Intraepidermal Nerve Fiber Densities in Chronic Inflammatory Autoimmune Diseases

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Background: Some patients with systemic lupus erythematosus have selective loss of small-diameter nerve fibers, while larger nerve fibers are unaffected.

Objective: To determine intraepidermal nerve fiber densities in patients with different chronic inflammatory autoimmune diseases.

Design: Cross-sectional study.

Setting: Stavanger University Hospital, Stavanger, and Haukeland University Hospital, Haukeland, Norway.

Patients: Sixty patients with systemic lupus erythematosus (SLE) (mean ± SD age, 43.2 ± 13.5 years), 61 patients with primary Sjögren syndrome (age, 57.1 ± 14.7 years), and 52 patients with rheumatoid arthritis (age, 57.4 ± 12.3 years) were compared with 106 healthy subjects (age, 49.0 ± 19.6 years).

Interventions: Skin biopsy specimens.

Main Outcome Measures: To evaluate small-diameter nerve fiber loss, intraepidermal nerve fiber densities were measured in skin punch biopsy specimens obtained from the distal part of the leg.

Results: The mean ± SD densities were 7.5 ± 3.8 fibers/mm in patients with SLE, 9.2 ± 3.8 fibers/mm in primary Sjögren syndrome, and 10.9 ± 5.4 fibers/mm in rheumatoid arthritis vs 12.4 ± 4.6 fibers/mm in healthy subjects. Densities were significantly less in patients with SLE vs patients with rheumatoid arthritis and vs healthy subjects (P<.001 for both), as well as in patients with primary Sjögren syndrome vs healthy subjects (P<.001). Eight patients (13%) with SLE, 2 patients (3%) with primary Sjögren syndrome, and 2 patients (4%) with rheumatoid arthritis had densities below the lower reference limit of 3.4 fibers/mm, consistent with small-diameter nerve fiber neuropathy.

Conclusion: The degree of loss of small-diameter nerve fibers differs among patients with these chronic inflammatory autoimmune diseases, likely reflecting differences in pathogenesis and organ affinity of the individual disease entities.

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Compared with healthy subjects, patients with SLE report more neuropathic symptoms, such as burning pain and aching, despite an absence of corresponding neurologic deficits, indicating that this phenomenon might be secondary to the involvement of small-diameter nerve fibers. In a study comparing patients with SLE, patients with RA, and healthy subjects, quantitative sensory testing revealed that the warmth sense and heat pain detection thresholds in patients with SLE were significantly higher compared with those in patients with RA or in healthy subjects, in agreement with a neuropathic process affecting small-diameter nerve fibers in patients with SLE. Two independent skin biopsy studies subsequently verified that small-diameter intraepidermal nerve fiber (IENF) densities are reduced in patients with SLE compared with normative values. In most of these patients with SLE and reduced density of IENF, the neuropathic process seems to be selective, sparing large-diameter nerve fibers.

In patients with PSS, IENF densities are reduced compared with those in healthy subjects, but (in contrast to SLE) large-diameter nerve fibers are also involved. This indicates that the loss of IENFs in PSS may be part of a generalized neuropathy.

The pathophysiological processes leading to small-diameter nerve fiber neuropathy are unclear but are likely directly or indirectly immune mediated. The immune-mediated process may be directed against different neuronal elements, or the neural elements may be affected secondary to an inflammatory process targeting the vasa nervorum.

To further investigate the effect of chronic inflammatory autoimmune diseases on small-diameter nerve fibers, we herein performed a comparative study among patients with SLE, PSS, and RA and compared the results with the findings among a reference population of healthy subjects from a previous study. Despite similarities among the various disease entities (such as chronic inflammation and genesis of autoimmunity), we hypothesized that small-diameter nerve fiber involvement (as expressed by IENF densities) could vary because of differences in immune profiles and organ-specific affinities, as well as high systemic inflammatory activity.

### METHODS

The patient cohorts with SLE and PSS have been previously described in detail. Briefly, the medical records of all patients and outpatients with a diagnosis of SLE from January 1, 1980, through December 31, 2003, or with a diagnosis of PSS from January 1, 1980, through December 31, 2004, at Stavanger University Hospital, Stavanger, Norway, were reviewed. Sixty patients who met the American College of Rheumatology’s revised criteria for the classification of SLE and 61 patients who met the revised international criteria for the classification of PSS were included. In addition, 32 consecutive patients from the outpatient clinic of the Department of Rheumatology, Haukeland University Hospital, Haukeland, Norway, who met the American Rheumatism Association’s (now the American College of Rheumatology) revised criteria for the classification of RA were recruited. All patients and healthy subjects were white. Demographic data are given in the Table. All patients and healthy subjects provided informed consent, and the study was approved by the regional research ethics committee. Disease activity was measured using the SLE Disease Activity Index and (for RA) using a disease activity score that covers 28 joints and the modified Stanford Health Assessment Questionnaire. To our knowledge, no validated SLE disease activity scoring system exists.

Skin biopsies were performed using a 3-mm disposable circular punch needle (Biopsy Punch; Stiefel Laboratories Ltd, Sligo, Ireland) under sterile conditions after application of local anesthesia (2% lidocaine hydrochloride and 1:200 000 epinephrine). Two biopsy specimens were obtained from each person on the same leg during a single procedure approximately 10 cm above the lateral malleolus. The specimens were obtained from the right leg unless the skin on that leg was inflamed or had scars. The biopsy specimens were immediately fixed and prepared as previously described. Intraepidermal nerve fiber density per millimeter was reported as the mean of counts in 6 sections, 3 from each of the 2 biopsy specimens. Intraepidermal nerve fiber densities less than 3.4 fibers/mm (representing the lower 1.96 SD from normative values) were considered abnormal.

Important variables such as IENF density and age were normally distributed and were subjected to parametric statistical analysis. Results are reported as mean ± SD. Analysis of variance was applied when testing for 3 groups or more of quantitative data, using IENF density or age as the dependent variable and patient group as the independent variable. P < .05 was considered significant and was Bonferroni corrected. The statistical analysis was performed using StatView version 5.0 software (SAS Institute Inc, Cary NC).

### RESULTS

The mean ± SD IENF densities were significantly less in patients with SLE (7.5 ± 3.8 fibers/mm), and patients with PSS (9.2 ± 3.8 fibers/mm) vs healthy subjects (12.4 ± 4.6...
nerve fiber density was not associated with age in the total group of patients (R²=0.02; P=.06) or in the individual groups of patients with SLE, PSS, or RA. The median disease activity score assessed using the SLE Disease Activity Index was 2.0 (mean, 2.4; range, 0.0-24.0). Among patients with RA, the mean disease activity score that covered 28 joints was 4.7±1.0 (range, 2.2-6.9), and 16 patients had a score exceeding 5.1, indicating high disease activity. There were no associations between IENF densities and disease activity in either group. In patients with PSS, surrogate markers for disease activity such as hemoglobin level, erythrocyte sedimentation rate, and number of disease classification criteria were used, but no associations with IENF densities were demonstrated.

There were no associations between IENF densities and disease duration, medication use, or routine hematological, biochemical, or immunologic variables, including anti-SSA and anti-SSB antibodies in any of the patient groups. Likewise, the use of corticosteroids and immunosuppressants, including hydroxychloroquine, did not affect densities. One patient with RA had diabetes mellitus, and 3 patients with PSS had glomerulonephritis (1 of whom had received a renal transplant). One patient with SLE had moderate renal failure (serum creatinine level, 1.9 mg/dL [169 µmol/L]).

Forty-four (79%) of 56 patients with PSS in whom a lip biopsy was performed had a focus score exceeding 1. We found no associations between the presence of positive findings on lip biopsy specimens and IENF densities. In patients with RA, there were no associations between IENF densities and duration of morning stiffness, modified Standard Health Assessment Questionnaire score, and the presence or absence of rheumatoid factor.

Distinct differences in IENF loss were observed among the 3 patient groups (Figure). Densities were not associated with drug treatment, disease duration, disease activity, or autoantibodies. Intraepidermal nerve fiber loss was significantly greater in patients with SLE compared with that in patients with RA. In patients with PSS, densities did not differ significantly from those of the other 2 patient groups, although it is likely that larger patient samples would have yielded significant differences in densities between the groups in the order shown in the Figure (ie, healthy subjects > patients with RA > patients with PSS > patients with SLE). In fact, similar densities were found in patients with RA and in healthy subjects. In patients with SLE, the proportion with IENF densities below the lower reference limit (<3.4 fibers/mm) was much higher than that in patients with PSS and in patients with RA. This indicates that there might be differences in pathophysiological processes affecting the small-diameter nerve fibers in the individual disease entities.

Although SLE, PSS, and RA are chronic inflammatory autoimmune diseases sharing common immune characteristics such as the presence of autoantibodies and rheumatoid factor production, the immunopathogenesis and targets for attack are different. Among patients with RA, an inflammatory erosive polyarthritis is the most prevalent finding, and extraarticular manifestations usually reflect severe RA, with increased levels of rheumatoid factor and signs of high inflammatory activity. Various cells contribute to the pathogenesis, with T cells and synovial fibroblasts playing crucial roles. In patients with PSS, chronic inflammation characterized by infiltration of CD4+ T cells in exocrine glands and by profound B-cell stimulation leads to failure of adequate tear and saliva production and to nonspecific phenomena such as fatigue. Systemic lupus erythematosus is a chronic inflammatory multiorgan disease characterized by general and excessive T-cell stimulation, polyclonal B-cell activation, and production of numerous diverse autoantibodies. Therefore, RA may principally be classified as a disease with predominantly mono-organ involvement, SLE as a disease with systemic or multiorgan involvement, and PSS as a disease somewhere in between.

The mechanisms underlying these abnormalities of small-diameter nerve fibers in autoimmune diseases are unclear. The diseases are characterized by different organ systems affected and by different methods of immunopathogenesis. Some patients with SLE may produce autoantibodies that react with constituents of small-diameter nerve fibers due to the nonspecific activation of B cells, or other factors associated with a more general immune activation in SLE may have deleterious effects on the small-diameter nerve fibers.

Among healthy subjects, IENF densities decrease modestly with age. No such association was found in this study among patients with autoimmune diseases. This is likely because of the narrow age range among the various patient groups in this study. However, the differences in IENF densities between disease entities are not explained by the differences in mean age.
REFERENCES