

Intraepidermal Nerve Fibre Density in Cancer Patients Receiving Adjuvant Chemotherapy

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Abstract. *Background:* Chemotherapy-induced neuropathy is a common adverse event in patients receiving vinca alkaloids, platinum derivatives and taxanes. However, the underlying pathogenetic mechanisms have not been completely elucidated. We set up a prospective pilot study on skin biopsies in newly diagnosed cancer patients receiving neurotoxic chemotherapeutic agents as adjuvant treatment in order to study the occurrence of small-fibre pathology and its relationship to clinical symptoms. *Patients and Methods:* Skin biopsies from distal leg were performed in 12 patients before, during and after chemotherapy. Using light microscopy, the intraepidermal nerve fibre (IENF) density was determined from the skin biopsies by counting morphometrically the immunopositive nerves per epidermal area. *Results:* Reduced IENF density was observed in eight patients at baseline. During the follow-up, the IENF density increased significantly in six patients and remained unchanged in two. In four patients, the IENF density was normal both at baseline and at the end of the follow-up period. Neuropathic symptoms were manifested in nine patients, but no association with the IENF count was found. *Conclusion:* During chemotherapy, results from patients revealed different evolutionary patterns of IENF density, but symptoms and IENF density were not related.

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Chemotherapy is one of the most effective therapies of cancer. It is used both as adjuvant therapy and as treatment for advanced cancer. Survival benefit of adjuvant chemotherapy has been demonstrated in breast, ovarian and colorectal cancer, and its role is under research for many other malignancies. In advanced cancer, chemotherapy prolongs survival and improves quality of life by preventing cancer-related symptoms. However, chemotherapy has many adverse effects: haematological and gastrointestinal toxicity, manifested as mucositis and diarrhoea, alopecia, nephrotoxicity, cardiotoxicity and neurotoxicity. Most of these toxicities are reversible, but some, such as neurotoxicity, can also be permanent. The long-term adverse effects of chemotherapy are becoming more important as more cancer patients are being cured.

The most neurotoxic chemotherapeutic agents are platinum derivatives, taxanes and vinca alkaloids (1, 2). The incidence and degree of neurotoxicity depend on the drug(s) used, the duration of administration and the cumulative dose applied (2, 3). The pathogenetic mechanisms of chemotherapy-induced neuropathy have not been fully clarified. Taxanes and vinca alkaloids are antimicrotubule agents which promote cell death. They bind to tubulin and induce metaphase arrest by altering microtubule dynamics at the end of the mitotic spindle formation, but they also bind with high affinity to axonal microtubules (3, 4). Oxaliplatin is a cisplatin derivative which inhibits DNA synthesis by forming DNA adducts. It also seems able to prevent microtubule formation by binding to proteins. Oxiplatin has been also shown to alter voltage-gated sodium channel kinetics in sensory neurons (3).

Sensory neuropathy presents itself as paraesthesia, numbness, and pain in the feet and hands, with symptoms generally appearing first in the toes and then in the fingers. Motor neuropathy presents itself usually as muscle weakness or difficulty in climbing stairs. The severity of both sensory and motor symptoms is mild to moderate and they are reversible if chemotherapy is discontinued or the dose is

reduced, but recovery lasts from months to years. Oxaliplatin-induced neurotoxicity may be acute or chronic. Acute neurotoxicity is frequent and consists of reversible cold-induced paresthesia, dysesthesia or pain of the hands or the face. These sensory symptoms may coexist with motor symptoms. Chronic sensory axonal neuropathy involves mainly the limbs (4, 5).

The patient's medical history and clinical neurological examination remain the cornerstone for detection of chemotherapy-induced neuropathy. Electroneuromyography (ENMG) and quantitative somatosensory testing can be used to assess large and small sensory fibres, respectively. Skin biopsy is a validated technique for determining intraepidermal nerve fibre (IENF) density and it has been shown to be useful in the diagnosis of small fibre polyneuropathy (6, 7). Only few studies, however, have addressed the role of small fibre dysfunction in cancer patients. This prompted us to set up a prospective pilot study on the IENF density in cancer patients receiving adjuvant chemotherapy.

Patients and Methods

Newly diagnosed cancer patients ranging in age from 18 to 70 years and starting their first adjuvant chemotherapy with platinum derivatives or taxanes at Tampere University Hospital were included. All patients were screened not to have diabetes, hypothyroidism, thiamine or B12 deficiency, excessive alcohol consumption, or any other known cause of neuropathy. History and clinical neurological examination were carried out before the commencement of chemotherapy and at the follow-up visits. Skin biopsies (diameter 3 mm) were taken from the distal leg 10 cm above the lateral malleolus before chemotherapy. Subsequent biopsies were obtained after three and six cycles of the therapy and after the end of chemotherapy, whenever possible.

Specimens were fixed in 10% formalin and then embedded in paraffin. Ten-micrometre sections were immunostained with a polyclonal panaxonal marker protein gene product 9.5 (PGP 9.5) (Ultraclone, Isle of Wight, UK). The number of IENF was counted with a light microscope at $\times 400$ magnification with the assessor blinded to the clinical status of the patients (MJK). Three adjacent skin sections were analysed to allow proper estimation of the IENF count. The estimation of epidermal area the point-counting was performed using a square lattice (Figure 1A). The fifth percentile of the normative range for the distal part of the leg (40 fibres per mm^2) was used as a cutoff point. The details of the method have been described previously (8). Our method follows the recently published skin biopsy guidelines except on reporting the IENF density per skin surface area (7).

The protocol was approved by the Ethics Committee of the Tampere University Hospital, and written informed consent was obtained from all participants.

Results

Twelve patients (8 males and 4 females; aged 37-66 years, mean 56 years) participated in the study. At the baseline visit, clinical neurological examination was normal in all patients and none of them presented any symptoms

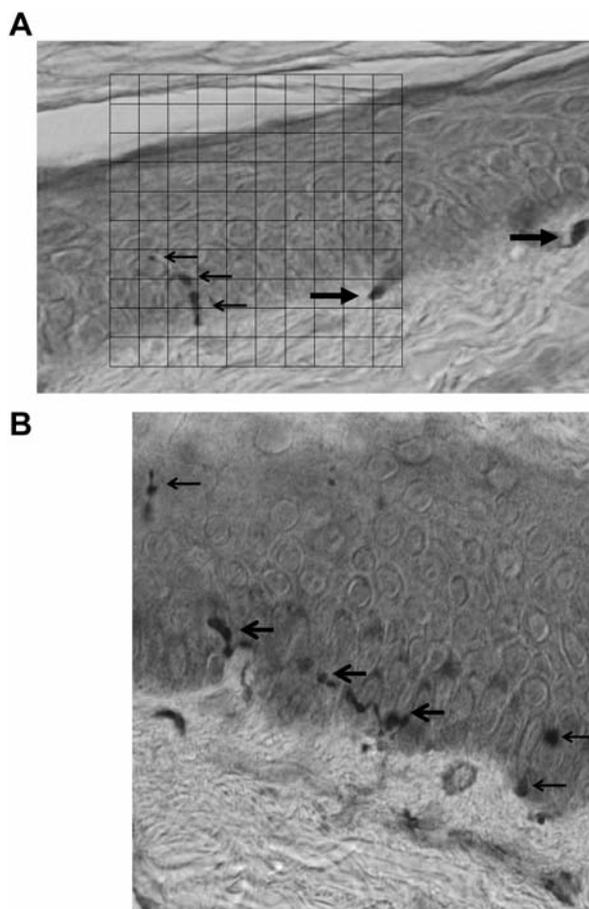


Figure 1. A: Demonstration of the microscope view using a 100-point square lattice. In this PGP 9.5-stained skin section of patient 5 before any treatment, there are altogether three PGP 9.5-positive axons: on the left, one axon in the epidermis (thin arrows), and two thicker axonal endings in the dermis on the right (broad arrows). According to the nerves per epidermal area index formula, there are approximately 97 axons in 1 mm^2 of epidermis. Formula: constant for individual microscope \times (number of nerves/number of lattice points) = $5917 \times (1/61) = 97$. B: In the skin section of patient 5 after 6 cycles of chemotherapy, three PGP 9.5-positive axons can be seen in the epidermis: two axons laterally (thin arrows) and a thicker horizontal axon in the middle (broad arrows).

suggesting neuropathy. Six patients had colorectal cancer and were treated with adjuvant oxaliplatin (cumulative doses of 500-780 mg/m^2). Four had prostate cancer and received an adjuvant docetaxel-based trial treatment (cumulative doses of 356-454 mg/m^2). Two patients with breast cancer received a docetaxel-based adjuvant treatment (cumulative doses of 228-386 mg/m^2). The demographic data of the patients, chemotherapy and the IENF densities at different time points are presented in Table I. None of the patients died during the study period, but metastatic spread of disease was observed in two (patients 3 and 12).

Table I. *Intraepidermal nerve fibre (IENF) density, symptoms and signs.*

ID	Age (years), gender	Type of cancer	Chemo-therapy	IENF* (n/mm ²)						Clinical presentation	
				Baseline	After 3 cycles (2nd)		After 5-6 cycles (3rd)		Final (no. of cycles) (4th)		
1	61 M	Colorectal	Oxaliplatin	237		ND	5 months (5 cycles)	76	22 months (6 cycles)	114	Spontaneous paraesthesia and cold allodynia in fingers after 3 cycles, recovered
2	59 F	Colorectal	Oxaliplatin	104	2 months	87	4 months (6 cycles)	64		ND	Dysaesthesia, cold allodynia and thermal hyperalgesia in hands after 3 cycles (recovered), and weakness and clumsiness of hands after 6 cycles, motor symptoms (persisted)
3	66 M	Colorectal	Oxaliplatin	24	2 months	124	4 months (6 cycles)	52	26 months (6 cycles)	133	Dysaesthesia and cold allodynia in fingers after 3 cycles, recovered
4	63 F	Colorectal	Oxaliplatin	21	2 months	78	4 months (6 cycles)	41	19 months (6 cycles)	80	Pain, spontaneous paraesthesia and pinprick hypoesthesia in hands after 6 cycles, persisted
5	37 M	Colorectal	Oxaliplatin	17	2 months	44	4 months (6 cycles)	70		ND	Dysaesthesia, cold allodynia and clumsiness in hands after 3 cycles, in addition spontaneous paraesthesia and warm allodynia of throat after 6 cycles, persisted
6	52 M	Colorectal	Oxaliplatin	22	2 months	20	4 months (6 cycles)	33		ND	Spontaneous paraesthesia, cold allodynia and pinprick hypoesthesia in hands after 3 cycles, recovered
7	58 M	Prostate	Docetaxel	30	2 months	145	20 months (6 cycles)	89		ND	Paraesthesia in feet after 3 cycles, recovered
8	64 M	Prostate	Docetaxel	26		ND	15 months (6 cycles)	72		ND	No symptoms
9	62 M	Prostate	Docetaxel	18	2 months	24		ND	20 months (8 cycles)	92	Spontaneous paraesthesia, dysaesthesia and thermal hypoesthesia in hands after 3 cycles, persisted
10	59 M	Prostate	Docetaxel	15	2 months	7	16 months (6 cycles)	0		ND	Spontaneous paraesthesia and clumsiness in hands and feet after 3 cycles, recovered
11	52 F	Breast	Docetaxel	68	2 months	89	4 months (6 cycles)	80		ND	No symptoms
12	39 F	Breast	Docetaxel	94	4 months	89	19 months (5 cycles)	125		ND	No symptoms

*Normal value of IENF >40/mm²; ND, not determined; M: male, F: female.

During follow-up, nine patients (75%) presented with clinical symptoms of neuropathy. Clinical neurological examination revealed concordant abnormalities. All symptomatic patients complained of paraesthesia or dysaesthesia. All oxaliplatin treated patients reported neuropathic symptoms. Five patients treated with oxaliplatin reported cold allodynia in hands; it remained persistent in only one of them. Altogether, in three oxaliplatin-treated patients, neuropathic signs and symptoms were detected at the last follow-up visit. In the docetaxel-treated group, three patients manifested with neurological symptoms, which were transient in two of them. Three patients did not develop any neuropathic symptoms or signs (Table I).

Separate IENFs were detected in skin samples immunostained with a polyclonal antibody to PGP 9.5. In

all 12 samples taken just before the chemotherapy, the morphology of the fibre was normal but the IENF count was lower than normal limits in eight. In oxaliplatin-treated patients, three different patterns in evolution of IENF counts were found. The IENF count remained within normal limits in all samples, but with a tendency to decline (patients 1 and 2). A second pattern was characterized by an abnormally low baseline IENF count and normalization in consecutive samples (patients 3, 4 and 5). Figure 1A and B show for the skin samples of patient 5 (baseline and after 6 cycles of chemotherapy, respectively). In patient 6, the IENF count remained below the normal range in all samples. Similar evolutionary patterns were also detected in docetaxel-treated patients (Figure 2).

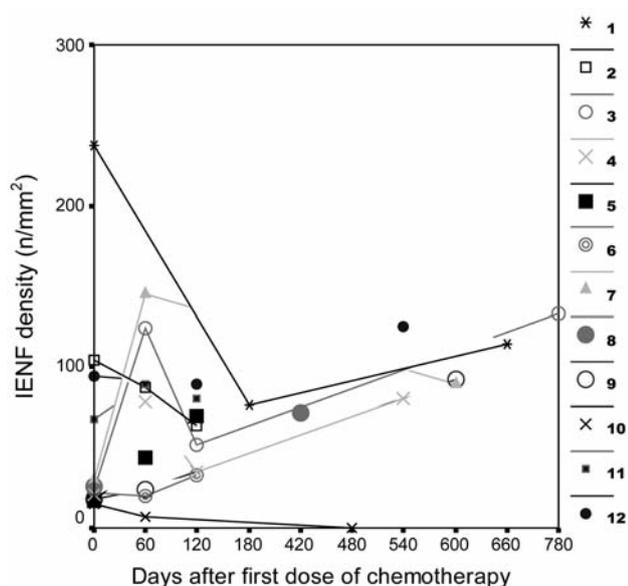


Figure 2. Intraepidermal nerve fiber (IENF) density at baseline and at different time points during follow-up period.

Discussion

The principal finding of this pilot study is that IENF densities can be markedly reduced in cancer patients, even prior to starting adjuvant chemotherapy. This suggests that neurotoxic chemotherapeutic agents cannot be solely blamed for the commonly encountered abnormalities of sensation or neuropathic pain. It would be tempting to assume that some patients develop paraneoplastic small fibre neuropathy, which may be subclinical at first. This condition may contribute to the occurrence of sensory symptoms by amplifying the impact of neurotoxicity and thus also predisposing to chronic neuropathy.

Lipton *et al.* (9) were first to demonstrate subclinical small fibre dysfunction in cancer patients. In their study, they determined thermal thresholds from 14 cancer patients who had not been treated with neurotoxic chemotherapeutic agents and did not have any identifiable risk factors for neuropathy. They found that 50% of patients had elevated thermal thresholds. Our results corroborate these earlier findings and indicate that small fibre neuropathy may be commonly associated with cancer.

Contrary to our preliminary hypothesis, neurotoxic chemotherapy does not always seem to induce loss of IENFs, but instead, different patterns may evolve, including the normalization of fibre count during chemotherapy. Recently, Burakgazi *et al.* (10) reported a significant reduction in distal leg IENF density in patients receiving oxaliplatin. The profile of the longitudinal IENF densities corresponded with our

results showing both decrease and increase of density during the first six months after baseline. In our study, no association between the IENF count and neurological symptoms was found. This may be partly due to the small sample size. The results of this pilot study need to be confirmed in a larger patient population with longer follow-up and combined IENF and quantitative somatosensory testing.

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