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Retrospective Review

Skin Biopsy in Complex Regional Pain Syndrome: Case Series and Literature Review

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Background: Accumulating experimental and clinical evidence supports the hypothesis that complex regional pain syndrome type I (CRPS-I) may be a small fiber neuropathy.

Objectives: To evaluate the use of commercially available standard biopsy methods to detect intradermal axon pathology in CRPS-I, and to ascertain if these structural changes can explain quantitative sensory testing (QST) findings in CRPS-I.

Study Design: Retrospective review of charts and laboratory data.

Setting: Outpatient clinic

Methods: Skin biopsies from 43 patients with CRPS-I were stained with PGP 9.5, and epidermal nerve fiber density, sweat gland nerve fiber density and morphological abnormalities were evaluated. Thirty-five patients had quantitative sensory testing.

Results: Alterations in skin innervation were seen in approximately 20% of CRPS-I patients with commercial processing. There were no patient characteristics, including duration of disease, that predicted a decreased epidermal nerve fiber density (ENFD). There was no consistent relationship between QST changes and ENFD measured by standard commercial skin biopsy evaluation procedures.

Limitations: Commercial processing of tissue does not utilize stereologic quantitative analysis of nerve fiber density. Biopsy material is utilized from a proximal and distal source only, and differences in denervation of a partial nerve territory may be missed. The functional attributes of small fibers cannot be assessed.

Conclusions: The negative results indicate that CRPS-I may be associated with changes in the ultramicroscopic small fiber structure that cannot be visualized with commercially available techniques. Alternatively, functional rather than structural alterations of small fibers or pathological changes at a more proximal site such as the spinal cord or brain may be responsible for the syndrome.

Key words: Complex Regional Pain Syndrome, CRPS-1, CRPS, skin biopsy, epidermal nerve fiber density, sweat gland nerve fiber density, quantitative sensory testing.

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Accumulating experimental and clinical evidence supports the hypothesis that complex regional pain syndrome type I (CRPS-I) may be a small fiber neuropathy (1-4). Many of the components of the syndrome required by the most recent International Association for the Study of Pain guidelines (5) can be attributed to C-fiber and A-delta fiber abnormal excitation and firing in association with the co-release of peptidergic neuropeptides (6-10). Particularly important for the synaptic plasticity

of pain transmission neurons, a postulated mechanism for the chronic pain in CRPS-I is the afferent barrage of mechano-insensitive C-fibers from the area of injury (11,12). There is no clinical distinction between CRPS type I and CRPS type II as both demonstrate similar signs and symptoms. Following an injury, inflammatory, nociceptive and immune processes evolve in parallel that induce and maintain the syndrome (13-15).

There is relatively sparse literature on the skin biopsy findings in CRPS-I patients and no consistent pattern of skin pathology has emerged from previous research (16,17). The only previous manuscript (3) which evaluated skin biopsy and quantitative sensory testing (QST) findings in the same group of patients found a decrease in the mean number of nerve dendrites in the epidermis over a painful area compared to control sites, in addition to previously described QST abnormalities. The decrease in epidermal nerve fiber density (ENFD) is a consistent feature of small fiber neuropathies. If a consistent association exists between small fiber pathology and CRPS-I, it could indicate that small fiber dysfunction plays a critical, maybe causal, role in CRPS-I pathogenesis (2). In addition, the verification of such a relationship may pave the way for a reliable laboratory test for CRPS-I. Previous research, including the above mentioned manuscript, has not elucidated the quantitative relationship of the severity of pathology in dermal structures and epidermal "C" and "A-delta" fibers to the QST findings in CRPS-I.

We analysed results of skin biopsies of CRPS-I patients done at our institution, the QST results of these patients, and correlated the severity of the QST abnormalities to skin biopsy testing results. The purpose of this study was to evaluate the use of commercially available standard biopsy methods to detect alteration of small fiber axons in the affected skin of CRPS-I patients, and to ascertain if structural changes in intradermal small nerve fibers or other dermal structures can explain the quantitative sensory findings in CRPS-I patients.

Methods

The study was a retrospective analysis of skin biopsies of 43 patients from the pain clinic of Drexel University College of Medicine. All patients were examined by the same neurologist (RJS) to assure they met the Budapest diagnostic criteria for CRPS-I (5). The study was approved by the Drexel University College of Medicine Institutional Review Board.

Inclusion Criteria

All patients met the Budapest criteria for CRPS-I and II (5). No patient had either clinical or electromyographic evidence of a specific nerve injury. The duration of illness and its severity (number of limbs involved as well as torso and face) were not determinants of entry. Patients between 17 and 80 years old were eligible. Eight patients were being treated for hypothyroidism, a common finding in long-standing CRPS-I, while demonstrating all the characteristic signs of CRPS-I I (17).

Exclusion Criteria

Patients with clear injury to a peripheral nerve, thus classifying them as CRPS-II, were excluded. Patients with a predominant history and laboratory evidence for a predominant small fiber neuropathy were also excluded.

Biopsy and Small Fiber Analysis

After subcutaneous administration of local anesthetic, biopsies were obtained from a symptomatic lower extremity in patients who either had CRPS-I limited to that limb or who had more generalized disease affecting all extremities, face, and torso. The lower limb with maximal pain and allodynia was chosen for biopsy. Biopsies were taken at a proximal site with no edema and a distal site with some edema that can theoretically lower axonal densities and were compared with the upper ipsilateral biopsy site (taken in an area without edema) and with standard control data. An earlier study did not demonstrate axonal density reduction in sites with edema (3). We did control for between-body-region variability (18). Two punch biopsies of 3 mm diameter were obtained, one at the level of the pubis on the thigh and the other at the calf 10 cm above the lateral malleolus.

Biopsy samples were processed and evaluated with light microscopy by a commercial laboratory (Therapath Laboratories, New York). Qualitative visual inspection rather than stereologic quantitation was utilized. The samples were fixed in 2% PLP solution (2% paraformaldehyde, .075 Molar lysine, 0.037 Molar sodium phosphate, 0.01 Molar periodates). All sections were stained with protein gene product 9.5 (PGP 9.5) which stains axons; hematoxylin-eosin, which identifies histologic abnormalities such as axonal swelling (typical of small fiber neuropathy [SFN]); and Congo red to rule out amyloidosis (19-21). ENFDs below the fifth percentile were classified as decreased.

ENFD was determined using a standard technique (22). Nerve fibers crossing the basement membrane

were counted in 5 different tissue sections. The total number of fibers was divided by the length of the epidermis in the 5 sections. The ENFD was compared to normative values from healthy volunteers obtained by Therapath. These data were collected by Therapath before offering skin biopsy testing commercially, and were used for the purposes of this manuscript with their permission. The use of normative data was done in accordance with published guidelines (22).

Sweat gland nerve fiber density (SGNFD) was measured in all biopsies studied after 2010 when the test became commercially available. The SGNFD was assessed utilizing standard techniques (23). A digitized image of the sweat gland was overlaid with a grid of circles. The number of circles that contained one or more axons was counted which was then divided by the total number of circles overlying the sweat gland. The result expressed as a percentage was compared to normative values from healthy volunteers.

Quantitative Sensory Testing

Quantitative sensory testing results were available for 35 patients. In all cases, QSTs were recorded on the same part of the limb (distal lower extremity) as the skin biopsy. In 28 patients, QST testing was done at a site at the same level as the skin biopsy. In 7 patients, the QST testing was done 10 cm distal to the skin biopsy.

Quantitative sensory testing was done using a previously described technique (1). Warm, cold, heat-evoked, and cold-evoked pain thresholds were performed using a Medoc TSA-2001 (Medoc, Ramat Yishai, Israel). The Peltier thermode surface was 3 × 3 cm (9 cm^2). Sufficient time was allowed for adaptation (e.g., such that the thermode felt neither cold nor warm to the patient). The temperature of the thermode was increased (warm and heat-evoked pain) or decreased (cold and cold-evoked pain) at a constant rate of 1°C/sec starting from the baseline. Baseline temperature (32°C) was the same for determination of cold, warm, cold-evoked, and heat-evoked pain thresholds. The temperature stimuli were limited to a range of more than -10°C to less than 50°C. The patient was instructed to press the response button when a temperature or a pain sensation was felt. The order of testing for each patient was as follows: cold threshold (3 trials); warm threshold (3 trials); cold-evoked pain (one trial); and heat-evoked pain (one trial).

Cold detection thresholds below 26.3°C, warm detection thresholds above 40.8°C, cold-evoked pain thresholds above 25°C, and heat-evoked pain thresholds below 41°C were considered abnormal. These cut-off values were based on a review of earlier QST studies in small fiber neuropathies (24,25).

Data Analysis

Data analysis utilized Stata: release 9.1 (StataCorp LP, College Station, TX). Mean and standard deviation were used to describe variables that are normally distributed. Median and range were used for variables with a skewed distribution. The unpaired Student's t-test was used to compare nerve densities between different sites.

Results

Patient Characteristics

Patient characteristics are described in Table 1. None of the patients had nerve conduction/electromyography-proven injuries to peripheral nerves from the inciting injury, and therefore were classified as CRPS-I. The distribution of age and duration of symptoms is further depicted in Fig. 1.

Skin Biopsy Findings

Epidermal nerve fiber density was measured in all patients. Mean ENFD at the thigh was 11.3 ± 3.8/mm, and at the calf was 7.7 ± 2.8/mm ($P < 0.01$). Compared to controls, the ENFD in CRPS-I patients was not significantly different at the thigh (ENFD in controls = 10.5 ± 2.1/mm, $P = 0.22$), or at the calf (ENFD in controls = 7.7 ± 1.7/mm, $P = 0.53$) (Fig.2).

Sweat gland nerve fiber density studies were done in 16 patients. The SGNFD could not be measured in 3 thigh biopsies and 6 calf biopsies due to the absence of sweat glands in the skin sample. Mean SGFND at the thigh was 56.7 ± 10.1, and at the calf was 51.6 ± 17.3 ($P = 0.39$). Compared to controls, the SGNFD in CRPS-I patients was not significantly different at the thigh (SGNFD in controls = 54.6 ± 10.7, $P = 0.59$), or at the calf (ENFD in controls = 49.5 ± 7.7, $P = 0.57$).

Table 1. *Patient characteristics at the time of biopsy.*

Age (Years)	43.1 ± 10.5 years
Sex (Females)	36 (83.7 %)
Duration of Symptoms (Years)	5.4 (0.2 - 22.8)
Inciting Soft Tissue Injury Preceding Symptoms	31 (72.1 %)

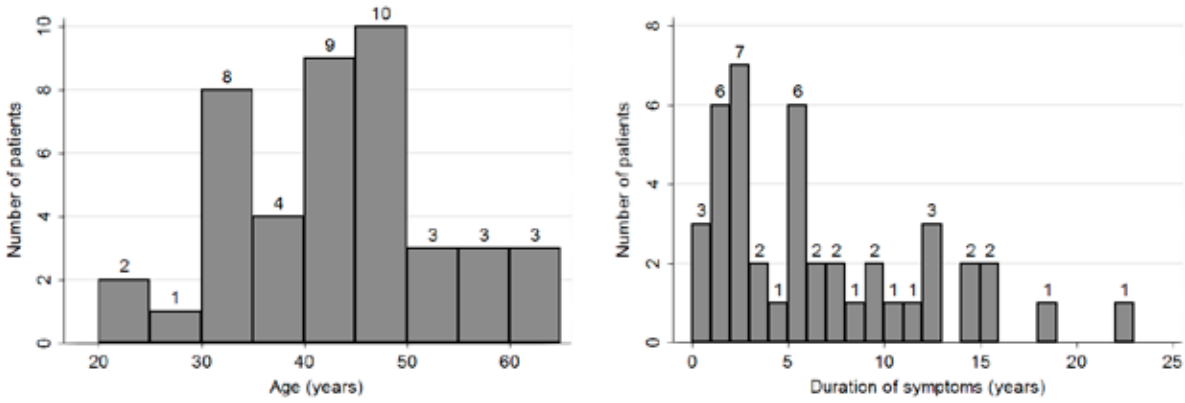

Fig. 1. *Distribution of age and duration of symptoms (in years).*

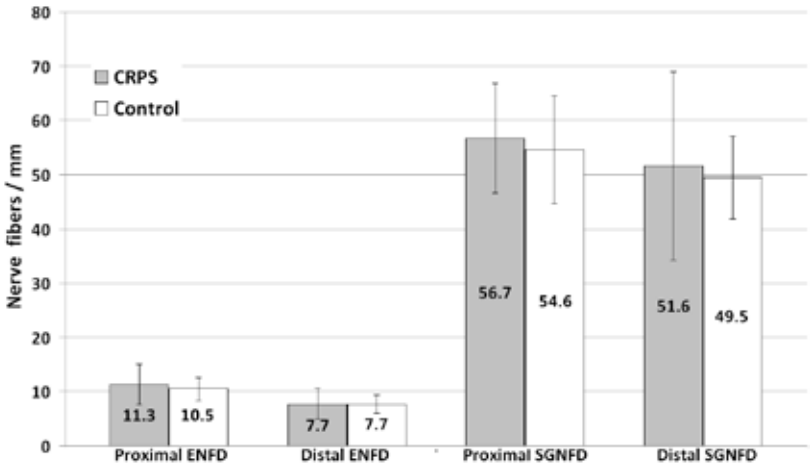

Fig. 2. *Mean epidermal nerve fiber density (ENFD) and sweat gland nerve fiber density (SGNFD) in CRPS-I patients and controls.*

Table 2. *Number (percentage) of patients with abnormal ENFD and SGNFD at the proximal and distal sites of skin biopsy.*

	Number (Percentage) of Patients with Abnormal ENFD (n = 43)	Number (Percentage) of Patients with Abnormal SGNFD (n = 16)
Proximal site	3 (6.9 %)	0*
Distal Site	9 (20.9 %)	2 /10* (20 %)
Both Sites	3 (6.9 %)	0

* Sweat glands were not found in 3 proximal and 6 distal skin biopsies, so the SGNFD could not be measured in these samples.

The number of patients who had ENFD and SGNFD below the 5th percentile cut-off value obtained from controls is noted in Table 2.

Correlation to Duration of Symptoms

There were no systemic variations in ENFD by duration of symptoms (Fig. 3).

The measured QST thresholds in CRPS-I patients and their interpretation after applying the cut-off criteria are detailed in Table 3. Cold-evoked pain thresholds could not be measured for 10 patients because they reported no pain even when the thermode was at the lowest temperature allowed by our protocol (-10°C). Similarly, heat-evoked pain could not be elicited in 4 patients even at the highest thermode temperature (50°C). These patients were noted to have "normal" evoked pain thresholds while calculating the prevalence of abnormality in Table 3.

Correlation between QST and ENFD

There was no linear relationship between any of the measurable QST thresholds and the ENFD (Fig. 4).

Heat-evoked pain thresholds that were too high (> 50 °C), or cold-evoked pain thresholds that were too low (< -10 °C) to be measured by our protocol could not be included in Fig. 4. Therefore, we compared the mean ENFD in patients with normal and abnormal QST thresholds (Fig. 5). There was no significant difference in the mean ENFD between patients with normal and abnormal QSTs.

Table 3. *Results of quantitative sensory testing.*

Threshold	Mean ± SD	Prevalence of Abnormality
Warm Detection	41.2 ± 4.0	12 (34.3 %)
Cold Detection	27.4 ± 3.4	8 (22.9 %)
Heat-evoked Pain	45.5 ± 3.6	3 (8.6 %)
Cold-evoked Pain	16.5 ± 13.9	13 (37.1 %)

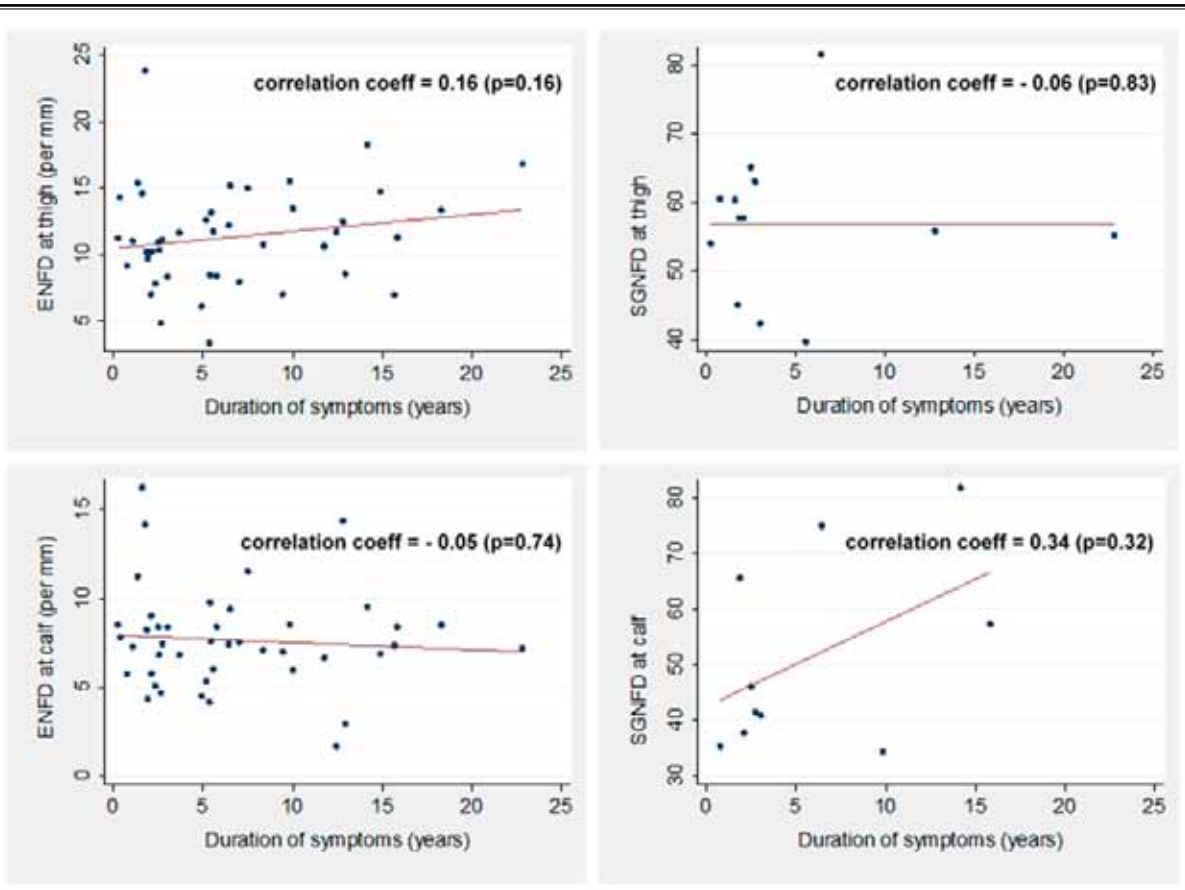

Fig. 3. Lack of correlation between ENFD/SGNFD and duration of symptoms (Spearman correlation).

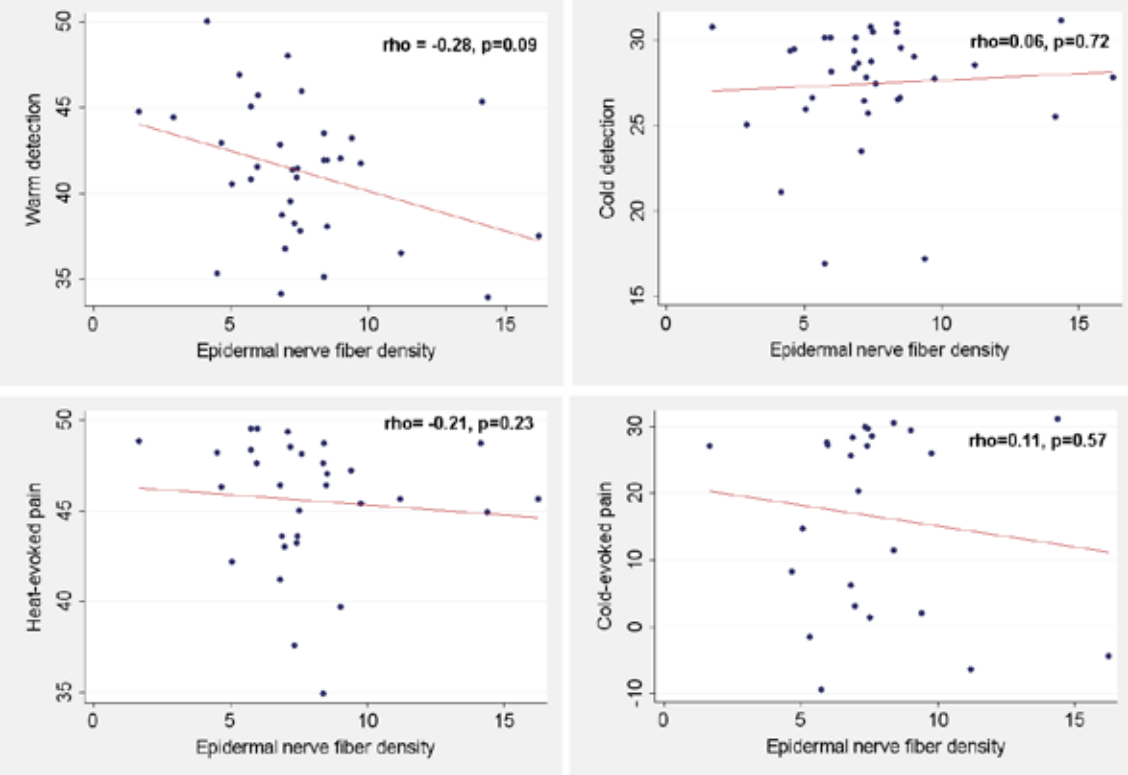

Fig 4. *Correlation between thermal thresholds and ENFD.*

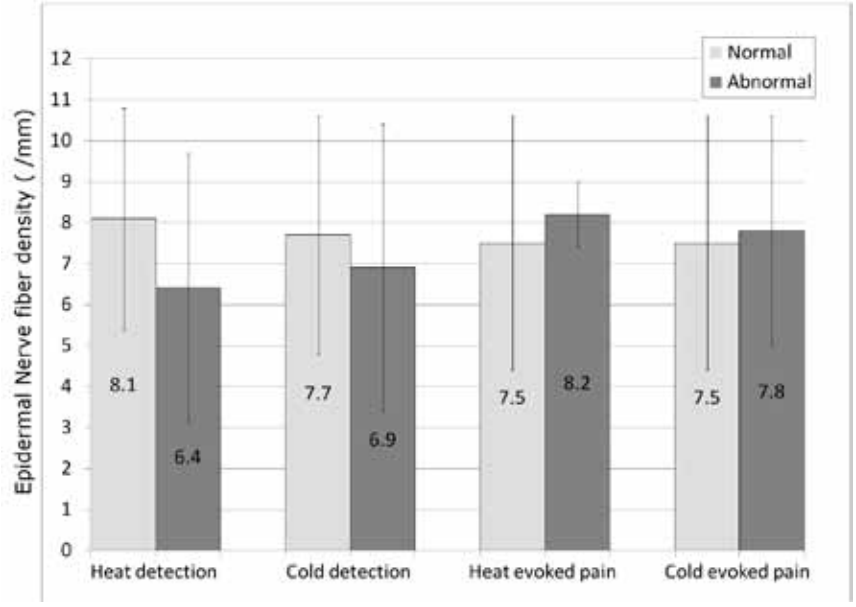

Fig. 5. *Mean ENFD in patients with normal versus abnormal thermal thresholds.*

Discussion

We found alterations in small fiber innervation of the skin with commercial processing in approximately 20% of CRPS-I patients. There were no patient characteristics, including the duration of disease that predicted a decreased ENFD. We found that CRPS-I patients frequently had abnormalities in QST. There was no consistent relationship between the severity of thermal hypoesthesia or the severity of thermal allodynia and the ENFD as measured by standard skin biopsy evaluation procedures.

Skin Biopsy

Skin biopsy is a validated technique for evaluation of SFN (22,26,27). The pan-axonal stain PGP 9.5 is commonly used to visualize nerve fibers in the epidermis. The commercial method of evaluation described in our study stains the same structural protein, PGP 9.5, as the earlier study by Oaklander et al (3). However, the method of counting nerve fibers is different. Commercial processing yields the density in terms of number of nerve fibers per linear mm. In contrast, Oaklander et al (2) describe a stereologic counting technique which accounts for thickness of the skin biopsy, and yields density in terms of number of dendrites per square mm. The method described by them could have a different, possibly greater, sensitivity than our method, and this factor may have caused an underestimation of ENFD abnormality in our study.

This study found minimal CRPS-I-related ENFD loss in CRPS-I patients. In our study, we found a lower ENFD at the calf compared to the thigh. This is consistent with normal skin innervation (19). Reduction in ENFD is a well described feature of SFNs, and has been shown to correlate with disease severity in many SFNs (28). Previous literature has not identified any consistent change in epidermal nerve fibers in CRPS-I patients (Table 4). An important consideration in the interpretation of the

Table 4. *Previous literature on small fiber dysfunction in complex regional pain syndrome (3,4,32,37).*

Study	Number of Patients	Biopsy Sites and Controls	Evaluation Technique	Results	Comments
Drummond 1996	17 9 patients had CRPS-I, 4 patients had CRPS-II. CRPS type could not be determined for 4 other patients.	1) Skin biopsies from affected limb in an area of mechanical hyperalgesia. In all but 4 patients, biopsy was done distal to the site of injury. 2) Control: corresponding area on the contralateral limb.	Light microscopy after immunostaining (neuron specific enolase, VIP{define}, CGRP, Substance P and somatostatin)	No significant differences in staining pattern among groups.	The qualitative nature of this study (rather than stringent quantification of findings) is possibly a cause of the negative results (3).
Van Der Laan 1998	8 CRPS-I patients	CRPS-I limbs which were amputated because of intractable pain. 1) Sural, tibial and common peroneal nerves examined. 2) Skeletal muscle. 3) Control: Nerve biopsies from age-matched patients with degenerative diseases with no evidence of neuropathy.	Light microscopy for all samples, and electron microscopy for all samples except tibial and common peroneal nerves. Nerves were stained using "standard techniques." Muscles were stained with hematoxylin and eosin and enzyme histochemistry.	Slight decrease of myelinated fiber density in 4/8 sural nerves. Electron microscopy showed pathology of the unmyelinated nerves (denervated parallel Schwann cell stacks, miniature axon sprouts, and increase in collagen packets) in these 4 patients. Decrease in type 1 muscle fibers in 8/8 patients.	50% of peripheral nerve biopsies were abnormal (4 of 8 patients) with extremely severe CRPS-I The authors concluded that there was "no consistent pathology of the peripheral nerves". The distal nerves (tibial and common peroneal) were evaluated only with light microscopy, not electron microscopy (3).

Table 4 (cont.). *Previous literature on small fiber dysfunction in complex regional pain syndrome (3,4,32,37).*

Study	Number of Patients	Biopsy Sites and Controls	Evaluation Technique	Results	Comments
Albrecht 2006	2 CRPS-I - I patients	CRPS-I limbs which were amputated for intractable pain. 1) Strips of skin approximately 3cm long and 1mm wide were analyzed. 2) Control: skin biopsies from 5 volunteers.	Light microscopy using immuno-staining (13 different antigens, including neurofilament, myelin basic protein and CGRP).	Reduction of normal A-delta and C - fibers in both patients. Abnormal presence of numerous thin-caliber axons. Numerous other morphological abnormalities in the innervation of hair follicles sweat glands, Meissner corpuscles, Merkel cells and vasculature.	The primary limitation of this extremely detailed study is the small number of patients (n = 2)
Oaklander 2006	18 CRPS-I patients	1) Punch skin biopsies taken from area of maximum pain. 2) Control: Skin biopsies from a nearby pain-free area (ipsilateral control) and a mirror image area on the contralateral limb (contralateral control).	Light microscopy after immunostaining using PGP 9.5	Decreased median density (approximate 20% decrease) of PGP 9.5 positive epidermal dendrites in painful area compared to ipsilateral control site and approximate 30% decrease compared to contralateral control site.	More stringent cut-offs for ENFD were used in this study. The authors compared median ENFDs, and neurites were counted. Conventional readings are in axon densities.

previous literature and our study is that the sensitivity and specificity of ENFD depends on the limits that have been established (22,29). At the commonly utilized below 5th percentile of normative data limit, the sensitivity is between 45%-90% and the specificity is 95% - 97% (22,26,29).

We did not find a consistent reduction in SGNFD in CRPS-I patients. The SGNFD has been found to be decreased in small fiber neuropathies (23,30), in some cases when the ENFD is normal (31). The lack of sweat glands in some of the biopsy samples was likely related to the shallow depth of the biopsies as most sweat glands are located in the deeper layers of the dermis. The only other histological study of sweat gland innervation in CRPS-I was published in 2006 (32). In 2 patients with severe CRPS-I, numerous sweat glands appeared to have no innervation. However the extent of this abnormality was not quantified in the paper and there was no comparison with normal skin. In our study, we found decreased SGNFD in about 20% of patients. SGNFD may be normal if the site of autonomic dysregulation is not at the level of the skin, such as in Multiple Systems Atrophy (34). SGNFD does not reflect functional changes in the nerves supplying sweat glands. Albrecht et al (33) described loss of normal calcitonin gene-related peptide (CGRP)-positive nerve fiber innervation of the superficial sweat glands, and abnormal expression of transient receptor potential type-1 cation channel (commonly known as TRPV1) by nerve fibers that innervate the deep sweat glands in the amputated limbs of 2 severe CRPS-I patients (33). The exact significance of these functional changes is not known.

The controls in this study were not matched by age and sex. Previous large studies have indicated that sex does not affect the ENFD, and there is no significant decrement of ENFD with increasing age, except higher values in patients from 10-19 years of age (33). However, recent studies indicate that ENFD is higher in women, and decreases with age (34). Studies suggested by recent guidelines (22) to establish normative values adjusted by age and sex are still being conducted (34), and the cut-off values derived from this research are still to be prospectively validated.

We do not believe our results are significantly confounded by the lack of age and sex matching. Firstly, there are no patients/controls from 10-19 years of age in this study. Secondly, a substantial portion of our analyses (including Table 1, Table 3, Fig. 3, Fig. 4, and Fig. 5) are not affected by this issue. The interpretation of Table 2 and Fig. 2 is potentially affected by the selection of controls. Previous research used to estimate ENFD sensitivity, and specificity does not utilize age and sex matched controls (35,36) like our present study, hence our study can be assumed to have similar sensitivity and specificity.

Quantitative Sensory Testing

There have been few published manuscripts on QST abnormalities in CRPS-I patients, however at least 2 of these manuscripts described QST findings in a very large number of CRPS-I patients (38-45) (Table 5). The results from previous studies have been variable.

Previous literature has described hypoesthesia to heat and cold in CRPS-I patients. An inverse relationship between ENFD and thermal detection thresholds has been described in small fiber neuropathies such as diabetes (46,47) and HIV (48), thus identifying loss of temperature sensitive nerve fibers/receptors as the cause for thermal hypoesthesia in these conditions. However, in the present study of CRPS-I patients, we did not find any significant correlation between ENFD and thermal thresholds.

Less consistently, previous literature has also identified that patients with CRPS-I frequently report thermal allodynia, pain at warm and cold temperatures that would not usually be interpreted as producing pain. Previous research has described an inverse relationship between the severity of thermal allodynia and ENFD in small fiber neuropathies such as diabetes (49), but this relationship is not consistent (50). We did not find any correlation between the severity of thermal allodynia and ENFD.

There is a growing body of literature that indicates that opioid use may have an effect on sensory pain thresholds. Since chronic pain patients are most often taking pain medication, including opioids, it is difficult to unequivocally ascertain whether the differences in thermal sensory thresholds between patients and controls are due to disease progression or medication use.

One recently published study addresses this issue. Chen et al (51) compared thermal quantitative sensory thresholds in 3 groups of people: controls, chronic pain patients not taking opioids, and chronic pain patients taking opioids. They found no significant differences among the 3 groups with respect to cool or warm detection thresholds. Pain patients (either on opioid treatment or not) had significantly more sensitive thermal pain thresholds and tolerance (for both cold- and heat-

Table 5. *Previous large studies on quantitative sensory testing in complex regional pain syndrome (NT = not tested) (38-45).*

Author, Year	Number of Patients	Hypoesthesia		Allodynia	
		Warm	Cold	Warm	Cold
Thimineur 1998	145	+	-	NT	NT
Birklein 2000	145 (57 had QST)	+	-	-	+
Tahmoush 2000	16	-	-	+	+
Eisenberg 2006	12	NT	-	+	+
Ucelyer 2007	42 (33 had QST)	+	+	-	-
Huge 2008 Acute CRPS-I	27	+	+	+	+
Huge 2008 Chronic CRPS-I	34	+	+	-	+
Huge 2011	118	+	+	+	+
Munts 2011	44	+	+	-	NT

evoked pain) than controls. However, the only significant differences between the 2 groups of chronic pain patients was that those taking opioids had lower heat pain thresholds and increased heat pain temporal summation compared to the patients not taking opioids.

This, we believe, would provide evidence that in our study, at least 3 of the 4 thermal QST parameters measured (cool detection, warm detection, and cold pain threshold) would most likely not have been significantly affected by opioid use. The heat pain thresholds might have been affected by opioid use, but in our study the presence of heat pain threshold abnormalities was minimal (8.6%, Table 3).

Our results can be interpreted in one of 3 ways. First, it is possible that standard skin biopsy procedures are not sufficiently sensitive for detecting structural changes in small fibers. The sensitivity of the standard procedure for evaluating skin biopsies in small fiber neuropathy is good to excellent (27). However, it is possible that changes in the ultramicroscopic structures or in the arborization of nerve fibers cannot be visualized using commonly utilized techniques. Second, it is possible that changes in small fibers in CRPS-I are of a functional rather than a structural nature. Previous research has indicated that there are numerous functional changes in small fibers, including changes in cytokine secretion and receptor mechanisms (8). Third, it is possible that both thermal hypoesthesia and thermal allodynia in CRPS-I are produced due to pathogenic changes at a more proximal site such as the spinal cord or brain (52,53).

The limitations of this study are those inherent in a retrospective analysis. The commercial processing of tissue does not utilize stereologic quantitative analysis of nerve fiber density. Biopsy material is utilized from a proximal and distal source only, and differences in denervation of a partial nerve territory may be missed (partial axonal denervation in a nerve territory). The functional attributes of remaining small fibers (connected to the dorsal root ganglion and dorsal horn) cannot be assessed. All of the signs and symptoms of CRPS-I may be modified by changes in nerve, blood vessels, muscle, and bone and therefore cannot be solely ascribed to small fibers. We utilized standard cut-offs for categorizing sensory thresholds. This categorization does not affect our primary analysis of correlating the severity of ENFD to the QST testing results (Fig. 3) since the thresholds are utilized as continuous variables for this analysis. However, the prevalence of QST abnormalities (Table 3 and Fig. 4) is dependent on the cut-offs used for categorizing the sensory thresholds. Lastly, we did not have local pain scores on these patients and hence could not correlate these with the sensory thresholds or ENFDs.

The results of this study need to be confirmed prospectively in a larger cohort of patients. In addition, the difference in small fiber density as measured by the stereologic methods described by Oaklander et al (2,3) and the commercial/standard method described in this study should be evaluated in the same group of patients. Our study should not preclude further studies evaluating small fiber dysfunction in CRPS-I. Future studies utilizing immunostaining of nerve fibers for components known to play an important role in CRPS-I morphology such as CGRP receptors may be more sensitive than morphological studies. Pathogenesis such as CGRP receptors, macrophages, and keratinocytes may be more sensitive than quantitative counts of dendrites.

Conclusion

There is accumulating evidence of an association between CRPS-I and damage to C and A-delta fibers. We did not find any difference between the ENFD/SGNFD in CRPS-I patients and controls using standard, commercially available skin biopsy procedures. QST abnormalities are common in CRPS-I There was no correlation between the severity of QST abnormalities and the epidermal nerve fiber density as measured by standard commercial skin biopsy evaluation procedures. The negative results indicate that CRPS-I may be associated with changes in the ultramicroscopic small fiber structure that cannot be visualized with commercially available techniques. Alternatively, functional, rather than structural, alterations of small fibers or pathological changes at a more proximal site such as the spinal cord or brain may be responsible for the syndrome.

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