Observational Studies

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Cutaneous nerve biopsy in patients with symptoms of small fiber neuropathy: a retrospective study

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Abstract

Objectives: We aimed to investigate to what extent small fiber tests were abnormal in an unselected retrospective patient material with symptoms suggesting that small fiber neuropathy (SFN) could be present, and to evaluate possible gender differences.

Methods: Nerve conduction studies (NCS), skin biopsy for determination of intraepidermal nerve fiber density (IENFD) and quantitative sensory testing (QST) were performed. *Z*-scores were calculated from reference materials to adjust for the effects of age and gender/height.

Results: Two hundred and three patients, 148 females and 55 males had normal NCS and were considered to have possible SFN. 45.3 % had reduced IENFD, 43.2 % of the females and 50.9 % of the males. Mean IENFD was 7.3 ± 2.6 fibers/mm in females and 6.1 ± 2.3 in males ($p<0.001$), but the difference was not significant when adopting *Z*-scores. Comparison of gender differences between those with normal and abnormal IENFD were not significant when *Z*-scores were applied. QST was abnormal in 50 % of the patients (48.9 % in females and 52.9 % in males). In the low IENFD group 45 cases out of 90 (50 %) were recorded with abnormal QST. In those with normal IENFD 51 of 102 (50 %) showed abnormal QST.

Conclusions: Less than half of these patients had reduced IENFD, and 50 % had abnormal QST. There were no gender differences. A more strict selection of patients might have increased the sensitivity, but functional changes in unmyelinated nerve fibers are also known to occur with normal IENFD.

Approval to collect data was given by the Norwegian data protection authority at University Hospital of North Norway (Project no. 02028).

Keywords: small fiber neuropathy; cutaneous nerve biopsy; intraepidermal nerve fiber density; quantitative sensory testing; gender differences; *Z*-score

Introduction

Small fiber neuropathy (SFN) is a disease of thin myelinated and unmyelinated somatic and in some cases autonomic nerve fibers [1]. When only these fibers are affected, the condition is recognized as pure SFN, but symptoms and signs of SFN may also occur in neuropathies involving myelinated nerve fibers [1]. Pure SFN, most often length-dependent in contrast to non-length dependent presentation with primary affection of dorsal root ganglia neurons, thus requires that nerve conduction studies (NCS) reflecting function of myelinated nerve fibers are normal [2]. Although there are also other methods such as for evaluation autonomic function and pain-related evoked potentials, quantitative sensory testing (QST) is commonly used. This psychophysical test may show sensory impairment suggestive of small fiber neuropathy, but the method depends strongly on the cooperation of the patient, and it also does not differentiate between lesions involving the peripheral or central sensory pathways. A definitely more objective method is skin biopsy at the ankle with quantification of small-diameter nerve

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fibers in the human epidermis (intraepidermal nerve fibers (IENFs)) for demonstration of nerve fiber loss indicating SFN [1, 3]. One draw-back of this method is that it is not commonly available, and in Norway only in our center. For classification of definite SFN, the following criteria have been proposed based on skin biopsy and QST: Decreased IENFD and/or abnormal QST in addition to symptoms and signs suggesting SFN, and normal sural nerve conduction studies (NCS) [4]. However, clinically painful SFN may also be connected with functional changes of small diameter nerve fibers without loss of IENFs in skin biopsies [5–7].

We present here the results of skin biopsies from patients that had been examined with NCS and in most cases also with QST. The purposes were to investigate to what extent cutaneous nerve biopsy and QST were abnormal in an unselected retrospective patient material with normal NCS and symptoms (like burning pain and coldness, pins-and-needles, pricks, tingling and numbness) suggesting that SFN could be present, and to evaluate possible gender differences. We also wanted to apply calculations of *Z*-scores taken age and gender in account in addition to using absolute values of IENFD.

Patients and methods

Patients

During 3 years skin biopsy for determination of IENFD were performed in 223 patients with symptoms of SFN. Only those with normal NCS (n=203) were included in this study (Figure 1). In addition to patients referred from the Neurology Outpatient Clinic, University Hospital of North Norway (UNN) or other hospitals, some patients were admitted from their family physicians. Most of those in the last-mentioned group did not get a full clinical examination by a neurologist, but they were screened with relevant clinical testing in the lower limbs (cotton wool and pin prick sensations with evaluation of either hypo- or hyperesthesia/allodynia, vibration sense, ankle tendon reflexes and muscle strength in the ankles and toes) by a specialist in clinical neurophysiology working in the field of neuromuscular disorders before final decision to perform a skin biopsy. Patients not selected were those with asymmetric symptoms and findings, for instance caused by radiculopathy or plexopathy or a contralateral hemispheric lesion, those with pain located in the joints and patients with tendinitis or myalgia.

Approval to collect these retrospective data was given by the Norwegian data protection authority at UNN (Project no. 02028).

Methods

Cutaneous nerve biopsy: The biopsies were most often obtained as the last test on the same day as NCS and QST. The method used in our laboratory has previously been described in detail [8]. In short, two punch biopsies were obtained laterally on the distal leg, and the specimens were fixed in cold fixative (2 % paraformaldehyde-lysine periodate (2 % PLP)) for up to 24 h at 4 °C. The specimens were then kept in a cryoprotective solution for one night. The next day the specimens were frozen in liquid nitrogen and stored in a –70 °C freezer for days up two weeks and thereafter serially cut in 50 µm sections using a cryostat. After immunohistochemical staining by hand for PGP 9.5, individual PGP 9.5 positive IENFs crossing the dermal–epidermal junction were counted at high magnification in bright field microscope in at least three nonconsecutive sections from each biopsy (Figure 2). The IENFD was then calculated as the mean of counts in these six sections. The same technician quantified all biopsies and participated in the calculation of the reference material. IENFD was defined as abnormal in a patient if Z-score was ≤2.

Nerve conduction studies: All patients underwent NCS. The investigations were performed by special trained technicians, and the results were controlled and interpreted by a clinical neurophysiologist. Surface electrodes were used for stimulation and recording; in most patients in at least one leg and one arm, in some patients in both legs. Motor NCS included the median, ulnar, peroneal and tibial nerves, and sensory NCS included the median, ulnar, radial, sural, superficial peroneal and medial plantar nerves. The following parameters were analyzed: distal latencies, amplitudes, conduction velocities, and for motor nerves F-M wave latencies. The NCS was classified as normal if abnormality was present in <2 nerves not including entrapments.

Quantitative sensory testing: QST was performed with SENSELab-THERMOTEST (Somedic AB, Sweden) using method of limits. A thermode of 50 × 25 mm was steadily applied to the skin. The temperature ranged from 5 to 52 °C. Skin reference temperature was set at 32 °C. While the temperature was decreased with 1 °C/s, the patient pushed a button changing the current in the thermode when perceiving cold and warmth respectively. Mean thresholds for perception from five successive tests of cold (CPT) and warmth (WPT) were recorded. Recordings were obtained from the distal part of the leg approximately 10 cm above the lateral malleolus at the same site as the biopsies were taken, and on the dorsal part of the foot. QST was classified as abnormal if one or more *Z*-scores of WPT and CPT were ≥2 on the leg and/or the dorsum of the foot. Patients were also asked about qualitative changes on thermal perception like unpleasant sensations.

Statistics: SPSS 28 for Macintosh was used for statistical analysis. The unpaired *t*-test and the Mann–Whitney test were used to compare normally and non-normally distributed variables. To analyze group differences Pearson´s chi-square test was applied. Simple regression analysis was performed to test associations between IENFD and WPT or CPT.

Calculations of *Z*-scores: The NCS results were compared with normal values available by the manufacturer where the degree of abnormalities is expressed as *Z*-scores (number of standard deviations between obtained and expected value dependent on age and height). To determine whether the values of WPT, CPT and IENFD were abnormal, we also used *Z*-scores. These *Z*-scores were calculated from our reference materials (QST: n=137 and IENFD: n=106 individuals [8]) after log transformations, taking age and gender (IENFD) and age and height (WPT and CPT) into account.

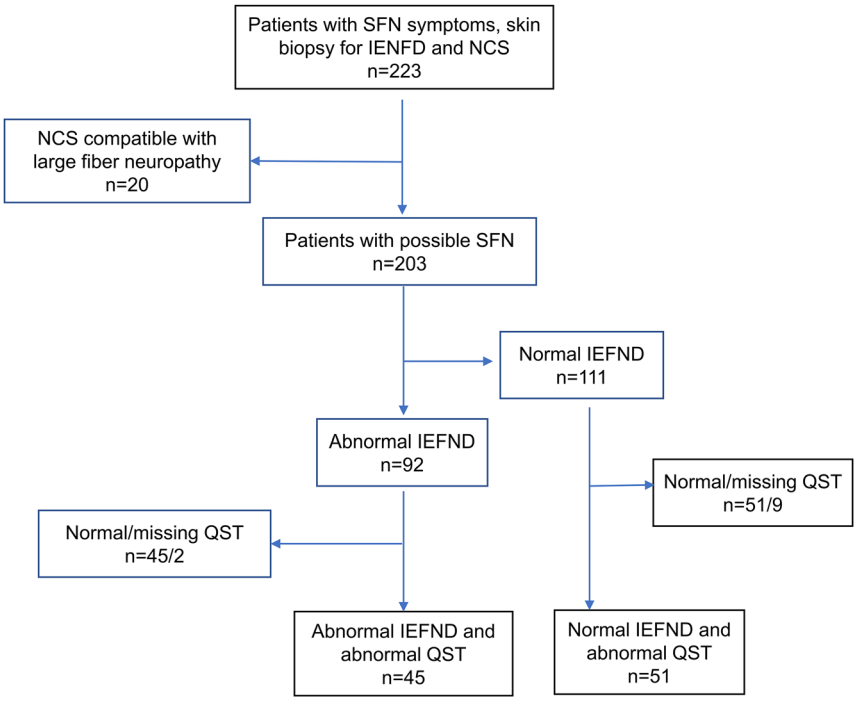

Figure 1: Diagram showing number of patients in the different groups of normal and abnormal NCS, QST and IENFD.

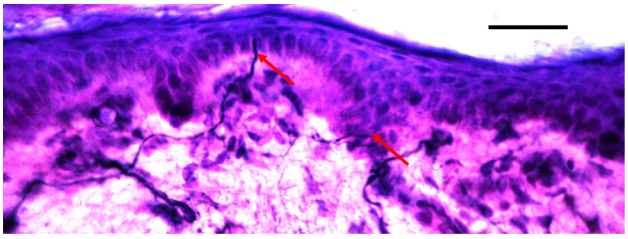

Figure 2: Frozen section processed for bright-field immunohistochemistry reacted with PGP 9.5. Counterstained with hematoxylin and eosin. Arrows show nerve fibers entering into the epidermal epithelium. Scale bar 50 µm.

Table 1: The most frequent underlying diseases known to or proposed to be connected with small fiber neuropathy in patients with symptoms of SFN (n=203).

	n (%)
Diabetes mellitus (1 and 2)	12 (5.9)
Cancer and treatment with cytostatics	11 (5.4)
Sarcoidosis	10 (4.9)
Fibromyalgia	8 (3.9)
Hypothyreosis	7 (3.4)
Sjögrens syndrome	6 (3.0)
Hereditary neuropathies	5 (2.5)
Celiaci	5 (2.5)
Restless legs syndrome	5 (2.5)
Other	21 (10.3)
No information of underlying disease	113 (55.7)

Results

Twenty cases had abnormalities in at least two nerves in the lower limbs and were classified as having large fiber neuropathy, and the remaining 203 were considered to have possible SFN.

Information about underlying diseases with potential to cause SFN was found in 90 out of the 203 (44.3 %) patients. The most prevalent were diabetes mellitus, cancer/cytostatics, and sarcoidosis (Table 1). Among other conditions known to be associated with SFN in addition to those listed in the table, were cold induced neuropathy, Ehlers–Danlos syndrome, and erythromelalgia. One hundred and thirteen (55.7 %) were considered to have possible SFN without established cause.

Of the patients with normal NCS, there was a female predominance with 148 females vs. 55 males, and the mean age of the females was higher (Table 2). Totally 92 (45.3 %) had reduced IENFD (Figure 1) when compared to our laboratory's normal material. The frequency of abnormal IENFD did not differ between genders (Table 2). Mean IENFD was lower in men than in women, but the *Z*-scores adjusting for gender and age were not significantly different. We also compared gender differences between those with normal and abnormal IENFD. The absolute values of IENFDs differed, but not when *Z*-scores were applied (Table 3).

Table 2: Gender differences of IENFD and QST parameters in patients, and comparison of these parameters between patients and reference materials.

IENFD	Patients			Reference materials	
			p-Value		p-Value[a]
Gender(n)	Females(148)	Males(55)		Total(106)	
Age, years	48.3 ± 13.8	43.0 ± 12.8	0.02	47.9 ± 18.4	ns
IENFD(Fibers/mm)	7.3 ± 2.6	6.1 ± 2.3	0.002	12.4 ± 4.6	<0.001
IENFD(Z-score)	−1.7 ± 1.0	−1.8 ± 1.2	Ns	−0.3 ± 1.0	<0.001
Abnormal IENFD n, %	64 (43.2)	28 (50.9)	Ns		
QST			**p-Value**		**p-Value[a]**
Gender(n)	Females(141)	Males(51)		Total(137)	
Age, years	48.5 ± 13.7	42.6 ± 12.9	0.008	42.3 ± 14.9	0.004
WPT leg °C	8.6 ± 4.1	11.1 ± 4.1	0.02	7.1 ± 3.1	<0.001
CPT leg °C	5.0 ± 3.3	6.6 ± 4.8	Ns	2.8 ± 1.6	<0.001
WPT foot °C	8.7 ± 4.0	10.1 ± 4.2	Ns	6.9 ± 3.1	<0.001
CPT foot °C	6.7 ± 5.5	8.7 ± 6.4	Ns	2.9 ± 1.7	<0.001
Abnormal QST n, %	69 (48.9)	27 (52.9)	Ns		

Values expressed as % or mean ± SD. [a]Comparison between the patient group and reference material. p-values of QST parameters are calculated from *Z*-scores.

Table 3: Gender differences between those with normal and abnormal IENFD.

		Females	Males	p-Value
Normal IENFD	Fibers/mm	8.85 ± 2.49	7.84 ± 1.84	p=0.016
	Z-score	−1.07 ± 0.73	−0.99 ± 0.69	Ns
Abnormal IENFD	Fibers/mm	5.28 ± 0.88	4.33 ± 0.92	p<0.001
	Z-score	−2.61 ± 0.48	−2.68 ± 0.90	Ns

Values expressed as mean ± SD.

QST was performed in 192 patients (141 females and 51 males). Ninety-six (50 %) had at least one abnormal QST threshold in the lower limbs. There were no gender differences of the QST parameters except higher WPT on the leg in males (Table 2).

In the abnormal IENFD group 45 cases out of 90 (50 %) were recorded with abnormal QST. In those with normal IENFD 51 of 102 (50 %) showed abnormal QST (Figure 1). There were no correlations between IENFD and thermal thresholds on the distal leg or dorsal foot.

Many patients, 90 out of 192 (46.7 %) experienced dysalgesia and/or pain when they were supposed to feel warmth or cold thresholds during QST. This experience was also reported by 17/59 individuals in our reference material, but less frequently (28.8 %, p=0.02). In the abnormal IENFD group they were 45 out of 90 (50 %), and when IENFD was normal 45 of 102 (44.1 %) (p=0.4). In those with abnormal QST thresholds in the lower limbs (n=96) 52 (54.2 %) had dysalgesia/pain, and when QST threshold was normal (n=96) 38 (35.5 %) reported dysalgesia and/or pain (p=0.04).

As expected both mean IENFD and mean QST parameters in the patients were significantly different from our reference materials (Table 2).

Discussion

The inclusion criterion of this retrospective study was possible SFN with relevant symptoms and that a skin biopsy for determination of IENFD had been performed. There was a striking predominance of females (72.9 %) in this population. This contrasts with results reported by Devigili et al. [2] with 52.3 % females, but there was a female predominance (67 %) in a recent report of 94 patients with SFN in Olmsted County, Minnesota [9]. Also, in the study of Weaver et al. [10] there were more females when they studied retrospectively gender differences in skin biopsy findings. It is therefore possible that females generally have more symptoms with pain syndromes including fibromyalgia, rising the suspicion of SFN. The explanation for this gender difference could be both social and psychological factors, but in addition genetic factors and biological differences in hormones and immune system may contribute [11–13]. Johnson et al. [9] found in addition to predominance of female gender that insomnia, use of opioid and non-opioid analgesics, obesity, hypertriglyceridemia, and diabetes were more common in patients than in controls. In the present study diabetes, cancer with treatment of cytostatics, sarcoidosis, fibromyalgia, hypothyreosis, and Sjögren syndrome were the most prevalent conditions that might have caused both symptoms of SFN and IENF loss.

Of the patients with normal NCS, 45.3 % had abnormal IENFD. The frequency did not differ between genders (43.2 % of the females and 50.9 % of the males). The absolute mean value of IENFD was lower in males than in females, but this difference was not present when *Z*-scores adjusting for age and gender were applied (Table 2). The study of Weaver et al. [10] reported that 44 %, including 33 % of the females and 63 % of the males, had low IENFD or sweat gland nerve fiber density. In contrast to the present study, however, reduced IENFD alone was strikingly predominant in the females (51.1 % vs. 7.8 % in males). In a retrospective study from Switzerland, 84/121 (69.3 %) of patients screened for SFN had abnormal IENFD (69.3 % females) [14]. In all patients a clinical investigation of a neurologist was required before skin biopsy was obtained. In a prospective study of 86 patients with suspected SFN, neurological examination was almost equally sensitive (62 %) as IENFD (70 %) [15]. It has also been reported that the diagnostic accuracy could be improved by using a symptoms inventory questionnaire [16]. In our patient material, a clinical examination and interview by a neurologist was not obligate, this would perhaps have increased the sensitivity. However, the decision of whether a skin biopsy should be obtained, was made by a skilled clinical neurophysiologist based on the patient's symptoms, a focused neurological examination of the feet and most often normal NCS results.

Both in the abnormal and in the normal IENFD group 50 % had abnormal QST. This may be explained by the fact that all patients had symptoms of SFN, and we suppose that involvement of IENFs can be present without necessarily reaching levels of abnormality in IENFD and warm and cold thresholds [5–7, 17]. In the present study we did not find correlations between IENFD and QST parameters, but we have previously reported in another patient material that such association was only present when NCS was abnormal, and thus seemed to be dependent of a more severe neuropathic process [18]. An association between function and density of fibers have however been reported in other studies, especially when tests were performed at the same site [19]. NCS were not systematically performed in these studies.

Many patients experienced dysalgesia/pain when they were supposed to feel warmth or cold thresholds during QST, both when IENFD was abnormal and in the normal group. In those with abnormal QST thresholds, dysalgesia/pain was more frequent than when QST was normal. This reflects that when thresholds for warm perception is abnormally increased and for cold decreased, there is more overlap with pain thresholds [20]. It is also known that polymodal nociceptors can be activated with temperatures above 45 °C [21].

The combination of reduced IENFD and abnormal QST is near the definition of the Besta criteria for definite SFN [22], but does in our study not include clinical signs of small fiber impairment although this was documented in the majority of the patients. The requirement of both abnormal IENFD and QST (and/or clinical signs) however, is stricter than the minimum NEURODIAB criteria for definite SFN with clinical signs, normal NCS, and abnormal QST or reduced IENFD [23].

In this retrospective study less than half of patients with symptoms of small nerve fiber neuropathy had reduced IENFD. This may reflect that there had been a too liberal practice of performing skin biopsy in some cases, and it has to be considered carefully when to take a biopsy as it does not have perfect diagnostic power and can be inaccurate. However functional changes in unmyelinated nerve fibers, for instance in sodium channelopathies and erythromelalgia, also occur with normal IENF density [5–7, 17]. We finally recommend to calculate *Z*-scores of IENFD instead of using absolute values to eliminate age and gender effects.

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Data availability: The raw data can be obtained on request from the corresponding author.

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