the present study. These data had never previously been reported. This study was carried out after having obtained approval from the Ethics Review Board of Hospital Universitari de Bellvitge. All participants provided written informed consent in accordance with the Declaration of Helsinki before study entry.

Neurological Assessment

Neurotoxic adverse effects secondary to chemotherapy were rated using National Common Institute-Common Toxicity Criteria, version 3 (NCI.CTCv3) sensory subscale.[15] Neurological examination was recorded according to the Total Neuropathy Score© (TNS), the clinical version of TNS (TNSc), and the reduced version of TNS (TNSr), all validated scales to assess CIPN.[17,18] Large sensory and motor fibers were assessed with nerve conduction studies by using standard methods and the widely accepted criteria for the identification of abnormalities as previously described.[14,15]

Symptoms related to CIPN were assessed with the submodule European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Chemotherapy-Induced Peripheral Neuropathy 20 (EORTC QLQ-CIPN20).[19] Patients had to indicate the degree to which they had experienced sensory, motor, and autonomic symptoms during the past week using a four-point Likert scale (1 = *not at all*, 2 = *a little*, 3 = *quite a bit*, and 4 = *very much*). Neuropathic pain in this questionnaire was defined according to answers obtained in Questions 35 (*Did you have shooting or burning pain in your fingers or hands?*) and 36 (*Did you have shooting or burning pain in your toes or feet?*). Ongoing neuropathic pain was defined in those patients reporting >1 score in EORTC QLQ-CIPN20 Questions 35 and/or 36 to include all patients with neuropathic pain despite of its severity. Emergent neuropathy was defined as worsening of one grade in NCI.CTCv3 and/or at least two points in TNSr.[14] Questionnaires were recorded at baseline (B) and at one month after the last dose of chemotherapy (F).

NGF Analysis

Blood collection was done concurrent with each clinical assessment as previously described.[16] Baseline

samples from all patients were obtained before receiving the first cycle of chemotherapy. Serum specimens were stored at −80°C until NGF analysis, which was performed by Enzyme-Linked ImmunoSorbent Assay (Labco®, Barcelona, Spain).

Skin Biopsy

Patients underwent skin biopsy from the distal leg (10 cm above the lateral malleolus), after local anesthesia using a 3 mm disposable punch under a sterile technique. A first biopsy was obtained at baseline and a second from the contralateral leg one month after finishing chemotherapy. Biopsy tissue was fixed in paraformaldehyde, refrigerated overnight, transferred to a sacarose-phosphate buffered saline solution, and thereafter cryoprotected until sectioned. Cryotome skin sections of 60 μm thick were washed free floating in phosphate buffered saline 0.3 Triton X100 and incubated with 1% normal goat serum for one hour to block nonspecific antibody adhesion. They were then incubated with primary antibodies to the pan-neuronal marker protein gene peptide 9.5 (PGP 9.5; AbD Serotec, Oxford, UK, 1/1000) and the basement membrane marker Type IV collagen (Chemicon, Billerica, MA, 1/800). After washes, sections were incubated using Cy2- and Cy3-conjugated secondary antibodies (Jackson Immunoresearch, Newmarket, Suffolk, UK). Sections were adhered to coverslips with agar, dehydrated with alcohol, and mounted with DPX mounting media (Sigma-Aldrich, Gillingham, Dorset, UK). Blinded to the clinical status of the patients and site of biopsy, an experienced researcher assessed the IENFD, which was calculated per linear millimeter of epidermis, according to the European Federation of Neurological Societies/Peripheral Nerve Society guidelines, in three sections randomly chosen from each biopsy.[4] IENFD was assessed with a light microscope at ×20 magnification. The fifth percentile of the normative data was used as a cutoff point, and IENFD was classified as reduced if below.[4] Digital images were acquired using confocal microscopy (Confocal Laser Scanning Microscope Zeiss LSM 700; Zeiss, Jena, Germany).

Statistical Analysis

Descriptive data analysis presented categorical variables as observed counts and weighted percentages and continuous variables as mean or median with the corresponding standard error or range, depending on the nature of the variable. Student t-test and Mann-Whitney *U* test were used to evaluate differences between patients at baseline, according to the nature of the variable. A repeated-measure analysis of variance was used to evaluate differences between groups (neuropathic pain, yes/no) and time. The association between clinical, biochemical, and pathological parameters was assessed using Pearson and Spearman correlation coefficient for continuous and nonparametric variables, respectively. Statistical analyses were performed using SPSS software package, version 18.0 (SPSS, Inc., Chicago, IL), and *P*-values <0.05 were considered significant.

Results

Sixty patients were included in the present study (Fig. 1). Forty eight patients had at least one NGF determination, being in 45 patients' data from paired (baseline and final) available and included in the analysis. Thirty-three patients of the whole series had paired skin biopsy. In 21 of these 60 patients, data from NGF and skin biopsy performed simultaneously were available. In 24 and 12 patients, data from only paired NGF or skin biopsy were available, respectively. Three patients only had one sample of NGF. No patient treated with BTZ had NGF determination. Demographic, clinical, and neurophysiological data are summarized in Table 1. Ten percent of patients presented evidence of neuropathy at baseline (Tables 1 and 2). Only in two (3.3%) patients, symptoms of burning neuropathic pain were present before chemotherapy.

Most patients ($n = 49$; 81.7%) developed some degree of CIPN according to NCI.CTCv3 and TNS scores. The severity of neuropathy according to the NCI.CTCv3 was as follows: Grade 1 (17 patients; 28.3%), Grade 2 (25 patients; 41.7%), and severe or Grade 3 (seven patients; 11.3%). Frequency and intensity of CIPN-related symptoms reported by patients according to EORTC-QLQ-CIPN20 at baseline and after finishing chemotherapy treatment are summarized in Table 2. Median TNSc and TNSr scores according to severity of neuropathy are shown in Figure 2.

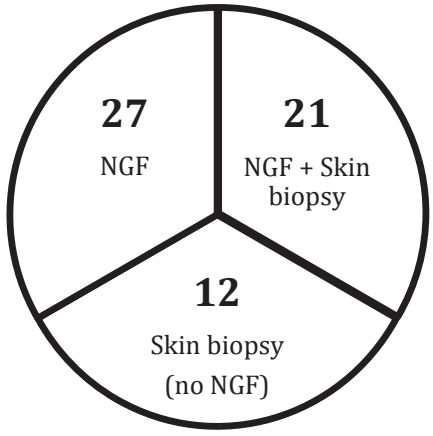

Fig. 1. Population included in the study. Numbers represent patients with samples of nerve growth factor (NGF) and/or skin biopsy.

Table 1
Baseline Clinical and Demographic Data of Patients Included in the Study

Whole Series, $n = 60$ (100%)	
Age (yrs)[a]	57.67 ± 12.43
Gender (F/M)	31 (51.7)/29 (48.3)
TNSc[b]	0 (0–5)
TNSr[b]	0 (0–9)
Baseline neuropathy	6 (10)
Diabetes mellitus	9 (15)
Type of cancer	
Colorectal	35 (58.3)
Gastric	3 (5)
Lung	1 (1.7)
Breast	16 (26.7)
Multiple myeloma	5 (8.3)
Chemotherapy agent	
PT	39 (65)
TX	16 (26.7)
BTZ	5 (8.3)
Cumulated dose (mg) at the end (no. of patients)[a]	
Oxaliplatin ($n = 35$)	1601.257 ± 301.86
Cisplatin ($n = 4$)	742.50 ± 130.75
Paclitaxel ($n = 8$)	1360.50 ± 85.90
Docetaxel ($n = 8$)	701.12 ± 239.00
BTZ ($n = 5$)	35.80 ± 5.40
NCS[a]	
Sural SNAP (μV)	12.27 ± 6.15
Radial SNAP (μV)	26.52 ± 9.96
Peroneal CMAP (Mv)	5.35 ± 2.85
Mixed median forearm SCV (meter/second)	65.44 ± 6.12
NGF levels (pg/mL)[a]	3.52 ± 5.49
IENFD distal leg[a]	8.68 ± 4.37

F = female; M = male; TNSc = Total Neuropathy Score, clinical version; TNSr = Total Neuropathy Score, reduced version; PT = platinum; TX = taxane; BTZ = bortezomib; NCS = nerve conduction studies; SNAP = sensory nerve action potential; CMAP = compound muscle action potential; SCV = sensory conduction velocity; NGF = nerve growth factor; IENFD = intraepidermal nerve fiber density.
Bortezomib was administered at initial dose of 1.3 mg/m^2 according to the accepted schedule: on Days 1, 4, 8, and 11, for up to eight 21-day cycles for up to eight cycles. Patients treated with oxaliplatin received a dose of 85–130 mg/m^2 intravenously once in two (oxaliplatin, fluorouracil, and leucovorin calcium [folinic acid] {FOLFOX}) to three (oxaliplatin and capecitabine [XELOX]) weeks for up to eight to 12 cycles; cisplatin was given between 25 and 100 mg/m^2 every three weeks for up to six cycles; paclitaxel was given in a dose of 80 mg/m^2 weekly for 175 mg/m^2 once in three weeks for up to 12 or four cycles, respectively; and docetaxel was administered every three weeks at doses of 75 mg/m^2 for four weeks.
[a]Results are expressed as mean ± SD.
[b]Median (range).

Significant correlations in the change of amplitude of the radial nerve and Questions 31 ($r = 0.539$, $P < 0.001$) and 34 ($r = 0.507$, $P < 0.001$) as well as mixed median forearm conduction velocity and Question 31 ($r = 0.349$, $P = 0.019$) were found.

Neuropathic pain was present in 13 patients (21.7%) at finishing chemotherapy. Nine (15%) and 12 (20%) patients reported shooting or burning pain in fingers or hands and toes or feet, respectively. Regarding the chemotherapy drug administered, 8 (20.5%) of PT, 3 (18.8%) of TX, and 2 (40%) of BTZ-treated patients reported neuropathic pain, respectively. Patients reporting neuropathic pain presented more complaints in the other QLQ-CIPN20 items (comparisons in Table 2). Among patients with diabetes mellitus or baseline peripheral neuropathy, only one (11%) and three (50%) patients reported neuropathic pain at finishing chemotherapy, although these frequencies were not statistically significant.

Nerve Growth Factor

At baseline, no differences in NGF serum levels were observed whether patients had diabetes mellitus or not (5.95 ± 10.12 vs. 3.02 ± 4.02 pg/mL, $P = 0.446$), preexisting or no peripheral neuropathy (3.51 ± 1.85 vs. 3.52 ± 5.61 pg/mL, $P = 0.997$), or whether developed neuropathic pain or not (4.70 ± 8.98 vs. 3.28 ± 4.6 pg/mL, $P = 0.673$). After finishing chemotherapy, patients suffering from painful CIPN presented higher NGF serum levels compared with those with painless CIPN (8.67 ± 11.91 vs. 2.55 ± 1.40 pg/mL, $P = 0.016$) (Fig. 3a). In contrast, patients without neuropathic pain or emergent neuropathy had NGF levels similar to baseline. Differences were also significant when patients without CIPN were excluded from the analysis (data not shown). When comparing the type of agent, patients developing TX-induced neuropathic pain had higher NGF serum levels than those without neuropathic pain (10.9 ± 16.06 vs. 2.91 ± 3.78, $P = 0.033$), whereas these differences did not reach significance in patients treated with PT (8.4 ± 10.2 vs. 3.3 ± 2.3, $P = 0.2$). Regarding the severity of CIPN, patients with worse CIPN presented higher NGF serum levels after treatment (no CIPN—Grade 1: 1.90 ± 1.18 vs. CIPN—Grade 2–3: 4.79 ± 6.38, $P = 0.037$) (Fig. 2b). At the end of the treatment, the serum level of NGF was correlated with the score in TNSc ($r = 0.271$; $P = 0.054$) and TNSr ($r = 0.340$; $P = 0.018$). In addition, a significant correlation was found between the change of NGF from baseline (NGF final/baseline) and the change of score in TNSc ($r = 0.382$; $P = 0.011$) and TNSr ($r = 0.433$; $P = 0.044$) (Fig. 4). The change of NGF (NGF final/baseline) was also significantly correlated with the intensity of neuropathic pain rated by patients in Question 35 ($r = 0.42$; $P = 0.004$) and Question 36 ($r = 0.314$; $P = 0.035$).

Skin Biopsy

At baseline, the mean IENFD was 8.49 ± 4.31, which is in line with normal reference values according to European Federation of Neurological Societies guidelines[5] (data from BTZ patients were excluded from this baseline counting because of not chemotherapy-naive nature). Thirty-three patients, treated with PT ($n = 20$), TX ($n = 8$), and BTZ ($n = 5$) had paired (baseline and final) biopsies and were included in this analysis. The mean IENFD in follow-up skin biopsies was similar to that at baseline (8.68 ± 4.37 vs. 7.77 ± 4.63, $P = 0.194$). After finishing chemotherapy, patients suffering neuropathic pain ($n = 9$; TX = 2, PT = 5, and BTZ = 2) presented slightly not

Table 2
Frequency of Sensory CIPN-Related Symptoms Reported by Patients According to the EORTC QLQ-CIPN20 Questionnaire at Baseline and After Treatment

EORTC-QLQ-CIPN20 Question		Baseline				After Treatment				
		1	2	3	4	1	2	3	4	P^a
31	Did you have tingling fingers or hands?	54	5	1	0	18	17	12	13	0.008
32	Did you have tingling toes or feet?	56	4	0	0	19	20	9	12	0.006
33	Did you have numbness in your fingers or hands?	54	6	0	0	18	20	12	10	0.047
34	Did you have numbness in your toes or feet?	53	7	0	0	21	16	11	12	0.02
35	**Did you have shooting or burning pain in your fingers or hands?**	**59**	**1**	**0**	**0**	**51**	**4**	**5**	**0**	—
36	**Did you have shooting or burning pain in your toes or feet?**	**58**	**2**	**0**	**0**	**48**	**8**	**3**	**1**	—
39	Did you have problems standing or walking because of difficulty feeling the ground under your feet?	55	5	0	0	32	19	9	0	0.003
40	Did you have difficulty distinguishing between hot and cold water?	60	0	0	0	48	6	5	1	0.001
48	Did you have difficulty hearing?	53	7	0	0	44	12	4	0	0.014

Bold text indicates $P < 0.05$.
Response: 1 = not at all, 2 = a little, 3 = quite a bit, and 4 = very much; results are expressed in the number of patients who answered the question.
[a]Chi-square test comparing frequencies in all no pain items scoring 1 (no symptoms) vs. >1 (symptoms present), with regard to the presence of neuropathic pain (Score 1 [painless] or >1 [painful] in Items 35 and/or 36).

significant lower IENFD compared with those without neuropathic pain ($n = 24$) (6.16 ± 3.86 vs. 8.37 ± 4.82, $P = 0.12$) (Fig. 5). No differences in IENFD according to severity of CIPN were observed. Regarding the chemotherapy received, patients treated with BTZ presented the largest decrease of IENFD, but differences were not significant. No correlation between changes of NGF and IENFD along treatment was found. Interestingly, microscopic examination indicated that six patients treated with TX ($n = 2$) and PT ($n = 4$) presented morphological changes consisting on fragmentation of the IENFs and axonal swellings (Fig. 6) considered degenerative changes associated with axonopathy. Three of these six patients showing structural changes on skin biopsy had neuropathic pain, with IENFD count within normal range in all three.

Discussion

The results of this study indicate that symptoms of neuropathic pain were reported by one-fifth of patients receiving TX or PT and nearly half of patients receiving BTZ, one month after finishing chemotherapy. This incidence is slightly lower than previously reported in the literature, which can be probably explained by the bias of previous studies including only patients with overt CIPN[3,20] and the different criteria used for neuropathic pain between studies. The main finding of the present work is that serum levels of NGF increase in solid cancer patients developing neuropathic pain, whereas they remain stable in solid cancer patients with painless or absent CIPN, suggesting the potential usefulness of NGF as a biomarker of CIPN-related pain and clinically relevant CIPN (Grade ≥2) in this population. In addition, the change of NGF with treatment was larger in patients with more severe neuropathic pain. Up to our knowledge, this is the first time that the relationship between the presence and severity of neuropathic pain in patients treated with PT or TX and serum levels of NGF has been reported.

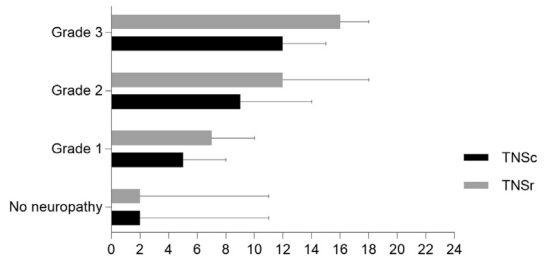

Fig. 2. Total Neuropathy Scores (TNSs) at finishing chemotherapy according to the severity of neuropathy measured by National Common Institute-Common Toxicity Criteria for Adverse Events scale (results are expressed as median and upper limits). TNSc = Total Neuropathy Score, clinical version; TNSr = Total Neuropathy Score, reduced version.

Although NGF was originally discovered as a trophic factor for sympathetic and small sensory neurons during development,[6] its role as mediator of pain has been extensively demonstrated.[21] NGF levels become elevated in several painful conditions, such as arthritis, migraine headache, fibromyalgia, peripheral diabetic neuropathy, and peripheral nerve injury.[22,23] In humans, subcutaneous injection of NGF evokes long-lasting mechanical hyperalgesia.[24] The increase in the level of normal firing in afferent neurons in neuropathic pain states has been attributed, at least in part, to altered expression of several types of voltage-gated sodium channels.[25] NGF is able to upregulate voltage-gated sodium channels expression in neuropathic pain states, which is critically linked to sensitization of peripheral nociceptors.[21]

The results of our study also support the concept that NGF may be a marker for the severity of CIPN in line with the reported other peripheral nerve injuries. Our data show an increase in serum NGF with increasing

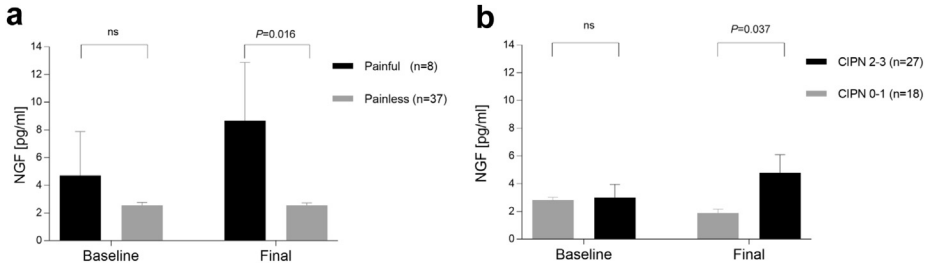

Fig. 3. Changes in circulating nerve growth factor (NGF) values during treatment with chemotherapy according to the presence of neuropathic pain. a) At finishing treatment. b) The severity of chemotherapy-induced peripheral neuropathy. Results are expressed as mean ± SEM. After finishing chemotherapy, patients suffering from painful or more severe chemotherapy-induced peripheral neuropathy (CIPN) presented higher NGF serum levels compared with those with painless or absent (0) Grade 1 CIPN. ns = not significant.

severity of neuropathy. In contrast, Cavaletti et al.[12] found a negative correlation between the decrease from baseline NGF and the severity of CIPN scored with TNS in 34 cervical cancer women receiving paclitaxel and cisplatin periodically assessed up to three months after the end of chemotherapy. They concluded that cisplatin-paclitaxel chemotherapy determines a clinically related decrease in NGF serum levels.[11] Another study of 23 patients treated with mixed schedules including PT and TX by determining circulating NGF at baseline and 24 hours after the end of the fourth and sixth chemotherapy courses showed a significant decrease in the NGF levels after six cycles in the nine patients assessed at this timepoint.[13] The authors found a significant correlation with severity of neuropathy after four but not after six cycles of treatment.[13] These two previous works suggested that production of NGF, which would increase as a response to nerve damage, may be impaired in patients developing CIPN. Importantly, no information regarding neuropathic pain was available from these studies. These contradictory results, at least in part, might be attributed to several reasons. First, the differences in the assessed cancer population. In the first study, all patients were women receiving schedules containing TX and PT, and in the second study, mixed treatment was administered to nearly half of the patients. Furthermore, no patients treated with oxaliplatin were involved in the previous studies, being the great population of our work. We identified that the increase of NGF was significant in the group of patients receiving TX and not in the PT group, when separately analyzed, despite similarity on the rate of CIPN-related neuropathic pain (18% and 20%). Whether such an increase of NGF in our series was only related with TX treatment remains unknown, and no formal conclusions can be stated on this regard from our work, but differences on NGF synthesis with each agent could underly the contradictory results. Second, the time of assessment of neuropathy and NGF was different, especially in the work of Bove et al. Results from the 19 of 24 patients analyzed after the fourth course were determined just 24 hours after the end of the cycle: what seems too early for detecting nerve damage and biological response to this, especially in the setting of treatment with PT, because of the well-known coasting effect that determines further progression of neuropathy after finishing chemotherapy during one to two months.[1] Furthermore, differences on criteria for defining CIPN severity, the statistical approach, and methodological differences in the determination of NGF could be also underlying the differences obtained with our results.

On the other hand, a reduction of IENFD has been consistently reported in almost all rodent models of

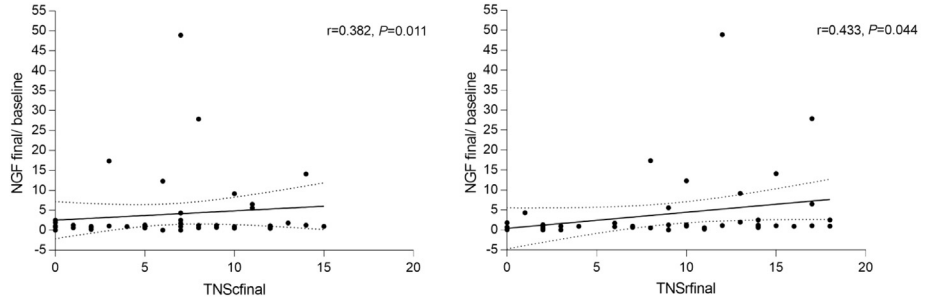

Fig. 4. Changes in circulating nerve growth factor values in relation to clinical neurological status, as scored by Total Neuropathy Score, clinical version (TNSc) and Total Neuropathy Score, reduced version (TNSr). 95% CI limits are presented as dotted line. NGF = nerve growth factor.

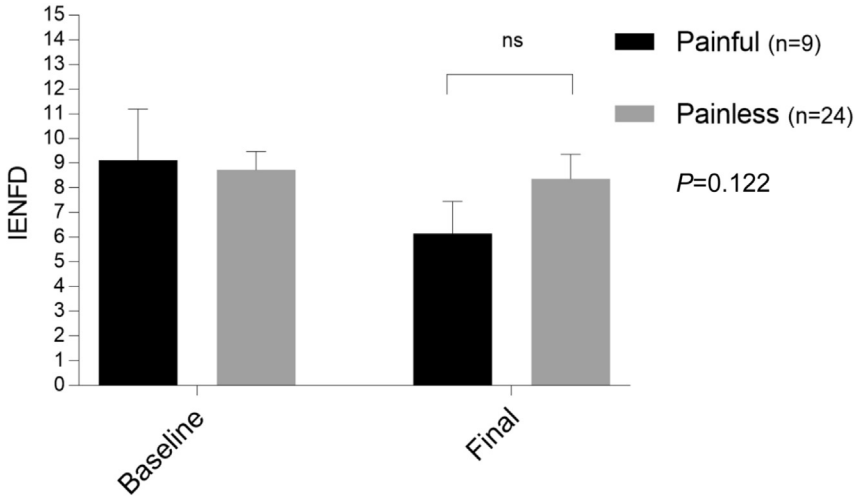

Fig. 5. Changes in intraepidermal nerve fiber density (IENFD). Results are expressed as mean ± SEM. ns = not significant.

CIPN tested, including treatments with cisplatin,[26] paclitaxel,[27,28] and BTZ,[29] especially restricted to the sensory axons terminal arbor without degeneration of the subepidermal nerve bundles.[30] However, contradictory results emerge from clinical studies, including our work, that do not allow establishing uniform conclusions regarding the involvement of small fibers by each drug (Table 3).[5,31–38] We observed trends toward reduced IENFD in the distal leg in patients displaying neuropathic pain, despite differences were not significant, what could be related with the low number of patients developing neuropathic pain or the nonhomogeneous population, and no definite association between loss of IENFD and neuropathic pain can be inferred from our results. In addition, we did not find differences in IENFD depending on severity of the neuropathy, in line with variable results, either increase or decrease, of the IENFD reported in the literature. In painful diabetic neuropathy, also conflicting results have been reported in this regard.[39] Because NGF seems to promote sprouting of tropomyosin-related kinase A-expressing nerve fibers, resulting in hyperinnervation of the epidermis,[39] it could contribute to the lack of congruency in reported data. However, we did not find a significant correlation between IENFD and serum levels of NGF. Noteworthy, whereas the painful characteristics of CIPN were described in several studies,[5,31–33,35–38] in only one, the relationship between IENFD changes and neuropathic pain was analyzed, showing negative

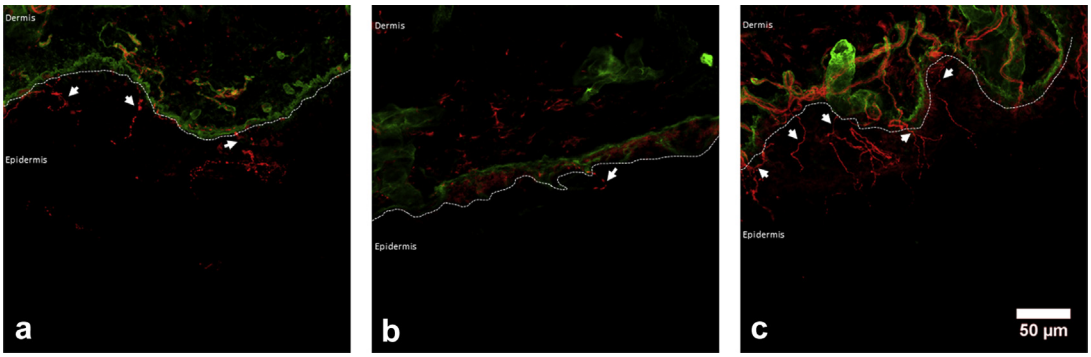

Fig. 6. Abnormalities of the intraepidermal nerve fibers. Confocal microscope double staining against protein gene peptide 9.5 (red) and Type IV collagen (green). Scale bar = 50 µm. a) Image from a 42-year-old woman with breast cancer who received 1448 mg of paclitaxel and developed painless taxane-induced neuropathy Grade 1 (Total Neuropathy Score, clinical version [TNSc]: 4). Sural sensory nerve action potential (SNAP): 3.00 µV; radial SNAP: 20.00. Intraepidermal nerve fiber density (IENFD) count was within normal range. Intraepidermal and dermal fibers showed fragmentation. b) Image from a 62-year-old diabetic man with gastric cancer who received 924 mg of cisplatin and developed Grade 2 painless neuropathy (TNSc: 7). Sural SNAP: 2.30 µV and radial SNAP: 16.30 µV. IENFD count was lower than normal reference value. c) Image from a 36-year-old woman with breast cancer who received 617 mg of docetaxel without CIPN (TNSc: 0). Sural SNAP: 14.30 µV and radial SNAP: 37.30 µV. IENFD was within normal range without morphological changes. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Characteristics of the Studies Evaluating Skin Biopsy on Patients With CIPN

Author, Year, Reference	N Type of Study	Tumor	Chemotherapy	Time of Skin Biopsy (*n*)	IEFND (Distal Leg)	Neurological Assessment	Findings
Lauria et al., 2003[31]	1		Paclitaxel	CIPN diagnosis months after	8.3, 5.4	Neurological, NCS, QST	• Patient showed a decrease in IEFND at the distal leg • Axonal swellings preceded the eventual loss of IENFD
Chaudhry et al., 2008[32]	27 Prospective	MM	BTZ Thalidomide	Baseline (27) After (14)	10.6 ± 6.0 9.1 ± 4.6	TNSr NCS, NCI	• Five patients had mild reductions in IEFND at the distal leg at baseline • A mild not significant decrease in distal leg IEFND was observed
Burakgazi et al., 2011[34]	8 Prospective	CRC	Oxaliplatin	Baseline (8) After six cycles (7) One year (4)	15.39 ± 6.75 12.89 ± 4.73 9.45 ± 3.92	TNSr NCS	• Significant decrease over time of IEFND • Change of IEFND more pronounced at distal leg than at distal thigh • Further loss of IEFND in three of four patients six months after chemotherapy • Increase of IEFND during treatment is observed in some patients
Koskinen et al., 2011[35]	12 Prospective	CRC Prostate Breast	Oxaliplatin Docetaxel	Baseline (12) After three cycles (10) After five to six cycles (10) Final (4)	56.3 ± 64.8 70.7 ± 45.8 63.8 ± 32.5 99.7 ± 16.6	Neurological examination	• No association between the IEFND and neurological symptoms • Baseline IEFND lower than normal limits in eight patients • Decrease and increase of IEFND is observed during the first six months • An increase of IEFND during treatment was observed in eight patients
Giannoccaro et al., 2011[36]	3 Case-control (15)	MM	BTZ	After treatment (3)	2.5 ± 1.3	Neurological examination NCS	• Autonomic small fiber involvement was documented in three patients • Significant reduction in IEFND compared with control in all patients
Boyette-Davis et al., 2013[37]	1 Case-control (7)	Breast	Paclitaxel	After treatment (1)	92 (finger) 71 (palm) 505 (forearm)	VAS Neurological examination QST	• A decrease in both Meissner's corpuscle and IEFND in the patient compared with those biopsies obtained from healthy control subjects • A 71% decrease in Meissner's corpuscles/mm^2
Krøigård et al., 2014[38]	40 Cross-sectional	CRC Breast	Oxaliplatin Docetaxel	After treatment (40)	1.7 (0.3–5.6) 3 (0.5–6.3)	TNSr, QST NSS, NIS	• Skin biopsy was the most sensitive method for diagnosing CIPN

								• Skin biopsy was abnormal in 80% of patients • Abnormal in 85% of oxaliplatin and 75% of docetaxel patients
Truini et al. 2014[5]	18 Cross-sectional	NA	NA	After treatment (18)	NA	NPSI NCS Laser		• No differences in IEFND among the three etiologies of PN (diabetes mellitus, hepatitis C virus, and chemotherapy) • No relationship of IEFND with neuropathic pain
Present work	33 Prospective	CRC Lung Gastric Breast MM	Oxaliplatin Cisplatin Paclitaxel Docetaxel BTZ	Before treatment (33) After treatment (33)	8.68 ± 4.37 7.77 ± 4.63	TNS NCI, NCS EORTC-QLQ CIPN20		• No reduction in mean IENFD compared with baseline • Patients with neuropathic pain presented a trend in less IEFND • No differences in IEFND according to the severity of CIPN • Fragmentation of IEFN and axonal swellings in patients with and without neuropathic pain

CIPN = chemotherapy-induced peripheral neuropathy; IEFND = intraepidermal nerve fiber density; IEFND in mm^2 (n/mm^2); NCS = nerve conduction studies; QST = quantitative sensory testing; MM = multiple myeloma; BTZ = bortezomib; TNSr = Total Neuropathy Score, reduced version; NCI = National Common Institute; CRC = colorectal cancer; VAS = visual analogue scale; NSS = Neurological Symptom Score; NIS = Neuropathy Impairment Score; NA = not applicable; NPSI = Neuropathic Pain Symptom Inventory; PN = peripheral neuropathy; EORTC-QLQ CIPN20 = European Organization for Research and Treatment of Cancer-Quality of Life Questionnaire Chemotherapy-Induced Peripheral Neuropathy 20.

results.[5] All these data would support the current view that reduction of IENFD may be observed in patients with peripheral neuropathy, irrespective of the presence or the absence of pain.[4] In fact, we observed morphological alterations such as axonal swellings and fragmentation of IENFs in patients with and without neuropathic pain. Axonal swellings, defined as focal axonal enlargements, are considered degenerative changes associated with degenerative-regenerative processes and ongoing neuropathic pain.[31] However, the presence of axonal swellings has been reported in a large proportion of patients with painless neuropathy or even healthy subjects.[5,40]

Based on our findings, the evaluation of IENFD based on PGP 9.5 immunoreactivity would not seem useful for predicting the course and severity of CIPN. Indeed, the pan-neuronal marker PGP 9.5 labels all IENFs in skin biopsies. Nociceptive IENFs are peptidergic or nonpepidergic based on their expression of neuropeptides. The peptidergic neurons are NGF dependent. Recently, a loss of Substance P and calcitonin gene-related peptide containing fibers in neuropathic pain after paclitaxel injection in an experimental model was demonstrated.[28] Up to our knowledge, no information regarding differences in peptidergic fibers in patients with CIPN has been reported in the literature and may deserve further investigation to better define the role of skin biopsy in this type of neuropathy.

A main limitation of the present study relies on the definition for neuropathic pain only based on burning and/or shooting symptoms of pain that may be an underrepresentation of patients with other pain features.[2] The use of other tools, such as visual analogue scale, would have been interesting but not considered because of the nature of the population of the study, with frequently associated pain from cancer that could have interfered with the objective of the study. Furthermore, lack of quantitative sensory tests in these patients to better explore the characteristics of pain is acknowledged. However, quantitative sensory tests are psychophysical tools not routinely used in clinical practice in which patient's collaboration is mandatory. The aim of the present study was not to evaluate the characteristics of pain of our patients but to identify a feasible objective biomarker of ongoing neuropathic pain. Currently, there is no available biomarker to objectively determine the presence and severity of neuropathic pain, and the clinical evaluation must rely on subjective reports concerning patients' experience of pain, which are frequently influenced by psychosocial conditions. The observation that a serum parameter could be an indicator identifying patients treated with neurotoxic chemotherapy and ongoing neuropathic pain is clinically important because of its feasibility as a routine test. The determination of

NGF levels might be considered in patients receiving drugs causing high prevalent pain and should be explored in larger sample studies including patients treated with BTZ.

Conclusion

An increase of serum levels of NGF was observed in patients receiving PT and TX compounds developing painful CIPN one month after finishing chemotherapy. This increase may be related to the biological response of denervated target tissue as a compensatory mechanism to the neurotoxic damage, and it might serve as a biomarker of the presence of neuropathic pain and the severity of nerve damage in this population. Long-term comprehensive and comparative studies to better define the course of NGF in relation to neurological outcomes and type of chemotherapy would be helpful in the design of therapies for CIPN-related pain.

Acknowledgments

The authors thank Jessica Jaramillo, Yolanda Martinez, and Marta Morell for their technical support, and all patients who took part in the study. This work was partially supported by PI1501303 grant from Instituto de Salud Carlos III and Fondo Europeo de Desarrollo Regional.

References

1. Argyriou AA, Bruna J, Marmiroli P, Cavaletti G. Chemotherapy-induced peripheral neurotoxicity (CIPN): an update. Crit Rev Oncol Hematol 2012;82:51–77.

2. Miaskowski C, Mastick J, Paul SM, et al. Chemotherapy-induced neuropathy in cancer survivors. J Pain Symptom Manage 2017;54:204–218.e2.

3. Geber C, Breimhorst M, Burbach B, et al. Pain in chemotherapy-induced neuropathy—more than neuropathic? Pain 2013;154:2877–2887.

4. Lauria G, Hsieh ST, Johansson O, et al. European Federation of Neurological Societies/Peripheral Nerve Society guideline on the use of skin biopsy in the diagnosis of small fiber neuropathy. Report of a Joint Task Force of the European Federation of Neurological Societies and the Peripheral Nerve Society. Eur J Neurol 2010;17:903–912. e44–e49.

5. Truini A, Biasiotta A, Di Stefano G, et al. Does the epidermal nerve fibre density measured by skin biopsy in patients with peripheral neuropathies correlate with neuropathic pain? Pain 2014;155:828–832.

6. Aloe L, Rocco ML, Bianchi P, Manni L. Nerve growth factor: from the early discoveries to the potential clinical use. J Transl Med 2012;10:239.

7. Hirose M, Kuroda Y, Murata E. NGF/TrkA signaling as a therapeutic target for pain. Pain Pract 2016;16:175–182.

8. Apfel SC, Arezzo JC, Lipson L, Kessler JA. Nerve growth factor prevents experimental cisplatin neuropathy. Ann Neurol 1992;31:76–80.

9. Aloe L, Manni L, Properzi F, De Santis S, Fiore M. Evidence that nerve growth factor promotes the recovery of peripheral neuropathy induced in mice by cisplatin: behavioral, structural and biochemical analysis. Auton Neurosci 2000;86:84–93.

10. Cavaletti G, Petruccioli MG, Marmiroli P, et al. Circulating nerve growth factor level changes during oxaliplatin treatment-induced neurotoxicity in the rat. Anticancer Res 2002;22:4199–4204.

11. Cavaletti G, Pezzoni G, Pisano C, et al. Cisplatin-induced peripheral neurotoxicity in rats reduces the circulating levels of nerve growth factor. Neurosci Lett 2002;322:103–106.

12. Cavaletti G, Bogliun G, Marzorati L, et al. Early predictors of peripheral neurotoxicity in cisplatin and paclitaxel combination chemotherapy. Ann Oncol 2004;15:1439–1442.

13. De Santis S, Pace A, Bove L, et al. Patients treated with antitumor drugs displaying neurological deficits are characterized by a low circulating level of nerve growth factor. Clin Cancer Res 2000;6:90–95.

14. Velasco R, Petit J, Clapés V, et al. Neurological monitoring reduces the incidence of bortezomib-induced peripheral neuropathy in multiple myeloma patients. J Peripher Nerv Syst 2010;15:17–25.

15. Velasco R, Bruna J, Briani C, et al. Early predictors of oxaliplatin-induced cumulative neuropathy in colorectal cancer patients. J Neurol Neurosurg Psychiatry 2014;85:392–398.

16. Velasco R, Santos C, Soler G, et al. Serum micronutrients and prealbumin during development and recovery of chemotherapy-induced peripheral neuropathy. J Peripher Nerv Syst 2016;21:134–141.

17. Cornblath DR, Chaudhry V, Carter K, et al. Total neuropathy score: validation and reliability study. Neurology 1999;53:1660–1664.

18. Cavaletti G, Frigeni B, Lanzani F, et al. The Total Neuropathy Score as an assessment tool for grading the course of chemotherapy-induced peripheral neurotoxicity: comparison with the National Cancer Institute-Common Toxicity Scale. J Peripher Nerv Syst 2007;12:210–215.

19. Postma TJ, Aaronson NK, Heimans JJ, et al. The development of an EORTC quality of life questionnaire to assess chemotherapy-induced peripheral neuropathy: the QLQ-CIPN20. Eur J Cancer 2005;41:1135–1139.

20. Smith EM, Pang H, Cirrincione C, et al. Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial. JAMA 2013;309:1359–1367.

21. Siniscalco D, Giordano C, Rossi F, Maione S, de Novellis V. Role of neurotrophins in neuropathic pain. Curr Neuropharmacol 2011;9:523–529.

22. Kim HC, Cho YJ, Ahn CW, et al. Nerve growth factor and expression of its receptors in patients with diabetic neuropathy. Diabet Med 2009;26:1228–1234.

23. Chang DS, Hsu E, Hottinger DG, Cohen SP. Anti-nerve growth factor in pain management: current evidence. J Pain Res 2016;9:373–383.

24. Svensson P, Cairns BE, Wang K, Arendt-Nielsen L. Injection of nerve growth factor into human masseter muscle

evokes long-lasting mechanical allodynia and hyperalgesia. Pain 2003;104:241–247.

25. Waxman SG, Zamponi GW. Regulating excitability of peripheral afferents: emerging ion channel targets. Nat Neurosci 2014;17:153–163.

26. Verdú E, Vilches JJ, Rodríguez FJ, et al. Physiological and immunohistochemical characterization of cisplatin-induced neuropathy in mice. Muscle Nerve 1999;22: 329–340.

27. Boyette-Davis J, Xin W, Zhang H, Dougherty PM. Intraepidermal nerve fiber loss corresponds to the development of taxol-induced hyperalgesia and can be prevented by treatment with minocycline. Pain 2011;152:308–313.

28. Ko MH, Hu ME, Hsieh YL, Lan CT, Tseng TJ. Peptidergic intraepidermal nerve fibers in the skin contribute to the neuropathic pain in paclitaxel-induced peripheral neuropathy. Neuropeptides 2014;48:109–117.

29. Bruna J, Udina E, Alé A, et al. Neurophysiological, histological and immunohistochemical characterization of bortezomib-induced neuropathy in mice. Exp Neurol 2010; 223:599–608.

30. Landowski LM, Dyck PJ, Engelstad J, Taylor BV. Axonopathy in peripheral neuropathies: mechanisms and therapeutic approaches for regeneration. J Chem Neuroanat 2016;76(Pt A):19–27.

31. Lauria G, Morbin M, Lombardi R, et al. Axonal swellings predict the degeneration of epidermal nerve fibers in painful neuropathies. Neurology 2003;61:631–636.

32. Chaudhry V, Cornblath DR, Polydefkis M, Ferguson A, Borrello I. Characteristics of bortezomib- and thalidomide-induced peripheral neuropathy. J Peripher Nerv Syst 2008; 13:275–282.

33. Richardson PG, Xie W, Mitsiades C, et al. Single-agent bortezomib in previously untreated multiple myeloma: efficacy, characterization of peripheral neuropathy, and molecular correlations with response and neuropathy. J Clin Oncol 2009;27:3518–3525.

34. Burakgazi AZ, Messersmith W, Vaidya D, et al. Longitudinal assessment of oxaliplatin-induced neuropathy. Neurology 2011;77:980–986.

35. Koskinen MJ, Kautio AL, Haanpää ML, et al. Intraepidermal nerve fibre density in cancer patients receiving adjuvant chemotherapy. Anticancer Res 2011;31:4413–4416.

36. Giannoccaro MP, Donadio V, Gomis Pèrez C, et al. Somatic and autonomic small fiber neuropathy induced by bortezomib therapy: an immunofluorescence study. Neurol Sci 2011;32:361–363.

37. Boyette-Davis JA, Cata JP, Driver LC, et al. Persistent chemoneuropathy in patients receiving the plant alkaloids paclitaxel and vincristine. Cancer Chemother Pharmacol 2013;71:619–626.

38. Krøigård T, Schrøder HD, Qvortrup C, et al. Characterization and diagnostic evaluation of chronic polyneuropathies induced by oxaliplatin and docetaxel comparing skin biopsy to quantitative sensory testing and nerve conduction studies. Eur J Neurol 2014;21:623–629.

39. Cheng HT, Dauch JR, Porzio MT, et al. Increased axonal regeneration and swellings in intraepidermal nerve fibers characterize painful phenotypes of diabetic neuropathy. J Pain 2013;14:941–947.

40. Wendelschafer-Crabb G, Kennedy WR, Walk D. Morphological features of nerves in skin biopsies. J Neurol Sci 2006; 242:15–21.