Skin Denervation and Cutaneous Vasculitis in Eosinophilia-Associated Neuropathy

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Background: Eosinophilia is frequently associated with peripheral neuropathy, and neuropathic pain is a major presentation. Little is known about the involvement of sensory nerve terminals and the vasculature in the skin of patients with eosinophilia.

Objectives: To investigate the skin innervation and the pathological abnormalities of the cutaneous vasculature and their clinical significance in eosinophilia-associated neuropathy.

Design: Case series.

Setting: National Taiwan University Hospital, Taipei, Taiwan.

Patients: Twelve patients with neuropathy and concomitant eosinophilia (with an eosinophilic ratio of white blood cell classification >10% or absolute eosinophil count of >1000/µL).

Interventions: Clinical assessments of neurological deficits, laboratory tests, nerve conduction studies, and a skin biopsy specimen 3 mm in diameter taken from the distal leg without active skin lesions.

Main Outcome Measures: Quantitation of epidermal innervation, immunopathological findings of the cutaneous vasculature, and motor disability grade.

Results: Six patients fulfilled the criteria of Churg-Strauss syndrome, and the other 6 patients were categorized as having primary eosinophilia. All of the 12 patients had mononeuropathy multiplex or polyneuropathy with sensory symptoms as the initial manifestation. Intrapidermal nerve fiber densities were reduced in 10 patients (83.3%), being significantly lower than in the controls (mean±SD, 2.12±2.30 vs 10.56±3.69 fibers/mm, respectively; P<.001) and negatively correlated with the disability grade (P=.003). Nine patients (75.0%), including all of the 6 patients with Churg-Strauss syndrome, had cutaneous vasculitis, and two-thirds of the 9 patients had perivascular infiltration of eosinophils.

Conclusion: Skin denervation with cutaneous vasculitis is a major manifestation of eosinophilia-associated neuropathy.

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OSINOPHILIA IS FREQUENTLY associated with inflammatory and autoimmune disorders, eg, necrotizing vasculitis in Churg-Strauss syndrome (CSS). Scattered reports have indicated the frequent association of eosinophilia with various types of neuropathies based on nerve biopsies. Traditionally, the demonstration of eosinophilic vasculitis in tissues depends on nerve or muscle biopsies. We recently demonstrated the feasibility of using skin biopsies to investigate cutaneous vasculitis in addition to exploring skin innervation; in vasculitic neuropathy and systemic lupus erythematosus, there was significant dermal vasculitis with infiltration of T cells and macrophages, suggesting the nature of the immune-mediated vasculopathy.

See also pages 935, 966, and 974

Skin innervation is reduced in inflammatory neuropathies such as Guillain-Barré syndrome, chronic inflammatory demyelinating polyneuropathy, and autoantibody-related neuropathy. Skin denervation has been demonstrated in systemic inflammatory diseases including vasculitis and systemic lupus erythematosus. In lupus, the degree of skin denervation is related to the extent
of cutaneous vasculitis. These findings raise the possibility that skin innervation is altered in eosinophilia. Eosinophilic syndrome is associated with neuropathies of diverse manifestations, ranging from focal to systemic neuropathies. In patients with CSS, sensory symptoms and neuropathic pain are major initial symptoms, accounting for 50% of presentations. Among different subtypes of neuropathies, the effects of eosinophilia on small fibers have received less attention, and to our knowledge, skin innervation has not been extensively evaluated in eosinophilia-associated neuropathy before.

In this study, we investigated the following: (1) whether eosinophilia could be demonstrated in cutaneous tissues; (2) whether there was associated dermal vasculitis; and (3) whether skin innervation was altered in eosinophilia-associated neuropathy.

METHODS

SUBJECTS

The criteria of eosinophilia-associated neuropathy included the following: (1) clinical evidence of symptomatic peripheral neuropathy; (2) eosinophilia according to an elevated ratio of white blood cell classification (>10%) or the absolute eosinophil count (>1000/µL); and (3) the absence of diabetes mellitus, renal impairment, malignancies, and specific autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome, and scleroderma. Patients fulfilling these criteria were recruited from National Taiwan University Hospital, Taipei, Taiwan, from January 1, 1997, to December 31, 2005.

In addition to detailed neurological examinations, patients also had laboratory tests carried out, including plasma glucose level, functional tests of the liver and kidney, and levels of antinuclear antibody, rheumatoid factor, anti-Sjögren syndrome A antigen, anti-Sjögren syndrome B antigen, anti-Smith antigen, antinuclear antibody antigen (Scl-70), C3 and C4 complement, and cryoglobulin. Possible malignancies were excluded by examining tumor markers (carcinoembryonic antigen and carbohydrate antigen 19-9), abdominal echo, and chest radiography results. Parasite infections were screened by stool ovum examinations. If patients had a history of asthma, chest radiography and computed tomography were performed to determine whether there was pulmonary infiltration. A diagnosis of CSS followed the definition of the American College of Rheumatology subcommittee. In this article, if patients with eosinophilia-associated neuropathy did not have CSS, malignancies, or parasitic infections, this subtype of eosinophilia was classified as primary eosinophilia.

The ability of ambulation was assessed by a disability grade from 0 to 6; grade 0 indicated normal neurological status; grade 1, minor signs or symptoms capable of running; grades 2 and 3, ambulation without and with assistance, respectively; grade 4, wheelchair bound or bed bound; grade 5, requiring mechanical ventilation; and grade 6, death.

This study was reviewed and approved by the ethics committee of National Taiwan University Hospital. Informed consent was obtained from each patient before the investigations. For comparison, age- and sex-matched subjects in the control group were retrieved from a previously described cohort.

NERVE CONDUCTION STUDIES

Nerve conduction studies were performed with a Viking IV Electromyographer (Nicolet, Madison, Wisconsin) in all of the patients following established methods. Studied nerves included sural, peroneal, tibial, median, and ulnar (motor and sensory) nerves. Abnormal results in nerve conduction studies were defined as having reduced amplitude of compound motor action potentials or sensory action potentials, prolonged distal latencies, or slowing of nerve conduction velocity.

SKIN BIOPSY AND IMMUNOHISTOCHEMISTRY OF THE SKIN

After informed consent was obtained, a skin biopsy specimen 3 mm in diameter was taken from the distal lateral leg 10 cm proximal to the lateral malleolar process. No active skin lesion was noted at the site of biopsy in any patient. If the sensory symptoms were discrepant between the right and left legs, the side with the more severe symptoms was sampled. The sampled skin tissue was fixed in 4% paraformaldehyde overnight. Sections 50 µm thick and perpendicular to the dermis were cut on a sliding microtome, quenched with 1% hydrogen peroxide in methanol, and blocked with 5% normal goat serum. Sections were incubated with rabbit antiserum to protein gene product 9.5 (1:1000; UltraClone, Isle of Wight, England) overnight. Protein gene product 9.5 is a ubiquitin carboxy-terminal hydrolase that labels myelinated and unmyelinated nerve fibers in the peripheral nervous system. Sections were then incubated with biotinylated goat antirabbit IgG (Vector Laboratories, Burlingame, California) for 1 hour and avidin-biotin complex (Vector Laboratories) for another hour. The reaction product was demonstrated using chromogen 5G (Vector Laboratories).

QUANTITATION OF EPIDERMAL INNERVATION

Epidermal innervation was quantified according to established criteria in a coded fashion. Observers were blinded to the clinical information. Protein gene product 9.5-positive nerves in the epidermis of each skin section were counted at ×40 magnification with a BX40 microscope (Olympus, Tokyo, Japan). Each individual nerve with branching points after crossing the basement membrane was counted as a single nerve. Epidermal nerves splitting below the basement membrane were counted as 2 nerves. The length of the epidermis along the upper margin of the stratum corneum in each skin section was measured using Image-Pro PLUS (Media Cybernetics, Silver Spring, Maryland). Intraepidermal nerve fiber (IENF) density was hence derived and expressed as the number of fibers per millimeter of epidermal length. In the distal leg, the mean ± SD (fifth percentile) normative values of IENF densities from our laboratory were 11.16±3.70 (5.88) fibers/mm for subjects younger than 60 years and 7.64±3.08 (2.50) fibers/mm for subjects aged 60 years or older.

PATHOLOGICAL ASSESSMENT OF VASCULITIS IN SKIN BIOPSY SPECIMENS

Biopsy specimens of the skin for evaluating vasculitis were fixed in 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin-eosin as described previously. Both perivascular inflammation and vascular injury (extravasation of red blood cells, fibrinoid necrosis, or disruption of vascular wall integrity by leukocytes) were required to definitively diagnose vasculitis. The vasculature in the skin belongs to the small vessels; this type of vessel is less likely to undergo fibrinoid necrosis, which is usually seen in medium-sized vessels. The disruption of vascular integrity, such as the discontinuity of the vas-
cular wall by infiltrating leukocytes or the extravasation of red blood cells, permits the diagnosis of vascular injury.\textsuperscript{16} Immunohistochemistry analysis was performed using the avidin-biotin-peroxidase complex technique as described earlier. Cell surface antigens were examined using the following monoclonal antibodies: T cells with CD3 (1:100; Ventana Medical System, Tucson, Arizona), B cells with CD20 (1:100; Dako, Glostrup, Denmark), and macrophages with CD68 (1:200; Dako).\textsuperscript{4,6} Sections were then incubated with biotinylated horse antimouse IgG (Vector Laboratories) for 1 hour and the avidin-biotin complex (Vector Laboratories) for another hour. The reaction product was shown by the chromogen 3,3'-diaminobenzidine (Sigma-Aldrich Co, St Louis, Missouri) and counterstained with hematoxylin. The skin pathological abnormalities and phenotypes of cellular infiltration were examined by 1 of us (C.-T.S.) in a blinded fashion.

### RESULTS

#### CLINICAL PRESENTATION AND LABORATORY DATA

There were 12 patients with eosinophilia-associated neuropathy (8 men and 4 women; mean ± SD age, 47.92 ± 11.65 years; age range, 27-71 years). The clinical presentations of these patients are listed in Table 1. Six patients (2 men and 4 women) fulfilled the criteria of CSS. In addition to eosinophilia and peripheral neuropathy, all of these 6 patients had adult-onset asthma (age at onset, 31-68 years) with involvement of the skin, lung, gastrointestinal system, and paranasal sinus. The

<table>
<thead>
<tr>
<th>Case No./ Sex/Age, y</th>
<th>Diagnosis</th>
<th>Eosinophils\textsuperscript{a}</th>
<th>Systemic Involvement Pattern of Neuropathy</th>
<th>Motor Weakness</th>
<th>Sensory Symptoms</th>
<th>Treatment</th>
<th>Disability Grade\textsuperscript{b}</th>
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</thead>
<tbody>
<tr>
<td>1/F/54</td>
<td>CSS</td>
<td>49.0</td>
<td>MM UL, left distal; LL, bilateral distal</td>
<td>Left foot, neuropathic pain</td>
<td>Steroid</td>
<td>3</td>
<td></td>
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<tr>
<td>2/M/59</td>
<td>CSS</td>
<td>44.0</td>
<td>MM LL, right distal</td>
<td>Right leg, neuropathic pain</td>
<td>Steroid</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3/F/50</td>
<td>CSS</td>
<td>63.0</td>
<td>MM UL, right distal; LL, bilateral distal</td>
<td>Bilateral feet, paresthesia</td>
<td>Steroid, cyclophosphamide</td>
<td>3</td>
<td></td>
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<tr>
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<td>14.5</td>
<td>MM LL, bilateral distal</td>
<td>Bilateral feet, neuropathic pain</td>
<td>Steroid, cyclophosphamide</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>5/F/40</td>
<td>CSS</td>
<td>40.0</td>
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<td>Left foot, neuropathic pain</td>
<td>Steroid</td>
<td>2</td>
<td></td>
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<tr>
<td>6/F/71</td>
<td>CSS</td>
<td>71.8</td>
<td>MM LL, left distal and proximal</td>
<td>Bilateral feet, paresthesia</td>
<td>Steroid</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7/M/39</td>
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<td>23.2</td>
<td>MM UL, bilateral distal and proximal; LL, bilateral distal and proximal</td>
<td>Bilateral hands, paresthesia</td>
<td>None</td>
<td>4</td>
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<td>8/M/36</td>
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<td>18.5</td>
<td>NA LL, bilateral distal and proximal</td>
<td>Bilateral feet, paresthesia</td>
<td>Steroid, PE</td>
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<td></td>
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<td>25.0</td>
<td>NA LL, bilateral distal and proximal</td>
<td>Right foot, neuropathic pain</td>
<td>Steroid</td>
<td>3</td>
<td></td>
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<tr>
<td>10/M/53</td>
<td>Primary eosinophilia</td>
<td>26.8</td>
<td>NA LL, bilateral distal and proximal</td>
<td>Right leg, neuropathic pain</td>
<td>Steroid, PE</td>
<td>3</td>
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<tr>
<td>11/M/50</td>
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<td>13.6</td>
<td>NA UL, bilateral distal; LL, right distal and proximal</td>
<td>Bilateral hands, paresthesia</td>
<td>Steroid</td>
<td>3</td>
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<tr>
<td>12/M/27</td>
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<td>NA LL, bilateral distal</td>
<td>Bilateral feet, neuropathic pain</td>
<td>Steroid</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CSS, Churg-Strauss syndrome; GI, gastrointestinal tract; LL, lower limb; MM, mononeuropathy multiplex; NA, not applicable; PE, plasma exchange; PN, polyneuropathy; UL, upper limb.

\textsuperscript{a} Differential indicates the differential count of eosinophils according to leucocyte classification; absolute, absolute eosinophil count.

\textsuperscript{b} The disability grade is according to the definition described in the "Methods" section.
other 6 patients (all men), apart from 1 patient with recurrent urticaria before the onset of neuropathy, were free from allergic diseases, parasitic infections, autoimmune diseases, and tumors.

All of the 12 patients presented with sensory and motor impairment of acute or subacute onset. Mononeuropathy multiplex was the major form of the neuropathy in 10 patients, whereas the other 2 patients had symmetrical sensorimotor polyneuropathy. In all of the patients, the initial neurological symptoms were paresthesia with various distributions: in the unilateral lower limb (5 cases), simultaneously in bilateral hands (2 cases), and in bilateral feet (5 cases). Neuropathic pain was present in 7 patients (4 with CSS and 3 with primary eosinophilia). Most patients had mild motor difficulties (disability grade 2 in 5 cases, grade 3 in 6 cases, and grade 4 in 1 case). There was no correlation between the eosinophil counts and disability. In nerve conduction studies, 6 patients had motor abnormalities in the upper and lower limbs and 6 had motor abnormalities in the lower limbs only; all of the patients had sensory abnormalities in the lower limbs. Eleven patients received immunomodulation therapy including corticosteroids, cyclophosphamide, and plasma exchange, and all of the patients showed remission of eosinophilia and significant improvement in ambulation (of at least 1 disability grade). One patient with primary eosinophilia experienced spontaneous remission in motor weakness (from an initial grade of 4 to a follow-up grade of 2). The neuropathy exhibited a remission-relapse course in 3 patients with CSS; the other 9 patients had a monophasic course. In summary, there were no obvious differences in neurological manifestations between the CSS and primary eosinophilia groups, and the responses to immunomodulating therapy were similar in both groups.

### DERMAL VASCULITIS IN EOSINOPHILIA-ASSOCIATED NEUROPATHY

Dermal vasculitis in the regions with no active skin lesion was demonstrated in all of the 6 patients with CSS and in the 3 patients with primary eosinophilia (Table 2). Additionally, in these 9 cases, perivascular infiltration of eosinophils was noted in 4 of the 6 patients with CSS and in 2 of those 3 patients with primary eosinophilia (Figure 1A). In addition to eosinophilic infiltration, most infiltrating cells were positive for the marker of macrophages and T cells but not for that of B cells (Figure 1B-D).

### SKIN DENERVATION IN EOSINOPHILIA-ASSOCIATED NEUROPATHY

In most patients with eosinophilia-associated neuropathy, the abundance of epidermal nerves was markedly reduced compared with that in the skin of control subjects (Table 2); in 4 patients, the epidermis was even completely denervated (Figure 2). Compared with age- and sex-matched control subjects (8 men and 4 women; mean±SD age, 49.00±11.47 years; age range, 31-72 years), the IENF densities in patients with eosinophilia-associated neuropathy were significantly lower (mean±SD, 10.56±3.69 vs 2.12±2.30 fibers/mm, respectively; *P* < .001) (Figure 3A), and the IENF densities were reduced in 10 patients (83.3%). The IENF densities in these patients were negatively correlated with the disability scores (*P* = .003) (Figure 3B).

### COMMENT

The major findings with eosinophilia-associated neuropathy in this study include the following: (1) marked

### Table 2. Pathological Findings of the Skin Biopsies

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Pattern of Neuropathy</th>
<th>RBC Extravasation</th>
<th>Perivascular Inflammation</th>
<th>Vasculitis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Perivascular Eosinophils</th>
<th>IENF Density, Fibers/mm</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>CSS</td>
<td>MM</td>
<td>+</td>
<td>+</td>
<td>Definite</td>
<td>−</td>
<td>0</td>
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<td>2</td>
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<td>MM</td>
<td>+</td>
<td>+</td>
<td>Definite</td>
<td>+</td>
<td>3.00</td>
</tr>
<tr>
<td>3</td>
<td>CSS</td>
<td>MM</td>
<td>+</td>
<td>+</td>
<td>Definite</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>CSS</td>
<td>PN</td>
<td>+</td>
<td>+</td>
<td>Definite</td>
<td>−</td>
<td>1.38</td>
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<tr>
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<td>CSS</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
<td>3.44</td>
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<tr>
<td>6</td>
<td>CSS</td>
<td>MM</td>
<td>+</td>
<td>+</td>
<td>Definite</td>
<td>+</td>
<td>5.98</td>
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<td>7</td>
<td>Primary eosinophilia</td>
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<td>−</td>
<td>+</td>
<td>Borderline</td>
<td>−</td>
<td>0</td>
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<tr>
<td>8</td>
<td>Primary eosinophilia</td>
<td>PN</td>
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<td>−</td>
<td>NA</td>
<td>−</td>
<td>6.39</td>
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<tr>
<td>9</td>
<td>Primary eosinophilia</td>
<td>MM</td>
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<td>−</td>
<td>NA</td>
<td>−</td>
<td>2.57</td>
</tr>
<tr>
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<td>Primary eosinophilia</td>
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<td>+</td>
<td>+</td>
<td>Definite</td>
<td>+</td>
<td>0.19</td>
</tr>
<tr>
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<td>Primary eosinophilia</td>
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<td>+</td>
<td>+</td>
<td>Definite</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>Primary eosinophilia</td>
<td>MM</td>
<td>+</td>
<td>+</td>
<td>Definite</td>
<td>+</td>
<td>2.49</td>
</tr>
</tbody>
</table>

Patients with abnormality, %

75.0 83.3 75.0 50.0 83.3

Abbreviations: CSS, Churg-Strauss syndrome; IENF, intraepidermal nerve fiber; MM, mononeuropathy multiplex; NA, not applicable; PN, polyneuropathy; RBC, red blood cell; +, positive; −, negative.

<sup>a</sup>Definite vasculitis indicates the presence of both RBC extravasation and perivascular inflammation; borderline vasculitis, the presence of either RBC extravasation or perivascular inflammation.
skin denervation; (2) dermal vasculitis with eosinophilic infiltration; and (3) correlated motor disability with skin denervation.

Many organ systems can be damaged by sustained over-activation of eosinophils, and the involvement of the peripheral nerves occurs in approximately 50% of patients with eosinophilia. The presence of paresthesia or neuropathic pain in eosinophilia-associated neuropathy raises the possibility of small-fiber involvement. Previously, this issue could only be examined with nerve biopsies to demonstrate a loss of unmyelinated nerve fibers. Because unmyelinated autonomic nerves are also present in sural nerves, our study using skin biopsies provides direct evidence that small-diameter sensory nerves are susceptible in eosinophilia-associated neuropathy.

The pathogenesis of eosinophilia-associated neuropathy includes vasculitic injury and eosinophilic neurotoxicity. Traditionally, vasculitis was diagnosed based on nerve or muscle biopsies in eosinophilia and other inflammatory diseases, such as primary Sjogren syndrome, lumbosacral plexopathies of the diabetic and non-diabetic types, and rheumatoid arthritis. The cutaneous vasculature provides an opportunity for evaluation of vasculitis. In a previous study of 9 CSS cases with active skin lesions and neuropathy, vasculitis was noted in the skin biopsies. In our series with no active skin le-
sessions, cutaneous vasculitis was present in 9 patients (75.0%), including all of the patients with CSS and half of those with primary eosinophilia. Additionally, perivascular eosinophilic infiltration was demonstrated in two-thirds of the patients in both subgroups. These findings suggest that cutaneous vasculitis indeed also occurs in clinically inactive skin tissues with eosinophilia.

The correlation of skin denervation and motor disability grade suggests that the concomitant injury to large and small fibers in eosinophilia-associated neuropathy and skin denervation in eosinophilia reflect generalized neurological deficits. This finding extends previous observations exploring the clinical significance of skin denervation. For example, skin denervation was found to be correlated with the disability grade in Guillain-Barré syndrome. In systemic lupus erythematosus, skin denervation was related to disease activities. Taken together, these findings provide therapeutic implications for clinical management. First, skin biopsy is a less invasive procedure to diagnose eosinophilia-related neuropathy compared with traditional nerve and muscle biopsies. In addition, skin biopsy has the benefit of exploring the effects on nerve terminals, which are among the parts of the nerves most vulnerable to vasculitis. Second, skin innervation may be an indicator for assessing the immunological derangements on neurological disabilities. This information is important for designing therapeutic planning or modifying current regimens because different levels of strategies are available, from immunomodulating to immunosuppression. Further studies applying this technique are required to test these implications for managing eosinophilia-associated neuropathy, such as different degrees of immunosuppression for different degrees of skin denervation.

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Author Contributions: Dr S.-T. Hsieh had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Chao, S.-T. Hsieh, and S.-C. Hsieh. Acquisition of data: Chao, S.-T. Hsieh, Shun, and S.-C. Hsieh. Analysis and interpretation of data: Chao, S.-T.
REFERENCES