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Follow-up psychophysical studies in bortezomib-related chemoneuropathy patients

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Abstract

Many frontline chemotherapeutic agents produce robust neuropathy as a dose-limiting side effect; however, the persistence of chemotherapy-related sensory disturbances and pain are not well-documented. We have previously investigated the qualities of bortezomib-induced pain, and now seek to determine the ongoing nature of this pain. Twenty-six control subjects and eleven patients who had previously been treated with bortezomib and who were experiencing ongoing pain consented to recurring quantitative sensory testing. A pilot immunohistochemistry study of skin innervation was also performed on patient obtained biopsies. Psychophysical testing in patients revealed persistent changes including decreased skin temperature in the area of pain, diminished touch and sharpness detection, increased pegboard completion times, and decreased sensitivity to skin heating. Additionally, the intensity of pain, as captured by the use of a visual analog scale and pain descriptors, was reported by patients to be unchanged during the retest despite similar morphine equivalent daily doses. The patient skin biopsies displayed a marked decrease in the density of epidermal nerve fibers and Meissner's corpuscles. These results signify a persistent and severe impairment of A β , A δ , and C fibers in patients with chronic bortezomib-induced chemoneuropathy. Further, this study reports a loss of both epidermal nerve fibers and Meissner's corpuscles.

Perspective—The results of this article indicate a persistent, painful peripheral neuropathy in patients treated with bortezomib. Pilot data indicates a loss of nerve fibers innervating the area of pain. This is the first paper to address the persistence, and potential contributing factors, of bortezomib chemoneuropathy.

Keywords

Peripheral Neuropathy; Velcade; Meissner's Corpuscles; Epidermal Nerve Fibers; Sensory Testing

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Chemotherapy treatment for cancer often produces sensory dysfunction and pain that persists long after therapy has ended. Between 50% and 100% of patients experience discomfort by a third treatment cycle with the chemotherapeutic agents vincristine³⁰, bortezomib⁶, paclitaxel¹³, or cisplatin⁷, or when exceeding a dose of 1.2 g of thalidomide¹⁷. Chemotherapies that cause neuropathic pain are the frontline agents used to treat thousands of cancer patients each year. At The M. D. Anderson Cancer Center in 2005, 40% of new patients received chemotherapy drugs associated with neuropathy (MD Anderson Pharmacy DataLog 2005), resulting in 20,000 patients at risk at this single center in one year alone.

Patients with chemotherapy-induced painful neuropathy present with a characteristic set of symptoms in a symmetrical stocking and glove distribution with an especial involvement of the glabrous skin of the hands and feet^{12,11,6}. Pain is often severe and refractory to management. For example, patients receiving bortezomib reported maximum daily pain ratings of 7.8 out of 10, despite taking a morphine equivalent daily dose of 37.5 mg⁶. Quantitative sensory examination reveals a marked impairment of A-beta and A-delta fibers but variable involvement of C-fibers.

This study set to determine the persistence of psychophysical deficits in chronic bortezomib chemoneuropathy patients and to gauge the effectiveness of pain management over time. In addition, a pilot immunohistochemistry study was conducted to determine whether chemoneuropathy-related sensory deficits were related to loss of epidermal nerve fibers as is frequently observed in diabetes¹⁹, small fiber sensory neuropathy²⁴ and post-herpetic neuralgia²⁹.

Materials and Methods

Patients

Eleven patients and twenty-six control subjects were included in this study. All patients were diagnosed with multiple myeloma, had been treated with bortezomib as a single frontline agent, and had bortezomib-induced pain. These patients were recruited through either the Pain Management Center or the Multiple Myeloma Clinic at the MD Anderson Cancer Center. Following confirmation of a chemoneuropathy diagnosis by a physician's clinical examination, patients provided informed consent and were then assessed using quantitative sensory analysis. The informed consent form and protocol were previously reviewed and approved by the Institutional Review Board of the M.D. Anderson Cancer Center. A mean of 13.3 ($\pm$ 2.7) months after the initial testing, these patients were brought back into the laboratory for a follow-up examination. The control group consisted of subjects recruited from faculty, staff, and volunteers of the institute who provided informed consent to participate in the study. No subjects in either the patient or control groups had known risk factors for peripheral neuropathy.

Testing procedure

The same testing procedures were used for both tests. Patients initially chose word descriptors from a previously validated list¹² that described their pain. On-going and daily maximum pain intensity was rated on a visual analogue scale (VAS) with prompts of 'no pain' at the bottom and 'most imaginable' at the top¹². Subjects reported symmetrical pain and sensory disturbances that presented in a stocking and glove type distribution as previously reported⁶. Accordingly, the data are organized into three areas as follows:

- A. *The painful area*: the zone of on-going pain was predominately located on the tips of the fingers and/or toes. The tip of the index finger was the site studied here.

- B. *The border area*: was adjacent to, but distinct from the painful area was a zone characterized by numbness and tingling. This area included the palms and/or soles of the feet. The thenar eminence was tested in this study.
- C. *The non-painful area*: an area proximal to the wrists and/or ankles that was adjacent to, but distinct from the border area and reported by the patient to be felt as 'normal'. The volar surface of the arm was chosen to represent this area.

Skin temperature—Skin temperature was measured using a radiometer, placed gently against the skin for approximately 2 seconds.

Touch detection thresholds and grooved pegboard test—Touch detection thresholds were determined using von Frey monofilaments (Semmes-Weinstein) in an up/down manner¹². Starting with a bending force of 0.05g, each von Frey monofilament was applied to the skin for approximately 1 sec. If the subject failed to detect the stimulus, the next higher force monofilament was applied to the same location. When the subject detected the presence of the stimulus, the next lower von Frey was administered. This continued until the same filament was detected for three applications and assigned as the touch detection threshold.

Manual dexterity was assessed with the grooved pegboard test³⁴. Patients were instructed to fill a five-by-five slotted pegboard in an ordered fashion, either across rows or down columns. The time subjects took to complete the board for both the dominant and the non-dominant hand was measured.

Sharpness detection threshold—Sharpness detection was determined using 8, 10, 16, 20, 32, 64 and 128g weighted needle devices¹⁴. Each stimulus was applied for about 1 sec in ascending order. The subjects were instructed to rate each stimulus as producing the sensation of touch, pressure, sharp, or pain. The sharpness detection threshold was defined as the mean force of the stimuli deemed “sharp” or “painful” from three trials that were separated by an average interval of 30 to 90s.

Heat and cold detection thresholds—Heat pain threshold was determined as previously described^{6,14}. Heat ramps were conducted using a 3.6cm by 3.6cm Peltier probe applied to the skin. The baseline temperature of the probe was set at 32°C and the temperature increased at a rate of 0.30°C/s. Subjects signaled when the probe was first perceived as warm (*warmth threshold*) and then painful (*heat threshold*). Upon determination of the heat threshold, the trial was immediately terminated and the probe returned to baseline temperature. The final threshold for each site was defined as the mean of three trials, which were separated by an average of 30 to 90s. If a subject failed to perceive warm or heat pain, the cutoff temperature of 52 °C was recorded as the default. The threshold to detection of cooling (*cool threshold*) and then cold pain (*cold threshold*) was similarly determined, except that the temperature was decreased at a rate of 0.50 °C/s. If a subject failed to perceive cold pain, the cutoff of 3°C was recorded as the default value.

Pilot study to investigate epidermal nerve fibers

One patient and seven healthy controls consented to having a 3 mm biopsy taken from each of the testing sites. Biopsied tissue was fixed in chilled Zamboni's fixative, refrigerated overnight, transferred to a sucrose-PBS solution and stored until sectioned. Antibodies to the pan-neuronal marker PGP 9.5 (AbD Serotec, Oxford, UK; 1/1000) and the basement membrane marker type IV collagen (Southern Biotech, Birmingham, AL; 1/25) were localized in 50 µm thick sections by staining using Cy2 and Cy3 labeled secondary antibodies (Jackson ImmunoResearch, West Grove, PA). Nonspecific antibody adhesion

was blocked by incubation with 5% normal donkey serum in diluent/wash solution (PBS, 0.3% triton X-100, 1% normal donkey serum). Sections were adhered to cover slips with agar, dehydrated with alcohol, cleared with methyl salicylate and mounted in DPX.

Confocal images were acquired with an Olympus DSU non-laser confocal microscope using a 20x plan apochromat objective (NA 0.75) to determine ENF and Meissner's corpuscle densities. Sampling for quantification purposes was carried out by collecting a montage of images across an entire section. Nerve fiber density was determined with Neurolucida Software (MicroBrightField, Colchester, VT) by tracing from the intersection of the nerve fiber (PGP-9.5-ir) with the dermal-epidermal junction basement membrane (type IV collagen-ir) to the ending within the epidermis. A counting unit was identified when a nerve crosses the basement membrane ¹⁹.

Statistical Analysis

The thresholds for touch detection were compared using non-parametric methods (Kruskal-Wallis test). The sharpness detection, thermal thresholds and the times in the grooved pegboard tests were compared using analysis of variance and post hoc comparison of the means with Tukey HSD. Comparisons of mechanical and thermal thresholds were performed between healthy subjects and patients for the different areas of the tested skin. For every comparison performed in the present study $p < 0.05$ was considered significant.

Results

Subjects

The patient group was composed of 7 men and 4 women with an average age of 58.2 ± 3.4 yrs, and the control group consisted of 16 males and 10 females with an average age of 52.7 ± 2.7 years. All patients were diagnosed with multiple myeloma and had been treated with bortezomib as a single frontline agent. The mean interval to re-test was 13.3 ± 2.7 months. During this testing interval time, four of the eleven patients experienced disease relapse which required treatment. One of these four patients was treated with additional bortezomib. The distribution of pain and sensory disturbances one year following drug treatment was identical for all subjects at the first and follow-up test, and occurred in a symmetrical stocking and glove type distribution as previously reported ⁶.

Pain intensity and quality

Pain intensity and pain quality, as reflected by VAS scores and the number of descriptive words chosen to express their pain, remained constant over the follow-up interval. Patients rated their daily maximum pain at 8.5 ± 0.69 in the first test session (test); this remained at 7.9 ± 0.78 at the follow-up exam (retest). Daily minimum pain, reflecting the best effect of all pain medications received, was 4.3 ± 1.0 at the first examination and 3.7 ± 0.58 at follow-up. At test, of the eleven patients, eight chose the descriptive word "numb", seven chose "burning", six chose "stabbing" and "tingling", five chose "sharp" and "shooting" and four or fewer chose the words "stabbing, dull, hot, cold, flashing, throbbing, itching and electric". At follow-up, of the eleven patients nine chose "sharp", eight chose "numb" and "tingling", seven chose "stabbing", and five chose "burning" or "electric". Four or fewer chose "squeezing, dull, hot, cold, spreading, flashing, throbbing, shooting, itching and electric". Pain medications also remained essentially constant with a mean morphine equivalent daily dose at the first test of 47.5 ± 21.5 mg compared to 44.2 ± 16.5 mg at the retest.

Skin temperature changes

Temperature of the fingertips in patients was significantly lower than in control subjects (Fig. 1). This effect was the same at the time of the follow-up, but was not seen in the border or non-painful areas.

Threshold for touch detection and pegboard performance

Patients showed greater than a two order magnitude increase in von Frey touch detection threshold compared to control subjects in the area of pain (fingertips) and in the border zone (thenar eminence) of sensory disturbance (Fig. 2). Touch detection deficits were also found in the volar forearm (non-painful area), though this deficit was not as pronounced as in the symptomatic areas of skin. Pronounced increases in grooved peg-board completion times were found at the time of the original test and during the follow-up examination (Fig. 2, right hand side).

Threshold for detection of sharpness

Deficits in sharpness detection were also observed in the chemoneuropathy patients (Fig. 3). Unlike the deficits to touch detection, which were present in all three testing areas, the threshold for sharpness detection was found to be increased in the area of pain (fingertips) only. The threshold remained elevated at the time of retest.

Thermal threshold

At the time of the initial test, increased warmth and heat thresholds were identified in all three testing areas (Fig. 4a). During the retest, warmth threshold within the border area was not significantly different from control subjects and the heat threshold was not significantly different from controls within the border zone and the non-painful area. Within the area of ongoing pain, deficits in both warmth and heat thresholds remained. In contrast, very little deficits in detection of skin cooling were noted (Fig. 4b).

Case report epidermal nerve fiber density changes

The patient from whom biopsies were taken was a 55 year old female, and the healthy control group consisted of 4 males and 3 females with an average age of 44.4 ± 2.19 years. Patient biopsies were obtained at the time of the retest examination. Pain location and intensity were similar for the patient at both the test and the retest. The patient reported that the most significant area of sensory disturbance and pain was the hands, with a maximum VAS of 9.6 at the first test and a 9.3 at the re-test time point. The minimum VAS scores were 6.5 (test) and 5.8 (re-test) despite a morphine equivalent daily dose of 45.0 mg. The quality of pain for this patient seemed to change. At the time of the first test, the patient identified “sharp”, “tugging”, “numb”, and “tingling”; at the re-test, “stabbing”, “sharp”, “dull”, “splitting”, “tingling”, “numb”, “throbbing”, “shooting”, and “electric” were all chosen by the patient to describe her pain. Touch detection thresholds at the first test for the area of pain, the border area, and the non-painful area were 1.81, .95, and .56 grams, respectively. At the time of the re-test, touch detection had severely deteriorated to 89.4 (area of pain), 89.4 (border area), and .95 (non-painful area). Deficits were also found for sharpness, warmth, and heat detection; these deficits remained relatively stable across time.

The patient’s ENF density was reduced as compared to biopsies obtained from healthy control subjects. The patient biopsy contained 38 ENFs/mm² within the 3mm biopsy from the area of pain (finger tip) (Fig. 5a), 118 ENFs/mm² within the border area (palm) (Fig. 5b), and 376 ENFs/mm² within the non-painful area (forearm) (Fig. 5c). This is in stark contrast to sample biopsies taken from a healthy volunteer (Fig. 5d, e, f). The average ENF density for biopsies obtained from healthy controls was 127.49 ENFs/mm² in the fingertip,

235.8 ENFs/mm² in the palm, and 633.09 ENFs/mm² in the forearm. Further, while control subjects had an average of 34.0 Meissner's corpuscles/mm² in the fingertip, this patient's fingertip biopsy was completely devoid of Meissner's corpuscles.

Discussion

The findings of this study extend the results of our earlier report on bortezomib-related neuropathy by showing that the pattern of sensory deficits remained almost identical at an approximately one year (13.3 ± 2.7 months) follow-up. Patients consistently had lower skin temperature in the area of ongoing pain. Deficits in touch threshold in all areas, in addition to bilateral increases in pegboard completion times, remained stable as did deficiencies in the area of pain for sharpness detection. Whereas significant impairments in heat threshold were found in all areas during the first test, at follow-up patients reported poorer detection of heat confined to the area of ongoing pain. Despite the fact that ten out of the eleven patients received no further bortezomib treatment, neuropathy-related pain scores and use of symptomatic pain medications were still present at the retest examination, providing evidence of the persistence of this condition. Contributing to this persistence may be a loss of epidermal nerve fibers, as was observed in biopsies taken from a patient in this study. Overall, the data indicate the presence of a persistent neuropathy that affects A β , A δ , and C fibers.

Diabetes produces similar symptoms of pain and sensory disturbances, and biopsies from patients with diabetic-related peripheral neuropathy show decreases in the density of epidermal nerve fibers¹⁹. Further, diminished ENF density has been found in animal models of paclitaxel³⁷, oxaliplatin³, and cisplatin²¹-induced hypersensitivity. Extensive depletion of ENF's was observed here in skin biopsies obtained from each of the three testing areas in one patient. These biopsies were taken at the time of the follow-up visit, indicating that the observed changes in ENF density may contribute to the genesis and/or persistence of neuropathic pain. Although some researchers have failed to find a relationship between ENF loss and pain^{38,29}, many others have found such an association. Examples include decreased ENF density that correlates with changes in warmth detection³⁶ and pinprick sensitivity⁴⁰. This hypothesis is further supported by findings in post-herpetic neuralgia that ENF loss is greatest in areas specific to neuropathic pain^{29,32,26}. While our biopsied patient reported significant pain in the hands, it is worth noting that many patients receiving bortezomib treatment experience pain in both the hands and the feet, often with the most severe pain localized to the plantar surface of the foot. All eleven patients in this study reported pain in both the hands and the feet, and future research may consider investigating QST responding and ENF density in both areas of the body. The patient in the current study had an incremental decrease in ENF density when comparing the area of pain (fingertip) to the border area (palm) and non-painful area (forearm). Compared to average ENF densities from control subjects, the patient had a 69.7% loss in the fingertip, a 49.9% decrease in the palm, and a 40.5% decrease in the forearm.

One other study has examined ENF density changes in humans as a result of chemotherapy³¹. ENF density within the calf region of patients following bortezomib treatment was lower than age-matched controls, especially for patients experiencing neuropathy. However, the biopsies were not matched to the sites where pain or sensory deficits measured by QST were defined, as was done here. Further, changes in the density of Meissner's corpuscles was not reported.

A possible mechanism for both the sensory changes and the alterations in ENF and Meissner's corpuscle density is found in consideration of the strong pro-inflammatory response seen with chemotherapeutic agents. Pro-inflammatory cytokines are released from

both animal and human tissues after exposure to chemotherapy drugs *in vitro*, and co-administration of chemotherapeutic drugs with cytokines, for example vincristine in combination with GM-CSF, markedly increases the severity and magnitude of treatment-induced pain and other neurological impairments³³. The cytokines IFN α/γ , TNF α , IL-1, and IL-6 are increased *in vitro* by cisplatin^{1,15,28} and paclitaxel^{41,25}. Both myelinating and nonmyelinating Schwann cells express receptors for TNF, IFN, IL-1, and IL-6, and activation of these receptors leads to activation of NF- κ B and c-jun pathways that in turn result in downregulation of myelin synthesis, increased expression of the p75 NGF receptor, dedifferentiation, extirpation of myelin and proliferation^{2,23,9,35}. As individual Schwann cells become activated and exposed to pro-inflammatory cytokines, these cells themselves may begin to synthesize and release pro-inflammatory cytokines³⁵, affecting neighboring Schwann cells and closing a positive feedback loop. Prolonged or high-dose exposure of Schwann cells leads to apoptosis, potentially explaining the transition to persistent pain in some patients². Finally, NGF, IGF, and NT-3, all of which offer defense against chemotherapy-induced pain in animals^{16,18,10}, inhibit NF- κ B signaling²⁷ and prevent or reverse the effects of inflammatory cytokines on Schwann cells^{39,8}. Minocycline, a broad spectrum tetracycline antibiotic that decreases cytokine expression and microglial activation^{20,22} prevents paclitaxel⁵ and oxaliplatin-induced hypersensitivity³ and ENF depletion in animals^{4,3}.

In summary, the results of this study on bortezomib-related chemoneuropathy indicate that pain intensity and deficits in sensory performance⁶ remain nearly constant over time and may be associated with a marked loss of ENFs and Meissner's corpuscles in affected skin. These data indicate that long-term strategy for the management of bortezomib-related chemoneuropathy should be focused more on protection of patients at or before onset as opposed to palliative strategies for management.

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Figure 1. Skin temperature in patients at the test and the retest

The bar graph shows mean ($\pm$ S.E.) skin temperature for control subjects (black bars) and from patients with bortezomib-related chemoneuropathy at the time of the initial test (Test, light grey bars) and the long-term follow-up examination (Retest, dark gray bars). The pain patients showed significantly cooler skin in the area of on-going pain (Pain Area), and a non-significant trend toward a decrease in the area of sensory disturbance, but not necessarily pain (Border Area) when compared to control subjects. There was no change over time in these data for the follow-up examination. ** = $p < 0.01$

Figure 2. Touch detection thresholds in patients at the test and the retest

The bar graph on the left shows the mean ($\pm$ S.E.) von Frey touch detection thresholds obtained from control subjects and from patients with bortezomib-related chemoneuropathy at the test and follow-up examinations. The pain patients showed significantly elevated touch detection within the area of on-going pain (Pain Area, glabrous surface of the index finger), in areas of sensory disturbance but not necessarily pain (Border Area, thenar eminence), and in areas of skin not perceived by the patients as symptomatic (Non-painful Area, volar surface of the forearm) at both test times. The bar graph at the right shows the mean ($\pm$ S.E.) slotted peg-board completion times for the dominant and non-dominant hands in control subjects (black bars) and pain patients at the test (light grey bars) and retest (dark grey bars) examinations. Patients took significantly longer times to complete the pegboard task in both hands and at both testing times. ** = $p < 0.01$

Figure 3. Sharpness detection thresholds for patients at the test and the retest

The bar graph shows the mean ($\pm$ S.E.) sharpness detection threshold for control subjects (black bars) and from patients with bortezomib-related chemoneuropathy at the test (light grey bars) and retest examination (dark grey bars). The pain patients showed significantly elevated sharpness detection within the area of on-going pain (Pain Area) during both the test and the retest when compared to control subjects. **= $p < 0.01$.

Figure 4. Thermal detection thresholds in patients at the test and the retest

The bar graph in (A) shows the mean ($\pm$ S.E.) temperature for detection of warming and for heat pain for control subjects (black bars) and for patients with bortezomib-related chemoneuropathy at the test (light grey bars) and retest examination (dark grey bars). The pain patients showed deficits in both the detection of skin warming and increased threshold to heat pain in all three areas tested during the test: the area of on-going pain (Pain Area), the area of sensory disturbance but not necessarily pain (Border Area), and the area of skin not perceived by the patients as symptomatic (Non-painful Area). During the retest, warmth detection in the border area was similar to control subjects. Also during this retest exam, impairments in heat detection were now only evident in the Pain Area. The bar graph in (B) shows the mean ($\pm$ S.E.) temperature for the detection of skin cooling and cold pain, in which there was little change. * = $p < 0.05$; ** = $p < 0.01$

Figure 5. Epidermal nerve fibers in a patient with chronic bortezomib-related neuropathy
 This figure shows immunohistochemistry staining with the pan-neuronal marker PGP9.5 (green) in the biopsies of a patient with bortezomib-related chemoneuropathy in the area of pain (finger tip) (A), the border zone (palm) (B), and the non-painful area (forearm) (C). These biopsies can be compared to similar biopsies from a control subject (D, E, F). Epidermal Nerve fibers (ENF) can be seen in the control subject crossing the dermal-epidermal junction (D/E J) into the epidermis (Epid) in all regions. Note the extensive decrease in epidermal nerve fibers in the patient biopsies. Also within the control subject biopsies from the fingertip and palm, Meissner's corpuscles (MC) can be clearly seen within the epidermis. These structures are notably absent in the patient biopsies. (scale: 100um)