Paper IV

Intraepidermal nerve fiber densities in chronic inflammatory autoimmune diseases

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Abstract

Background: Some patients with systemic lupus erythematosus have a selective loss of small-diameter nerve fibers while larger nerve fibers are left unaffected. Little is known about these densities in other chronic inflammatory autoimmune diseases.

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

Design: Cross sectional study.

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

Patients: Sixty patients with systemic lupus erythematosus, aged 43.2 $\pm$ 13.5 years (mean $\pm$ SD), 61 patients with primary Sjögren's syndrome, aged 57.1 $\pm$ 14.7 years, and 52 patients with rheumatoid arthritis, aged 57.4 $\pm$ 12.3 years were included, and compared to a group of 106 healthy subjects, aged 49.0 $\pm$ 19.6 years.

Interventions: Skin biopsy specimens.

Main outcome measures: Intraepidermal nerve fiber densities were measured in skin punch biopsy specimens taken from the distal part of the leg to evaluate small-diameter nerve fiber involvement.

Results: The intraepidermal nerve fiber densities in patients with systemic lupus erythematosus was 7.5 $\pm$ 3.8 fibers per millimeter (mean $\pm$ SD), in primary Sjögren's

syndrome 9.2 $\pm$ 3.8 fibers per millimeter, and in rheumatoid arthritis 10.9 $\pm$ 5.4 fibers per millimeter as compared to 12.4 $\pm$ 4.6 fibers per millimeter in healthy subjects. The densities were significantly less in patients with systemic lupus erythematosus compared to rheumatoid arthritis ($P < 0.0001$) and healthy subjects ($P < 0.0001$), and also in patients with primary Sjögren's syndrome compared to healthy subjects ($P < 0.0001$). Eight patients with systemic lupus erythematosus (13%), and 2 patients in each of the groups of patients with primary Sjögren's syndrome (3%) and rheumatoid arthritis (4%) had intraepidermal nerve fiber densities below the lower reference limit (3.4 intraepidermal nerve fibers per millimeter), consistent with small-diameter nerve fiber neuropathy.

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

It is well known that patients with chronic inflammatory autoimmune diseases have involvement of their central and peripheral nervous system,[1] and also that the prevalence and pattern of involvement differ between individual disease entities. Although immunologic and inflammatory features are shared in these conditions, such observations nevertheless point to distinct differences in pathogenesis and pathophysiology.

However, comparing the extent of neuropathy between different diseases is hindered by the use of various diagnostic criteria, and also by the selection of patients. Based on nerve conduction studies, we recently demonstrated an 18% prevalence of polyneuropathy in an unselected cohort of patients with systemic lupus erythematosus (SLE) consisting of 10% sensory, 7% sensorimotor, and 2% motor neuropathy.[2] In primary Sjögren´s Syndrome (PSS) the main findings have reportedly been a symmetric sensorimotor neuropathy followed by symmetric sensory neuropathy.[3,4] Adopting the new international criteria for PSS,[5] which ensures that subjects with non-autoimmune causes for sicca phenomena are excluded, we recently disclosed a prevailing subclinical motor neuropathy based primarily on increased F-wave latencies on NCSs in an unselected cohort of PSS.[6] In patients with rheumatoid arthritis (RA), compression neuropathies are considered common,[1] although a controlled study disputed this by disclosing more carpal tunnel syndromes in healthy subjects compared to patients with RA.[7] A mild distal symmetric sensory neuropathy is considered a late complication to the disease, and rarely, mono- and multiple mono-neuropathies are reported in association with the very serious condition of rheumatoid vasculitis.[1]

Patients with SLE complain of more neuropathic symptoms like burning pain and aching compared to healthy subjects despite a lack of corresponding neurologic deficits indicating that this phenomenon might be secondary to the involvement of small-diameter nerve fibers.[8] In a study comparing patients with SLE, RA, and healthy subjects, we found by quantitative sensory testing that the warmth sense and heat pain detection thresholds in

patients with SLE were significantly higher compared to patients with RA or healthy subjects in agreement with a neuropathic process affecting small-diameter nerve fibers in the SLE patients.[9] In 2 independent studies we thereafter performed skin biopsy studies and verified that the intraepidermal small-diameter nerve fiber (IENF) densities are reduced in patients with SLE compared to normative values.[2,10] In most of these patients, the neuropathic process seems to be selective, sparing large-diameter nerve fibers.[2]

We also discovered that the IENF densities in patients with PSS are reduced compared to 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.[6]

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 a variety of neural elements, or the neural elements may be affected secondary to an inflammatory process affecting the vasa nervorum.[11,12]

To further investigate the impact of chronic inflammatory autoimmune diseases on small-diameter nerve fibers, we performed a comparative study in patients with SLE, PSS, and RA and compared the results to a reference population of healthy subjects.[13] We hypothesized that despite similarities between the various disease entities like chronic inflammation and autoimmune genesis, the involvement of small-diameter nerve fibers as expressed by IENF densities could vary due to differences in the immune profiles and specific organ affinities and possibly could be influenced by high systemic inflammatory activity.

Patients and methods

The patients with SLE and PSS have previously been described in more detail.[2,6] Briefly, the medical records of all inpatients and outpatients with a diagnosis of SLE or PSS from January 1, 1980, through December 31, 2003, for SLE and through December 31, 2004, for PSS at Stavanger University Hospital were reviewed. Sixty patients with SLE, fulfilling the revised

American College of Rheumatology criteria for the classification of SLE,[14] and 61 patients with PSS fulfilling the revised international classification criteria for primary SS[5] were included. In addition 52 consecutive patients from the outpatient clinic of The Department of Rheumatology, Haukeland University Hospital, fulfilling the American College of Rheumatology criteria for RA[15] were recruited. All controls and patients were white. Demographic data are shown in Table 1. All patients and healthy subjects gave their informed consent, and the study was approved by the regional research ethics committee. The disease activity of SLE was measured according to the SLE Disease Activity Index,[16] and the disease activity in RA by the Disease Activity Score including 28 joints and the modified health assessment questionnaire.[17,18] In PSS, no validated disease activity scoring system exists.

Skin biopsies were performed with a 3 mm disposable circular punch needle (Biopsy Punch, Stiefel Laboratories Ltd., Sligo Ireland) with a sterile technique after local anesthetizing with 2% lidocaine and epinephrine. Two biopsy specimens were obtained from each person on the same leg in the same procedure approximately 10 cm above the lateral malleolus. The specimens were taken 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.[10] The densities of IENFs per millimeter were reported as the mean of counts in 6 sections, 3 from each of the 2 biopsies. The IENF densities were considered abnormal when less than 3.4 fibers per millimeter (the lower 1.96 SD from normative values).[13]

Statistics

Important variables like IENF densities and age were normally distributed and subjected to parametric statistics. These results are thus reported as mean $\pm$ SD. Analyses of variance was applied when testing for three or more groups of quantitative data with IENF density or age as the dependent variable, and patient groups as the independent variables. $P < .05$ was

considered significant, and were Bonferroni corrected. The statistical analysis was performed using the StatView packages.

Results

The mean IENF density was significantly less in patients with SLE (7.5 ± 3.8 fibers/mm), and PSS (9.2 ± 3.8) compared to healthy subjects (12.4 ± 4.6), and also in SLE compared to RA (10.9 ± 5.4) (Figure 1). Eight patients with SLE (13%), and two patients in each group with PSS (3%) and RA (4%) had less than 3.4 IENFs per millimeter. The IENF density was not associated with age in the total group of patients ($P = 0.06$, $R^2 = 0.02$), nor in the individual groups of patients with SLE, PSS, and RA.

The median disease activity index assessed by SLE disease activity index in patients with SLE was 2.0 (mean 2.4), range 0.0 – 24.0. The mean DAS28 in patients with RA was 4.7 ± 1.0, range 2.2 – 6.9, and 16 patients had a score > 5.1, defined as high disease activity.[17] There were no associations between IENF densities and disease activity in either group. In patients with PSS, surrogate markers for disease activity like hemoglobin, erythrocyte sedimentation rate, and number of classification criteria were used, but no associations to IENF densities were demonstrated.

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

Forty-four out of 56 patients with PSS in which a lip biopsy was performed had a focus score ≥ 1 (79%). We found no associations between the presence of positive lip biopsies

and IENF densities. In patients with RA, there were no associations between IENF densities and duration of morning stiffness, modified health assessment questionnaire, and the presence or absence of rheumatoid factor.

Comment

Distinct differences in small-diameter nerve fiber loss appeared between the three groups of patients (Figure 1). These densities were not associated with drug treatment, autoantibodies, disease activity, or disease duration. The loss of IENFs in patients with SLE was significantly larger compared to patients with RA. In PSS the densities did not differ significantly from the two other groups of patients, although it is likely that a higher number of patients in each group would have yielded significant differences between the groups in the following density order: healthy controls > RA > PSS > SLE. In fact, patients with RA had the same densities as healthy subjects. In SLE the proportion of patients with IENF densities below the lower reference limit (< 3.4 fibers per millimeter) were much higher than in PSS and RA. This indicates that there might be differences in pathophysiologic processes affecting the small-diameter nerve fibers in the individual disease entities.

Although SLE, PSS, and RA all are chronic inflammatory diseases sharing common immune characteristics like the presence of autoantibodies and rheumatoid factor production, the immunopathogenesis and the targets for attacks are different. In patients with RA, an inflammatory, erosive polyarthritis is the most prevalent finding, and extra-articular manifestations usually reflect severe RA with high levels of rheumatoid factor and signs of high inflammatory activity.[19] Various cells contribute to the pathogenesis of which T-cells and synovial fibroblasts play crucial roles.[20] In patients with PSS, a chronic inflammation characterized by infiltration of CD4+ T-cells in exocrine glands and a profound B-cell stimulation leads to failure of adequate tear and saliva production and general and unspecific phenomena like fatigue.[21] SLE is a chronic inflammatory multi-organ disease characterized

by a general and excessive T-cell help, polyclonal B-cell activation, and the production of numerous autoantibodies of diverse specificities. Principally, RA may therefore be classified as a disease with predominantly mono-organ involvement, SLE with systemic or multi-organ involvement, and PSS somewhat in-between.

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

The IENF densities decrease, although only modestly, with age in healthy subjects.[13] However, no such association was found in this study of patients with autoimmune diseases. This is likely due to the narrow age range in 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.

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Legend to Figure 1

Mean number (± 95% confidence interval) of intraepidermal nerve fibers (IENF) in punch biopsy specimens from the distal part of the leg in healthy subjects, rheumatoid arthritis (RA), primary Sjögren´s syndrome (PSS), and systemic lupus erythematosus (SLE).

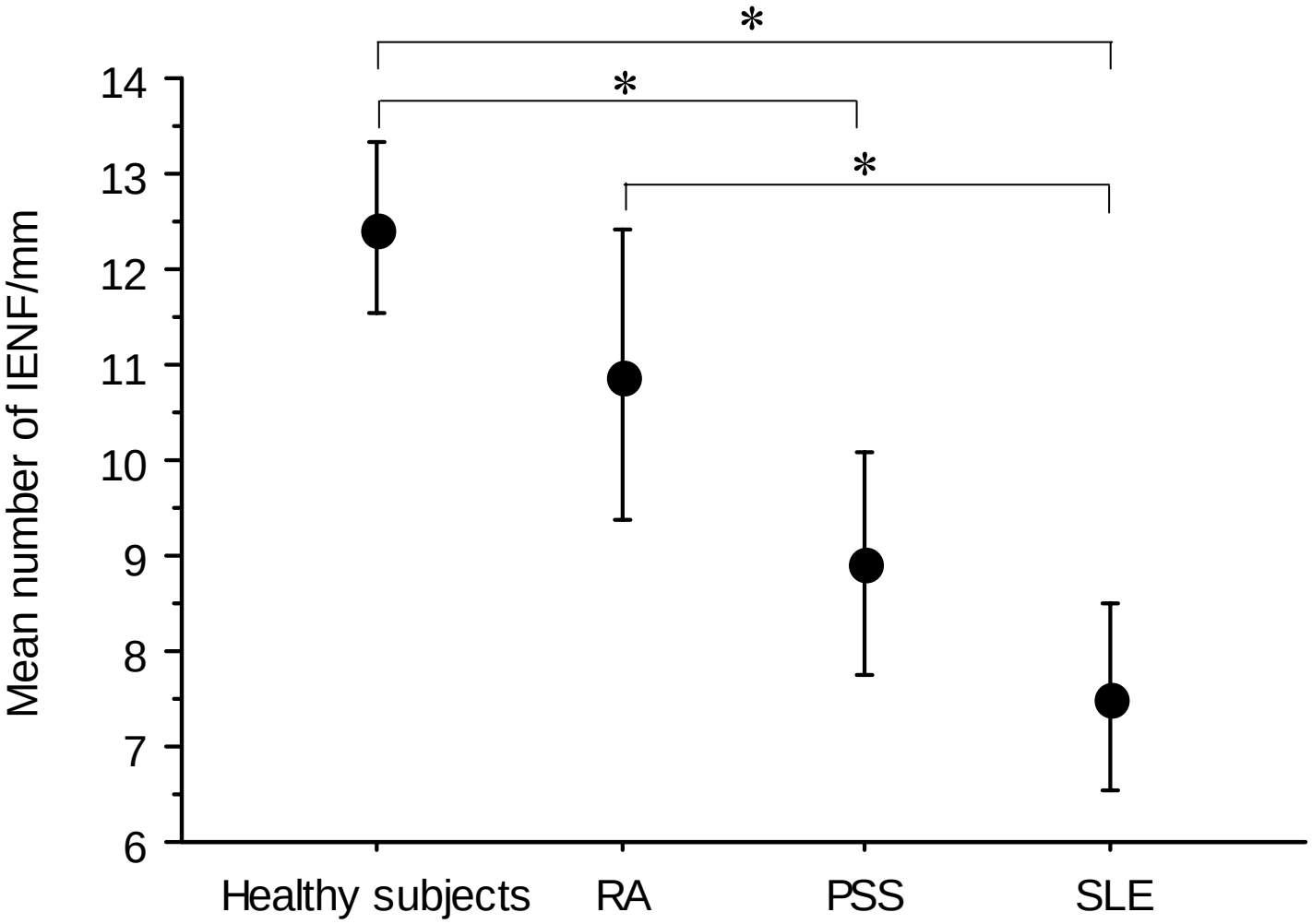
* $P < 0.0001$.

Legend to Table 1

Demographic data for patients with systemic lupus erythematosus (SLE), primary Sjögren´s syndrome (PSS), rheumatoid arthritis (RA), and healthy subjects.

Table 1

	SLE	PSS	RA	Healthy subjects
No. of patients	60	61	52	106
Female (%) Male (%)	51 (85) 9 (15)	53 (87) 8 (13)	43 (83) 9 (17)	66 (62) 40 (38)
Age mean (SD) years	43.2 (13.5)	57.1 (14.7)	57.4 (12.3)	49.0 (19.6)
Disease duration median (range) years	10.0 (1.0 – 52.0)	12.1 (0.0 – 48.0)	11.0 (0.8 – 35.0)	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Mean number of IENF/mm
Healthy subjects
RA
PSS
SLE