The handle [http://hdl.handle.net/1887/25983](http://hdl.handle.net/1887/25983) holds various files of this Leiden University dissertation.

**Author:** Swartjes, Maarten  
**Title:** Neuropathic pain and its treatment with ARA 290 and ketamine: overlapping pathways  
**Issue Date:** 2014-06-12
Corneal nerve density predicts the severity of symptoms in sarcoidosis patients with painful neuropathy

Michael Brines, Maarten Swartjes, Martijn Tannemaat, Ann Dunne, Monique van Velzen, Paolo Proto, Elske Hoitsma, Ioannis Petropoulos, Xin Chen, Marieke Niesters, Albert Dahan, Rayaz Malik, Anthony Cerami.

Technology 2013; 1(1): 1-7
Corneal nerve density in painful neuropathy

Innovation

Currently, a definitive diagnosis of small fiber neuropathy (SFN) requires a skin biopsy that demonstrates small nerve fiber loss. However, quantifying IENFD in skin biopsies is an invasive, labor-intensive process that has a low sensitivity for diagnosing SFN and does not correlate with the pain that patients report. Alternatively, CCM is a rapid non-invasive clinical ophthalmic technique for in vivo imaging of corneal nerve fibers. Here we show that CCM is a useful diagnostic tool to evaluate small fiber damage and that corneal nerve fiber density is inversely related to symptoms in patients with sarcoid neuropathy. This technology expands the role of CCM as a surrogate marker for both nerve fiber damage and pain in clinical trials of novel therapeutics in sarcoid and perhaps other small fiber neuropathies.

Introduction

Loss of small, unmyelinated nerve fibers, i.e., small fiber neuropathy (SFN), is an increasingly recognized feature of a wide range of neuropathies. It is a major cause of pain and poor quality of life with an inability to work. SFN is characterized by spontaneous pain, dysesthesiae, paresthesiae, and altered thermal sensory thresholds. Additionally loss of post-ganglionic autonomic nerve fibers leads to a wide variety of symptoms including anhidrosis, orthostasis, and a range of other manifestations depending upon the organs affected.

Routine electrodiagnostic studies, such as electromyography and nerve conduction studies, in conjunction with tendon reflexes and strength testing evaluate large nerve fibers. Consequently, these tests remain normal in small fiber neuropathy and pure small fiber damage is not easily evaluated. Based on data from preclinical models, inflammation has been suggested to be a common mechanism for the reduction in small nerve fibers and a recent study has confirmed that pro-inflammatory cytokines are elevated in patients with SFN and pain.

Curative therapy for SFN is lacking. Current therapy is directed towards symptomatic pain relief which is generally not satisfactory. Although reduced nerve fiber density as determined by skin biopsy is the hallmark of SFN, sensitivity appears suboptimal in sarcoidosis, and to date no study has shown that nerve fiber density obtained by skin biopsy directly relates to patient symptoms, e.g., pain. Hence, no biological marker for pain has yet been established and therefore the outcome of clinical trials have been based upon patient-reported outcomes that are highly variable and subjective.
SFN is difficult to diagnose as complaints of pain and autonomic dysfunction are variable and standard electrophysiological testing cannot directly assess the function of the small nerve fibers involved. Additionally, the natural history of SFN is poorly understood and fluctuates over time. The current diagnostic standard for SFN requires the presence of symptoms, a clinical examination consistent with the loss of small nerve fiber function, and a skin biopsy that documents reduced small, unmyelinated and thinly myelinated nerve fibers (Aδ and C). Because normative values have been derived for the distal leg, this site is typically used for diagnosis. Sarcoidosis is an inflammatory disease that is associated with SFN. The prevalence of SFN in patients with chronic sarcoidosis is not precisely known, but may be as high as ~75%. Several questionnaires (the Small Fiber Neuropathy Screening List as well as an autonomic symptom assessment) have been developed to aid in the diagnosis of SFN in patients with sarcoidosis. Although they are useful in screening for patients with SFN, diagnostic confirmation requires a 3 mm skin biopsy and immunohistochemistry to quantify IENFD. Several recent studies have shown that IENFD is reduced in patients with sarcoidosis and neuropathic symptoms. However, SFN of sarcoidosis has been described as a non-length dependent process that occurs in a “patchy” distribution and therefore it is possible that a biopsy obtained from the distal leg might not reflect the presence of reduced small nerve fibers at other locations. Thus a majority of patients with symptoms of SFN have an ankle IENFD that is not below the 0.05 quantile level of normal that has been suggested as required for a definitive diagnosis of SFN. Furthermore, skin biopsy, although well-tolerated with minimal potential adverse effects, is an invasive procedure and sample processing requires a dedicated laboratory for fixation, sectioning, staining, and nerve fiber counting that is time and labor intensive and fraught with significant potential artifacts. Additionally, innervation of the skin is not equally distributed and follow-up biopsies cannot be taken at the exact same location. Hence, skin biopsies are not ideal for following the progression of disease and to assess the potential beneficial effects of therapeutic interventions.

The cornea has the highest density of nerve fibers of any tissue (up to 600 times more than the skin) and therefore any process that targets small nerve fibers may be especially prominent in the eye. Corneal nerve fibers originate from the ophthalmic branch of the trigeminal nerve and distribute radially towards the apex of the cornea parallel to the surface. Corneal innervations consists of predominantly C fibers, i.e., small, unmyelinated fibers that are polymodal nociceptors, that respond to a wide range of mechanical, thermal, and chemical stimuli. For these reasons the corneal nerve fibers may be more reflective of the pain that patients report. Over the last decade, a confocal microscopic technique has been developed to directly, and non-invasively visualize nerve fibers that innervate the cornea, termed corneal...
confocal microscopy (CCM)\textsuperscript{16}. This technique allows direct visualization of a narrow slice of tissue containing nerve fibers running parallel to the surface. The utility of this methodology has been evaluated in a range of neuropathies including, diabetic neuropathy, Fabry’s disease, Charcot-Marie-Tooth disease 1A, and chemotherapy induced neuropathy\textsuperscript{17-20}. Corneal nerve fiber number is directly related to the severity of neuropathy derived from a neurological examination that tests both small and large nerve fiber function\textsuperscript{19}, as well as cooling detection thresholds, axon reflex-mediated neurogenic vasodilatation in response to cutaneous heating by laser Doppler imaging flare technique (LDIFLARE), heart rate variability (HRV)\textsuperscript{21} and IENFD\textsuperscript{22}. It is currently unknown whether corneal confocal microscopy may aid in identifying nerve fiber loss and severity of pain in patients with sarcoid neuropathy.

**Methods**

**Study Criteria and Patient Population**

The results reported here are derived from a study population with chronic sarcoidosis and debilitating symptoms of painful neuropathy (protocol NTR3575 in the International Clinical Trials Registry Platform). After Ethics committee approval and informed consent according to the Declaration of Helsinki, patients were recruited according to the following inclusion criteria:

- Diagnosis of sarcoidosis according to accepted international criteria\textsuperscript{23}.
- Spontaneous pain level (“pain now” of the Brief Pain Inventory) $\geq 5/10$ or Small fiber neuropathy screening list score (SFNSL) > 37/84.
- Pain defined as distal pain plus one of the following: dysesthesia, burning/painful feet worsening at night, or intolerance of sheets/clothes touching the legs/feet.

Exclusion criteria were:

- Clinically relevant abnormal history of physical and/or mental health.
- A semi recumbent systolic blood pressure of $>150$ mmHg and/or diastolic blood pressure of $>90$ mmHg at screening.
- History of alcoholism or substance abuse within three years prior to screening.
- Positive pregnancy test.
- Male patients habitually using more than 21 units of alcohol per week and female patients using more than 14 units of alcohol per week.
- Male patient unable/unwilling to use a medically acceptable method of contraception throughout the entire study period. Female patient not using oral contraceptives, or not postmenopausal.
- History of severe allergies, or an anaphylactic reaction or significant intolerability to prescription or non-prescription drugs or food.
Table 1: Patient characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (+ SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>38</td>
</tr>
<tr>
<td>Males/females</td>
<td>20/18</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.1 + 2.6</td>
</tr>
<tr>
<td>Age</td>
<td>49.5 + 1.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>177.6 + 1.8</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>25.8 + 0.5</td>
</tr>
<tr>
<td>Years since diagnosis of sarcoidosis</td>
<td>8.4 + 1.3</td>
</tr>
<tr>
<td>Use of NSAIDs</td>
<td>14</td>
</tr>
<tr>
<td>Use of neurological/psychological drugs</td>
<td>11</td>
</tr>
<tr>
<td>Use of oral steroids</td>
<td>13</td>
</tr>
<tr>
<td>Use of opioids</td>
<td>7</td>
</tr>
<tr>
<td>Use of systemic anti-inflammatory drug</td>
<td>10</td>
</tr>
<tr>
<td>Prior use anti-TNF therapy</td>
<td>2</td>
</tr>
<tr>
<td>High sensitivity C-reactive protein (mg/L)</td>
<td>2.2 + 0.5</td>
</tr>
<tr>
<td>Angiotensin converting enzyme (normal 23-67 nmol/min/ml)</td>
<td>49.3 + 5.1</td>
</tr>
<tr>
<td>Elevated ACE (number)</td>
<td>11</td>
</tr>
<tr>
<td>SFNSL score baseline</td>
<td>43.4 + 2.1</td>
</tr>
<tr>
<td>BPI total pain score (range 0-40)</td>
<td>20.6 + 0.9</td>
</tr>
<tr>
<td>BPI pain interference (maximum 70)</td>
<td>34.1 + 1.7</td>
</tr>
<tr>
<td>6 Minute Walk Test (meters)</td>
<td>473.4 + 15.5</td>
</tr>
<tr>
<td>Predicted 6 Minute Walk Test (meters)</td>
<td>692.6 + 9.6</td>
</tr>
</tbody>
</table>

• Vaccination or immunization within the last month.
• Participation in an investigational drug trial in the 3 months prior to study.
• Major surgery within three months prior to screening.
• Donation or loss of blood (> 500 mL) within 3 months prior to screening.

Thirty eight patients (18 females, 20 males) of mean age 49.5 years (range 28-65) satisfying inclusion criteria were evaluated (Table 1). The duration of sarcoidosis was 8.4 ± 1.3 (SEM) years. No patient was using capsaicin topical cream that is known to reduce intraepidermal nerve fiber density. None had serious or progressive lung disease. The mean score of the Brief Pain Inventory Short Form (BPI) was 54.7 (out of 110 total) and the mean SFNSL score was 43.4 (out of 84 total).

Clinical Testing

Quantitative Sensory Testing was accomplished according to the protocol of the German Pain Network. A 6 Minute Walk Test (6 MWT) was performed according to published protocols. Predicted 6 MWT distance for normal individuals as a function of age, gender, and height was calculated using the formula of Troosters et al.
Nerve Fiber Quantification

Skin biopsies (3 mm) were obtained from the proximal thigh (20 cm below the anterior superior iliac spine) and the distal leg (10 cm above the lateral malleolus) and processed following established guidelines. Free floating 50 µm thick sections were cut and stained using rabbit anti-protein gene product 9.5 antibody (Dako Netherlands bv) visualized using a goat anti-rabbit Alexa fluor 488 antibody (Invitrogen, Life Technologies, Grand Island, NY). A minimum of 3 sections selected from the ends and the middle of each biopsy series was evaluated using a Leica M5500 fluorescence microscope (Leica Microsystems, Rijswijk, The Netherlands), magnification 1000x. The nerve fibers were counted manually. Images were recorded with Leica Application Suite, magnification 400x and epidermal lengths were measured using ImageJ (NIH, Bethesda, MD, USA). Normative data of nerve fiber density used for the distal leg was that of Lauria et al. and for the thigh from Umapathi et al.

Corneal confocal microscopy was carried out using the Rostock Cornea Module with the Heidelberg Retina Tomograph III using established methodology. A minimum of 6 images containing nerve fibers (i.e., to be within Bowman’s layer) were evaluated using computer software as previously described. Corneal nerve fiber data obtained from Twenty two healthy volunteers (gender (M/F-9/13), age 49.0 ± 2.7, height 167.3 ± 2.3, weight 71.1 ± 3.1, BMI 25.3 ± 0.9) had a mean nerve fiber density = 31.6 ± 6.4 (SD) per mm²; mean nerve fiber length = 21.7 ± 3.6 mm/mm²; and mean nerve branch density = 54.6 ± 23.4/mm².

Statistics

Statistical analysis was performed using JMP (SAS, Inc, Cary, NC). Stepwise linear regression modeling, analysis of covariance, unpaired t-test, or Mann-Whitney U test were carried out where appropriate.

Results

Almost all patients had a significant reduction in the distance they could walk in 6 minutes as estimated from the normative predictive data generated for older individuals by Troosters et al. which was 693 meters. The mean reduction in expected 6 MWT distance in the sarcoidosis patients was 219 meters (95% confidence interval: 186-253 meters).

Quantitative sensory testing showed that the majority of patients exhibited significant small nerve fiber dysfunction as evidenced by alteration in thermal thresholds (Table 2). The most common abnormality was a decrease by more than 2 SD below the mean of normal volunteers in the cold and warm detection thresholds in ~ 80%
Chapter 7

Table 2: Results of quantitative sensory testing. Patients showed functional impairment of both small nerve fibers (Aδ and C) as well as larger sensory nerve fibers (Aβ). Data are expressed as number of patients deviating beyond the 95% confidence interval of a sex- and age-matched normal populations as reported by Rolke et al24.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nerve fibers involved</th>
<th>Change</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold detection threshold</td>
<td>Aδ &amp; C</td>
<td>Decrease</td>
<td>30 (79)</td>
</tr>
<tr>
<td>Warm detection threshold</td>
<td>Aδ &amp; C</td>
<td>decrease increase</td>
<td>30 (79) 1 (3)</td>
</tr>
<tr>
<td>Thermal sensory limen</td>
<td>Aδ &amp; C</td>
<td>decrease increase</td>
<td>8 (21) 2 (5)</td>
</tr>
<tr>
<td>Paradoxical heat sensation</td>
<td>Aδ</td>
<td>Decrease</td>
<td>15 (40)</td>
</tr>
<tr>
<td>Cold pain threshold</td>
<td>Aδ &amp; C</td>
<td>Increase</td>
<td>3 (8)</td>
</tr>
<tr>
<td>Heat pain threshold</td>
<td>C</td>
<td>decrease increase</td>
<td>4 (11) 5 (13)</td>
</tr>
<tr>
<td>Mechanical detection threshold</td>
<td>Aβ</td>
<td>Decrease</td>
<td>21 (55)</td>
</tr>
<tr>
<td>Mechanical pain threshold</td>
<td>Aβ</td>
<td>decrease increase</td>
<td>15 (40) 6 (16)</td>
</tr>
<tr>
<td>Mechanical pain sensitivity</td>
<td>Aβ + C</td>
<td>decrease increase</td>
<td>3 (8) 5 (13)</td>
</tr>
<tr>
<td>Dynamic mechanical allodynia</td>
<td>Aβ</td>
<td>Increase</td>
<td>14 (37)</td>
</tr>
<tr>
<td>Windup ratio</td>
<td>Aδ &amp; C</td>
<td>Increase</td>
<td>6 (16)</td>
</tr>
<tr>
<td>Vibration detection threshold</td>
<td>Aβ</td>
<td>Decrease</td>
<td>35 (92)</td>
</tr>
<tr>
<td>Pressure pain threshold</td>
<td>Aδ &amp; C</td>
<td>decrease increase</td>
<td>4 (11) 17 (45)</td>
</tr>
</tbody>
</table>

of the patients. Additionally, > 90% of the patients showed a decrease in the vibration detection threshold.

Corneal nerve fiber images of patients with sarcoidosis typically showed reduced corneal nerves compared to healthy controls (Figure 1A and B). Quantification showed that the mean corneal nerve fiber density (CNFD; patients: 21.6 fibers/mm² ± 5.9 SD versus controls: 31.6 fibers/mm² ± 6.4; P<0.0001) and Length (CNFL; patients: 13.2 mm/mm² ± 4.0 versus controls: 21.7 mm/mm² ± 3.6 P<0.0001) of patients with chronic sarcoidosis were significantly reduced compared to normal controls (Figure 1C and D). In contrast, mean corneal nerve branch density was not significantly different from controls (patients: 51.2/mm² ± 30.5 SD versus controls 54.6/mm² ± 23.4 SD).

The median intra-epidermal nerve fiber density of the distal leg was significantly reduced compared to age and gender matched normal controls (Figure 2A). The average difference between the normal population age and sex dependent median values and the patient population was 4.7 fibers/mm² (P<0.0001; Mann-Whitney Test). Stepwise linear regression modeling determined that age, height, and gender
were covariates of IENFD. In contrast, these variables were not covariates for IENFD of the proximal thigh, for which the density was also reduced approximately 50% compared to normal individuals (mean of patients: 11.0 fibers/mm (confidence interval 9.9-12.0) versus age-matched normal controls: 20.6 fibers/mm (confidence interval 19.8-21.5)).
The mean ratio of IENFD of the proximal thigh to the distal leg was 3.9 ± 1.5 SEM, with one patient equal to 0.9 and the others > 1.0. The patients in this study, therefore, had a peripheral neuropathy of a length-dependent nature.

Figure 2: A: The distal leg intraepidermal nerve fiber density of patients with sarcoidosis and symptoms of SFN is reduced compared to normal population. The horizontal lines correspond to the median value of each gender. The dashed line represents the age-dependent median normative value. B: There is a significant linear relationship between CNFL and IENFD of the distal leg (P=0.009). A similar finding was observed for CNFD (data not shown). C: Gender, age, height, and CNFL (or CNFD) are covariates for IENFD of the distal leg. A linear model constructed using these variables provides the relationship between CNFL and IENFD. Here, the least mean squares predicted values of the distal leg IENFD are plotted versus CNFL, showing that the slope of the relationship is the same for female (95% CI: 0.12 to 0.25) and male (95% CI: 0.16 to 0.29) patients.

interval 17.8-23.4). The mean ratio of IENFD of the proximal thigh to the distal leg was 3.9 ± 1.5 SEM, with one patient equal to 0.9 and the others > 1.0. The patients in this study, therefore, had a peripheral neuropathy of a length-dependent nature.
IENFD of the proximal leg was not significantly correlated to that of the distal leg (Pearson’s correlation coefficient = 0.20; P = 0.22).

However, the IENFD of the distal leg, which is typically employed for diagnosis of SFN, was significantly correlated to CNFL (Figure 2B) and to CNFD (data not shown). Linear regression modeling showed that age and gender were covariates and that a good predictive model incorporating CNFL (Figure 2C) or CNFD (not shown) could be constructed.

**Figure 3:** Corneal nerve fiber length and number are correlated with patient related symptoms. A: CNFL is inversely correlated with the Brief Pain Inventory pain interference score (P = 0.0005) B: CNFD are inversely correlated with the Brief Pain Inventory pain interference score (P = 0.012, respectively). C: In contrast, IENFD has no relationship with the BPI pain interference score.
Additionally, both CNFL (Figure 3A) and CNFD (Figure 3B) were negatively correlated with the pain interference component of the Brief Pain Inventory. Specifically, the relationship between the CNFL and BPI pain interference score for individual patients without controlling for additional variables was described by the linear function BPI pain interference score = \(-1.38 \times \text{CNFL (mm/mm}^2) + 52.3\) (slope 95% CI: \(-2.1\) to \(-0.7\); Pearson’s correlation coefficient of \(-0.54\); \(P = 0.0005\)) and for CNFD: BPI interference score = \(-0.71 \times \text{CFND} + 49.4\) (slope 95% CI: \(-1.3\) to \(-0.2\); Pearson’s correlation coefficient of \(0.4\); \(P = 0.012\)). In contrast, there was no relationship between the BPI pain interference score and IENFD at the ankle (Figure 3C) or proximal thigh (data not shown). A weaker correlation (Pearson’s coefficient \(-0.33\); \(P = 0.04\)) was also noted between CNFL and the “average pain” score of the BPI, whereas no relationship was evident with IENFD (data not shown). No correlation was found between IENFD, CNFL, or CNFD with the “worst”, “least”, or “now” pain components of the BPI.

Stepwise linear regression analysis including other potential covariates of the BPI pain interference score showed that height, weight, and 6 MWT difference from expected were also inversely related to the pain interference score. Construction of a linear model with CNFL as the dependent variable in addition to weight, height, and 6 MWT deficit accurately predicted BPI pain interference (Figure 4; prediction formula with a slope of 1; Pearson’s correlation coefficient = \(-0.78\); \(P < 0.0001\)).

Figure 4: Corneal nerve fiber length can be used to predict the Brief Pain Inventory pain interference score. A linear model constructed with CNFL as the independent variable, with height, weight, and 6 MWT as covariates predicts with high accuracy the BPI interference score (Pearson’s correlation coefficient = \(-0.78\); \(P < 0.0001\)).
Corneal nerve density in painful neuropathy

Discussion

The main findings of this study conducted in a population of sarcoidosis patients having pain consistent with SFN are two-fold: Corneal nerve fiber quantification 1) provides the same information as intra-epidermal nerve fiber densities obtained from the distal leg and can therefore be used for diagnosis and 2) in contrast to IENFD, CCM data is highly predictive of the pain that patients report. Secondary results show that the neuropathy documented by the skin biopsies in this population is of a length-dependent phenotype. The majority of the patients also appear to have involvement of larger nerve fibers based on an elevated vibration detection threshold, and in addition most patients show a significant reduction in functional exercise capacity, as evaluated by the 6 MWT.

Skin biopsies are an invasive procedure with a significant technical threshold of preparation and analysis to overcome. If a follow up biopsy is required, a different region of the skin is examined. Additionally, as previously reported in normal individuals and in patients with sarcoidosis and neuropathy, age and gender are strong covariates of IENFD obtained from the distal leg\textsuperscript{7,10,27} and thus potentially complicate interpretation. These problems could potentially be avoided by utilizing CCM-derived corneal nerve fiber data which can be obtained repeatedly from the same central location of the eye.

Notably, the pattern of nerve fiber involvement in our patients was not consistent with a non-length dependent process as reported by some clinicians\textsuperscript{2,30}. Here, both biopsy sites on the leg provided equivalent evidence of reduced nerve fiber densities, although the values were not significantly correlated. It should be noted that in diabetes, characterized by an accepted length-dependent neuropathic process, the much shorter corneal nerves also reflect the same pathology as the longer fibers innervating distal extremities\textsuperscript{19}. These observations give reassurance that sampling corneal nerve fibers alone is sufficient to yield a diagnosis of reduced numbers of small nerve fiber in the setting of SFN.

There are currently no data available to explain why the corneal nerve fibers are so prominently affected in SFN. One important factor may be the very dense innervation of the cornea predominantly by C fibers. The patients in this study were selected in part by having pain, a primary function of the C fibers, and it is possible that processes that affect these fibers may cause changes more evident against a background of high fiber density, such as in the cornea. Another speculative hypothesis is that since there is no resident blood supply (corneal metabolism relies on diffusion of oxygen from the surrounding tear layer) the small nerve fibers are particularly prone to hypoxic and/or inflammatory injury, similar to what has been proposed to explain the preferential involvement of longer fibers in many neuropathic processes such as diabetes\textsuperscript{31}.
It is notable that similar to other studies evaluating IENFD, the current study found no relationship between IENFD from thigh and ankle biopsies with the pain that patients reported. In contrast, there was a strong inverse relationship between corneal nerve fiber density and the extent to which the patients reported that pain was interfering with the activities of daily living. Current research has identified a prominent role for inflammation as an inducer of chronic pain states in the central nervous system\textsuperscript{32}. Perhaps the central processes of the ophthalmic nerve are more direct participants in a central pain promoting processes, than fibers in the distal extremities that synapse centrally in the spinal cord.

The inverse relationship noted between corneal nerve fiber quantification and the patient reported outcome of pain interference in this study is potentially useful in the clinical assessment of patients reporting pain-related limitations in activities of daily living. The linear model that relates the pain interference score with CNFL and the additional covariates of height, weight, and performance on the 6 MWT allows an objective means by which to corroborate self reported data. Deviations from predicted values would alert a clinician to search for a confounding factor, e.g., pain reports from a malingering.

Finally, the patients in this study had a clearly reduced functional capacity as indicated by the 6 MWT, in spite of the fact that none of the patients had documented significantly reduced cardiopulmonary status. This finding has recently been observed in patients with chronic sarcoidosis\textsuperscript{33}. Although the investigators concluded that poor muscle strength, fatigue, and exercise intolerance were the primary defect, it is also possible that a number also had SFN, as previous studies have shown a high incidence of SFN in the chronic sarcoidosis population\textsuperscript{11}. In the present study, the patients were selected for having pain as well, and it is also possible that discomfort contributed significantly to the observed decrease in function.

One limitation of this study is that only patients with pain were evaluated. It is well known that SFN can be present without complaints of pain\textsuperscript{3} and it remains to be determined whether the corneal nerve fibers will reflect the neuropathic process in these patients. Additionally, the variable autonomic symptoms that accompany SFN often include xerophthalmia, which has been recently shown to be associated with a decrease in corneal nerve density\textsuperscript{34}. In spite of these limitations, we anticipate that the results of this study showing that CCM-derived nerve fiber data reflect with good fidelity that obtained from skin biopsies could apply to other diseases associated with SFN.
Conclusions

Corneal and cutaneous nerve fibers are reduced in the majority of sarcoidosis patients selected for pain and symptoms of SFN. The painful symptoms of SFN are inversely related to corneal nerve fiber density in this population of patients. CCM appears a useful, non-invasive method to quantify nerve density for the diagnosis of SFN. Additionally, CCM may prove to be a non-invasive, repeatable method to follow the natural history of SFN and to test efficacy of therapeutic interventions.
References


32. Leung L, Cahill C: TNF-alpha and neuropathic pain - a review. Journal of neuroinflammation 2010; 7: 27