Epidermal Nerve Fiber Quantification in Patients With Erythromelalgia

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IMPORTANCE Erythromelalgia is a clinical diagnosis based on intermittent warmth, erythema, and pain in the distal extremities. One problem facing physicians is how to objectively test for this disease. Given that other painful conditions of the distal extremities (ie, neuropathy related to human immunodeficiency virus, diabetes, or Fabry disease) can be evaluated with a skin biopsy to visualize pathologically decreased densities of the small nerve fibers that innervate the epidermis, one hypothesis is that erythromelalgia could similarly be associated with a loss of epidermal nerve fiber density (ENFD).

OBJECTIVES To examine whether erythromelalgia is associated with a structural loss of small fibers using the ENFD technique and to compare this with functional studies of small nerve fibers.

DESIGN, SETTING, AND PARTICIPANTS In a retrospective study of 52 consecutive patients with erythromelalgia who were seen between September 1, 2010, and September 22, 2015, patients were interviewed and examined and their conditions clinically diagnosed by a board-certified dermatologist at a large tertiary referral center, where ENFD testing became a routine part of evaluating erythromelalgia in 2010. Thus, all 52 consecutive patients were included solely based on their clinical diagnosis of erythromelalgia. For quantification of ENFD, observers were masked to all patient information except for name and clinic number.

MAIN OUTCOMES AND MEASURES The hypothesis that patients with erythromelalgia would have decreased ENFD was formulated before data collection. Epidermal nerve fiber density, the primary outcome, is a measurement of the density of small nerve fibers within the epidermis. Secondary measures included functional small fiber evaluation, such as autonomic (heart rate, blood pressure, and sweat testing) and subjective testing of pain.

RESULTS In this cohort study, 52 consecutively seen patients were identified (female, 42 [80%]; median age, 44 years; age range, 13-82 years). Whereas only 5 of 52 patients (10%) had ENFD at or below the fifth percentile of healthy control individuals, most patients had functional abnormalities of these small fibers; 29 patients (60%) had abnormal sweat test results, 21 (42%) had abnormal pain thresholds, and 20 (38%) had abnormal blood pressure or heart rate control.

CONCLUSIONS AND RELEVANCE Unlike other diseases of the small nerve fibers that cause acral pain syndromes, erythromelalgia is not characterized by loss of ENFD. However, most patients have impaired function of these small fibers. Physicians would benefit from performing functional rather than structural small fiber studies when evaluating erythromelalgia.
Epidermal Nerve Fiber Quantification in Erythromelalgia

Erythromelalgia is a clinical diagnosis based on the presence of intermittent heat, pain, and redness of the extremities, with the feet being more commonly involved than the hands. Symptoms are classically provoked by heat, exercise (especially use of the involved extremities), or emotional stress and are relieved by cooling. Symptoms are bilateral and occasionally even involve the lips, nose, and ears. Patients with this condition may become severely functionally impaired, and quality-of-life scores are lower and mortality is higher in these patients compared with age- and sex-matched control individuals.1

Because erythromelalgia is a clinical diagnosis and includes a heterogeneous group of patients, there are similarly a diverse set of diseases thought to cause erythromelalgia. One of the most well-studied underlying pathologic findings is the presence of arteriole thrombi in the distal extremities of patients with hematologic malignant neoplasms associated with thrombocytosis,2 and aspirin has been used with some success in these patients, highlighting the role of platelet aggregation and vessel occlusion in one form of this disease.3 However, this pathophysiologic finding accounts for only approximately 9% of cases. An even smaller number of erythromelalgia cases have been associated with underlying infections, systemic lupus erythematosus, or drugs.1 Most cases of erythromelalgia, however, arise without any known cause.

The mechanism for the observed red, hot feet has been studied.4-6 One theory is that there is primary dysregulation of distal vasculature. Several studies4,5 have found an increase in blood flow and temperature in symptomatic areas. Another study6 suggested that the opening of arteriovenous shunts in the extremities may be responsible for symptoms; these shunts effectively steal oxygenated blood from the capillaries that normally deliver tissue oxygen, causing tissue hypoxia and pain.

An alternative mechanism is dysfunction of small nerve fibers, which are the small unmyelinated and thinly myelinated nerves that sense pain and are part of the autonomic sympathetic nervous system, which controls sweating, heart rate, and blood pressure. Most small fiber neuropathies have a length-dependent pattern and so selectively involve the terminal ends of the longest nerve fibers (those serving the feet). Consequently, small fiber neuropathies will usually present as painful feet, often with burning, sharp stabbing, and aching pain. Because erythromelalgia similarly involves pain in a length-dependent distribution, several studies4,7 have examined whether this disease is also caused by small fiber neuropathy. Testing of sweating, blood pressure, and heart rate control in patients with erythromelalgia has revealed abnormalities in more than 80% of these patients, which reflects dysfunction of small nerve fibers.4,7 Patients with this condition also have abnormally low heat pain thresholds, also suggesting that small fiber pathologic findings are a major driver in this disease's pathogenesis.8

Although autonomic and pain testing are good functional tests of small fiber neuropathies, one diagnostic tool to visually confirm a small fiber neuropathy is the skin biopsy stained for neural markers. With this skin biopsy technique, one essentially quantifies the small fibers within the epidermis that may be involved in the experience of pain. This technique of quantifying epidermal nerve fibers (ENFs) has emerged as a useful way to diagnose and study small fiber neuropathies9; US and European guidelines agree that ENF density (ENFD) evaluation is a reliable and efficient technique to establish the diagnosis of small fiber neuropathy.10,11 For example, the dysesthetic and painful small fiber neuropathy related to diabetes, human immunodeficiency virus (HIV), or Fabry disease reveals decreased ENFD.12-15 Thus, one largely unresolved issue is if the pain in erythromelalgia is indeed largely caused by a small fiber neuropathy; then there should be a similar decrease in the ENFD in patients with erythromelalgia. Given the consistent findings of abnormal functional studies of small fibers, one would expect that skin biopsy results would reveal decreased ENFD.

Methods

Patients
We collected data (not deidentified) on 52 consecutive patients diagnosed with erythromelalgia who underwent ENFD testing between September 1, 2010, and September 22, 2015, at Mayo Clinic, Rochester, Minnesota. The date of September 1, 2010, was chosen because at that time ENFD testing became a routine part of the diagnostic workup for patients with erythromelalgia. This study was approved by Mayo Clinic’s Institutional Review Board, and written informed consent was obtained from each participant in this study.

Inclusion and Exclusion Criteria
Patients were included if they met the diagnosis of erythromelalgia. This study was approved by Mayo Clinic’s Institutional Review Board, and written informed consent was obtained from each participant in this study.

Skin Biopsy, Histologic Processing, and ENF Quantification
Three-millimeter skin punch biopsy specimens were systematically obtained at standard sites from the distal leg, at 10 cm proximal to the lateral malleolus on the dorsum of the foot, on the buttocks, and on the middle finger. The specimens were immediately placed in OCT (optimal cutting temperature) compound and frozen at −80°C until sectioning. The sections were stained for neural markers. With this skin biopsy technique, one essentially quantifies the small fibers within the epidermis that may be involved in the experience of pain. This technique of quantifying epidermal nerve fibers (ENFs) has emerged as a useful way to diagnose and study small fiber neuropathies9; US and European guidelines agree that ENF density (ENFD) evaluation is a reliable and efficient technique to establish the diagnosis of small fiber neuropathy.10,11 For example, the dysesthetic and painful small fiber neuropathy related to diabetes, human immunodeficiency virus (HIV), or Fabry disease reveals decreased ENFD.12-15 Thus, one largely unresolved issue is if the pain in erythromelalgia is indeed largely caused by a small fiber neuropathy; then there should be a similar decrease in the ENFD in patients with erythromelalgia. Given the consistent findings of abnormal functional studies of small fibers, one would expect that skin biopsy results would reveal decreased ENFD.

Key Points

Question Is the evaluation of abnormally decreased epidermal nerve fiber density a useful tool in the diagnosis of erythromelalgia?

Findings In this cohort study of 52 consecutive patients with a clinical diagnosis of erythromelalgia, most patients had abnormalities on functional nerve testing, but less than 10% of patients had decreased epidermal nerve fiber density.

Meaning Skin biopsy for evaluation of epidermal nerve fiber density is not useful in the diagnosis of erythromelalgia; instead, physicians may wish to focus on functional nerve testing, which more reliably identifies this disease.
above the lateral malleolus with the patient under local anesthesia. The biopsy specimens were fixed and reacted with protein gene product 9.5 by standard approaches that have been extensively described previously.16,17 Fifty-micrometer serial sections were cut at right angles to the surface of the skin.

Evaluation of numbers of ENFs crossing the dermal-epidermal interface was facilitated by the Imaging System for Nerve Morphometry program. With use of a cursor and a programmed evaluation developed by Mayo Clinic staff, the lengths of the dermal-epidermal interface minus openings of glands and hair were measured. A standard reference microscope reticle was used to correct for microscopic and imaging magnification. High-dry microscopy was used for counting ENFs. Counts of ENFs per millimeters were based on ENFs crossing the dermal-epidermal boundary. The ENFs were counted by 2 peripheral nerve quantification technicians (including J.K.E.) at our institution who were masked to provisional diagnosis, symptoms, neuropathic findings, neurophysiologic test results, and the reason for the biopsy. The only information available to these technicians was patient name and clinic number. The ENFD was reported as the number of ENFs per 1-mm length of a 0.05-mm-thick section. Ten serial skip sections were available per patient and averaged to obtain a final ENFD result. Then, ENFD in patients with erythromelalgia was compared with that of age- and sex-matched controls at our institution.

Portions of the skin biopsy were fixed in buffered paraformaldehyde-lysine-periodate, and sections were stained with hematoxylin-eosin and Congo red stain. Clinical neuromuscular histologists (including J.K.E.) examined the epidermis and dermis to evaluate for the presence of inflammatory cells or amyloid deposition.

**Vascular Studies**
Local vasculature procedures before and during symptoms included laser Doppler flowmetry, measurement of skin temperature, and transcutaneous oximetry. Details of the noninvasive vascular procedures used are outlined in previous publications.4,5

**Neurophysiologic Testing**
In addition to nerve conduction studies and needle electromyography to assess large-fiber function, the thermoregulatory sweat test (described extensively elsewhere) and a quantitative sweat measuring system (Q-Sweat, WR Medical Electronics Co), a test to evaluate the postganglionic sympathetic sudomotor axon reflex (measured on the forearm, proximal lateral leg, distal medial leg, and dorsum of the foot), were performed to evaluate small-fiber function. In concordance with the Q-Sweat test, cardiovagal and adrenergic function were examined to assess for more global loss of autonomic function relating to heart rate and blood pressure, respectively.20

**Quantitative Sensory Testing**
Quantitative sensory testing was performed to assess a patient’s response to touch pressure and pain from heat. These tests were performed in accordance with previously well-described methods and published reference values corrected for applicable variables.21,22 The site of testing was the dorsal aspect of the foot. A subset of our patients also had testing performed at the lateral leg.

Assessment of hyperalgesia or hypoalgesia was performed by assessing a painful response to mechanical or heat stimuli. To evaluate hyperalgesia or hypoalgesia, we measured 3 heat and mechanical pain thresholds: (1) the minimum intensity of stimulus that produced a pain response in the patient greater than 50% of the time, (2) the stimulus that produced a pain rated as a 5 of 10, and (3) the difference in intensity between these 2 pain stimuli thresholds. If a patient’s thresholds occurred at or below the fifth percentile of healthy controls, the patient was classified as having hyperalgesia. On the other hand, if a patient reported pain at only the 95th percentile or above that of healthy controls, the patient was classified as having hypoalgesia.23

**Results**

**Patient Demographics**
Fifty-two consecutively seen patients were identified (female, 42 [80%]; median age, 44 years; age range, 13-82 years). Regarding location of symptoms, 50 patients (96%) had symptoms in the bilateral lower extremities, with another 21 (50%) having involvement of the face, nose, or ears.

**Quantitative Analysis**
Of the 52 patients with clinically diagnosed erythromelalgia, 5 (10%) had ENFD at or below the fifth percentile for age- and sex-matched controls (biopsies performed at the distal leg). In other words, 10% of our patients compared with 5% of the healthy population database had ENFD below the fifth percentile. Table 1 and Table 2 list the neurophysiologic characteristics of all patients with a clinical diagnosis of erythromelalgia and patients with ENFD at or below the fifth percentile, respectively.

The 5 patients with decreased ENFD had other conditions known to cause a decrease in ENFD. Namely, 2 patients had diabetes, 2 patients had numbness of the involved extremities that predated the symptoms of erythromelalgia by years (with 1 of these patients having a family history of idiopathic peripheral neuropathy), and 1 patient had ulcerations of the lower extremities attributable to near-continuous immersion of their feet in ice water.

When the skin biopsy specimens were examined for inflammatory changes or amyloid deposition, only 4 of 52 patients (8%) had abnormalities; these were all nonspecific changes. These 4 patients had collections of mononuclear cells in abnormal locations: 2 patients had collections of these cells around hair follicles, 1 had collections of inflammatory cells associated with capillary stasis, and 1 patient had inflammatory cells scattered nonspecifically within the dermis. No abnormalities were seen on Congo red stain. Only 1 of these patients had ENFDs less than the fifth percentile for age- and sex-matched controls.
Discussion

The aim of our study was to evaluate whether ENFD was abnormal in erythromelalgia; ENFD was rarely abnormal. Our major finding—that 47 of 52 patients (90%) with erythromelalgia have preserved ENFD at the distal leg—is an important but unexpected result for 2 major reasons. First, when other disorders with acral predominant pain, such as neuropathy related to diabetes, HIV, or Fabry disease,13-15 are considered, these disorders are characterized by a marked decrease in ENFD. Second, most of our patients had a functional small fiber neuropathy abnormality. A total of 29 patients (60%) had ENFD analysis performed because this is now part of the standard workup at our institution. However, in the previous study,25 patient information was collected sporadically during a 27-year period, increasing the effect of selection bias. Another important difference between the 2 studies is how patient information was collected. In our study, each patient with a clinical diagnosis of erythromelalgia had ENFD analysis performed (because this is now part of the standard workup at our institution). However, in the previous study,25 patient information was collected sporadically during a 27-year period, increasing the effect of selection bias.

The patients in our study who had evidence of a small fiber neuropathy based on loss of ENFD probably had other causes of their neuropathy, such as diabetes, preexisitng peripheral neuropathy with numbness predating the symptoms of erythromelalgia by years (likely inherited neuropathy), or long-term, excessive cold water immersion of the leg, leading to ulceration. These patients also had clear abnormalities on nerve conduction studies, and 3 of 4 who underwent quantitative sensory testing had decreased sensation to mechanical touch, both findings suggesting a more widespread neuropathy that involved large, myelinated fibers. Of note, there was little overlap between patients with decreased ENFD and inflammatory cells present in the skin; only 1 patient (the patient with immersion foot syndrome) had decreased ENFD with accompanying inflammatory cells. Thus, patients with decreased ENFD probably had loss of these ENFs because of other conditions separate from erythromelalgia.

One previous study,25 from our institution, examined patients with erythromelalgia and found that 13 of 16 patients (81%) had significantly decreased ENFD. This difference may be attributable to different locations of the skin biopsy; the prior study25 included skin biopsy specimens from the fingers and toes, whereas the present study examined skin biopsy specimens from the distal leg. Because the more distal sites are more painful compared with the more proximal sites, this result is not completely unexpected. However, skin biopsies performed on the fingers and toes are technically more difficult, and standard reference values for ENFD at those locations have not been established. Another important difference between the 2 studies is how patient information was collected. In our study, each patient with a clinical diagnosis of erythromelalgia had ENFD analysis performed (because this is now part of the standard workup at our institution). However, in the previous study,25 patient information was collected sporadically during a 27-year period, increasing the effect of selection bias. If one is to accept the results of the 2 studies as valid, a potential conclusion is that erythromelalgia may denervate only the most acral extremities while relatively preserving more proximal extremities. Even our functional tests revealed a tendency for distal predominant impairment that improved more proximally. For example, in 15 patients who had heat pain abnormalities at their dorsal foot who also happened to have heat

Table 1. Test Results for Patients With a Clinical Diagnosis of Erythromelalgia

<table>
<thead>
<tr>
<th>Test</th>
<th>Total No. of Patients Tested</th>
<th>No. (%) of Patients With Abnormal Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENFD at or below the fifth percentile compared with healthy age- and sex-matched control individuals (measured at distal leg)</td>
<td>52</td>
<td>5 (10)</td>
</tr>
<tr>
<td>Presence of epidermal or dermal inflammatory cells on H&amp;E staining</td>
<td>52</td>
<td>4 (8)</td>
</tr>
<tr>
<td>Nerve conduction studies and electromyography</td>
<td>50</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Thermoregulatory sweat test</td>
<td>48</td>
<td>29 (60)</td>
</tr>
<tr>
<td>Q-Sweat</td>
<td>49</td>
<td>23 (46)</td>
</tr>
<tr>
<td>Cardiovascular function</td>
<td>52</td>
<td>14 (27)</td>
</tr>
<tr>
<td>Adrenergic function</td>
<td>52</td>
<td>11 (21)</td>
</tr>
<tr>
<td>Quantitative sensory testing (measured at dorsal foot)</td>
<td>49</td>
<td>25 (51)</td>
</tr>
</tbody>
</table>

Abbreviations: ENFD, epidermal nerve fiber density; H&E, hematoxylin-eosin; TcPO2, transcutaneous measurement of blood PO2.

Table 2. Test Results for Patients With ENFD at the Distal Leg at or Below the Fifth Percentile

<table>
<thead>
<tr>
<th>Test</th>
<th>Total No. of Patients With ENFD at or Below the Fifth Percentile*</th>
<th>No. (%) of Patients With Abnormal Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of epidermal or dermal inflammatory cells on H&amp;E staining</td>
<td>5</td>
<td>1 (20)</td>
</tr>
<tr>
<td>Nerve conduction studies and electromyography</td>
<td>5</td>
<td>5 (100)</td>
</tr>
<tr>
<td>Thermoregulatory sweat test</td>
<td>4</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Q-Sweat</td>
<td>4</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Functions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>4</td>
<td>2 (50)</td>
</tr>
<tr>
<td>Adrenergic</td>
<td>4</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Quantitative sensory testing (measured at dorsal foot)</td>
<td>4</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Heat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperalgesia (pain threshold &lt;5th percentile)</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Hyperalgesia (pain threshold &lt;1st percentile)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hypoalgesia (pain threshold &gt;95th percentile)</td>
<td>4</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Mechanical decreased sensation</td>
<td>4</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Vascular Doppler ultrasonography and TcPO2</td>
<td>5</td>
<td>4 (60)</td>
</tr>
</tbody>
</table>

Abbreviations: ENFD, epidermal nerve fiber density; H&E, hematoxylin-eosin; TcPO2, transcutaneous measurement of blood PO2.

* Compared with ENFD in healthy age- and sex-matched control individuals.
pain testing performed at their distal leg, 4 (27%) had resolution of their abnormalities at the more proximal site. However, functional impairment still outnumbered structural impairment, even when both tests examined the same site. Thus, despite there being a distal to proximal improvement in some of our patients, there was a mismatch between functional nerve impairment and structural nerve abnormalities. Although the reason for this mismatch is unclear, other small fiber neuropathies also reveal similar discrepancies. For example, in hypohidrosis caused by a variety of small fiber neuropathies26 or in patients with small fiber neuropathies related to HIV,27 there is only a loose correlation between functional and structural nerve loss.

An important recent advance in understanding the pathophysiological mechanisms of erythromelalgia is the observation that a subset of patients with this disease have small fiber neuropathies attributable to a mutation in the sodium channel Nav1.7.28 In addition to the ganglia of the sympathetic and sensory nervous system,29,30 this sodium channel isoform is highly expressed along the axons of pain-sensing neurons and their intraepidermal nerve endings.31 A gain-of-function mutation in Na1,7, which makes the channel hyperexcitable, has been found to be causative in certain cases of erythromelalgia32,33 and in other idiopathic cases of small fiber neuropathies.34,35 In patients with small fiber neuropathies with these gain-of-function mutations, several studies have found axonal damage and decreased ENFD,34,36 potentially the result of sodium dysregulation and associated calcium toxicity.37 In our study, we did not perform genetic testing on our patients. Future studies would provide much-needed information on how Na1,7 affects ENFD and, more generally, the underlying neuronal pathophysiologic mechanisms in erythromelalgia.

Conclusions

Skin biopsy results at the distal leg for ENFD are abnormal in less than 10% of cases, whereas functional tests of small fiber neuropathies (ie, sweating, heat pain testing at the distal foot, blood pressure, and heart rate) will reveal at least 1 abnormality in almost all patients. Our findings indicate that skin biopsies for ENFD evaluation are not useful for the diagnosis of small fiber neuropathy associated with erythromelalgia.

REFERENCES


