

# Correlation between serum E-selectin levels and panoramic nailfold capillaroscopy in systemic sclerosis

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## Abstract

E-selectin is expressed by the activated endothelium and its plasma levels are increased in patients with systemic sclerosis. Eighteen patients fulfilling the American Rheumatism Association criteria for systemic sclerosis, 15 females and 3 males, 42-70 years old, 9 with diffuse and 9 with limited forms, were sequentially recruited for this study. Serum E-selectin levels were determined by commercially available ELISA and their association with nailfold capillaroscopic abnormalities was investigated. Nailfold capillaries were analyzed by 16X magnification wide-field capillaroscopy. Two parameters on capillaroscopy were used to correlate to serum E-selectin: deletion and ectasia. Data were analyzed statistically by the Student *t*-test and Spearman correlation. Two-tailed P values below 0.05 were considered significant. E-selectin range was 38 to 200 ng/ml ( $80 \pm 39.94$ ). There was a correlation between serum E-selectin levels and the deletion capillaroscopic score ( $r = 0.50$ ,  $P < 0.035$ ). This correlation was even stronger within the first 48 months of diagnosis ( $r = 0.63$ ,  $P < 0.048$ ). On the other hand, no association was observed between selectin and ectasia. Patients with diffuse disease presented higher serum E-selectin levels than patients with limited disease, although the difference was not statistically significant ( $96.44 \pm 48.04$  vs  $63.56 \pm 21.77$  ng/dl;  $P = 0.08$ ). The present study is the first showing a correlation between soluble serum E-selectin levels and alterations in capillaroscopy. The stronger correlation of deletion score in capillaroscopy in early disease suggests that serum E-selectin levels might be a useful biochemical marker of disease activity in systemic sclerosis.

## Key words

- Systemic sclerosis
- Nailfold capillaroscopy
- E-selectin
- Adhesion molecules

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## Introduction

Systemic sclerosis is a disease of connective tissue characterized by thickening and fibrosis of the skin and by involvement of internal organs. In contrast to other rheumatic autoimmune diseases, such as systemic lupus

erythematosus and rheumatoid arthritis, there is no reliable disease activity marker for systemic sclerosis. Early histologic changes include microvascular abnormalities and deposition of increased amounts of extracellular matrix. Endothelial cell activation appears to be one of the earliest features. Therefore, the

endothelium is believed to play a central role in the vascular pathophysiology of the disease (1).

Early events in a standard inflammatory process induce the expression of cellular adhesion molecules which coordinate the migration of leukocytes to the extravascular sites involved (2). Soluble forms of adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1), leukocyte function associated molecule-3 (LFA-3), vascular adhesion molecule-1 (VCAM-1), and E-selectin have been detected in the plasma of patients with systemic sclerosis as well as in other autoimmune disorders, representing potentially useful clinical markers (3,4).

*In vitro* cultures of scleroderma-derived fibroblasts express significantly higher amounts of surface ICAM-1 than normal fibroblast cultures (5). Increased expression of the VCAM-1 integrin ligand has also been observed on the endothelium and stratum granulosum on the skin of patients with early systemic sclerosis (6).

E-selectin contains a lectin-like N-terminal domain capable of recognizing the tetrasaccharide sialyl-Lewis<sup>x</sup> moiety in monocytes and neutrophils (7). E-selectin mediates a neutrophil adhesion pathway distinct from that mediated by ICAMs and leukocyte integrins and participates in early steps of neutrophil binding to the endothelium, before transendothelial migration (8). E-selectin is particularly interesting because it is found only on the activated endothelium in contrast to other adhesion molecules which have a wide tissue distribution (9). A retrospective study indicated that its circulating form is found at low levels in healthy individuals and at elevated levels in the sera of patients with systemic lupus erythematosus, polyarteritis nodosa, and systemic sclerosis (10). Furthermore E-selectin serum levels were higher among those patients with recent onset active disease (10). Increased tissue expression of E-selectin has been used to demonstrate endothelial cell activation on the skin of patients with systemic sclerosis (11).

Nailfold capillaroscopy is a routine procedure in the investigation of patients with Raynaud's phenomenon and systemic sclerosis. Nailfold capillaries can be assessed by microscopy, video capillaroscopy (12) and ophthalmoscopy (13). Probably morphological alterations can reflect disease activity. In a study, 22 patients who had Raynaud's phenomenon, abnormal findings on capillaroscopy and high soluble serum E-selectin developed characteristics that satisfied classification criteria for systemic sclerosis within a 1- to 7-year follow-up (14).

E-selectin seems to be a unique marker for endothelial activation, being transiently expressed on cultured endothelial cells 2-8 h after *in vitro* stimulation and substantially lost from the surface within 24 h (15). Since microvascular disease is an important feature of systemic sclerosis, it is possible that circulating E-selectin levels may reflect the degree of *in vivo* endothelial cell activation and the extent of capillaroscopic abnormalities in systemic sclerosis.

In the present study we determined soluble E-selectin levels in sera from patients with systemic sclerosis and attempted to correlate E-selectin levels with nailfold capillaroscopy.

## Patients and Methods

Eighteen patients fulfilling the American Rheumatism Association criteria for systemic sclerosis (16), 15 females and 3 males, 42-70 years old, 9 with diffuse and 9 with limited forms, were sequentially recruited for this study from the Systemic Sclerosis Spectrum Outpatient Clinic at the Medical School Hospital of the São Paulo Federal University. The medical charts of all patients were reviewed to obtain age, sex, disease subtype, and disease duration.

Nailfold capillaries were analyzed by 16X magnification wide-field capillaroscopy concomitantly with blood sampling for E-selectin determination. For each patient the nailfolds of 4 fingers (excluding the thumb) on each

hand were determined. Capillary abnormalities were rated according to the protocol proposed by Andrade et al. (17) with emphasis on quantitative and semiquantitative assessment of capillary enlargement and devascularization. The total number of enlarged loops was divided by the number of examined fingers. Focal devascularization was rated according to the deletion score proposed by Lee et al. (18) as follows: grade 0 = no deletion areas, grade I = 1 or 2 discrete deletion areas, grade II = more than two discrete deletion areas, and grade III = extensive and confluent deletion areas. Deletion score was determined by the mean of the deletion grades obtained in the fingers.

Two parameters on capillaroscopy were used to correlate to serum E-selectin: deletion score (as described above) and ectasia (total number of enlarged loops per finger mean).

Serum samples were obtained and stored at -70°C until analysis. Circulating E-selectin levels were determined by a commercially available ELISA system (R & D, Oxford, UK), according to the manufacturer's protocol.

Data were analyzed statistically by the Student *t*-test for comparison of E-selectin levels in the diffuse and limited forms of the disease. Spearman's coefficient was used to assess possible correlations between the nailfold capillaroscopy parameters and serum E-selectin concentration. Two-tailed *P* values below 0.05 were considered significant. All analyses were carried out using the SPSS 8.0 software.

The study protocol was approved by the University Ethics Committee and all patients signed an informed consent document.

### Results

E-selectin range was 38 to 200 ng/ml (mean = 80 ± 39.94) and disease duration was 64.94 ± 61.57 months. We observed correlation between serum E-selectin levels and the deletion score capillaroscopic parameter (*r* = 0.50, *P* = 0.035; Figure 1A). This correlation was even stronger within the first 48 months (median of disease duration) of diagnosis (*r* = 0.63, *P* = 0.048; Figure 1B). On the other hand, no association was observed between selectin and ectasia. Patients with diffuse disease tended to present higher serum E-selectin levels than patients with limited disease, although the difference was not statistically significant. The same was observed when comparing patients with

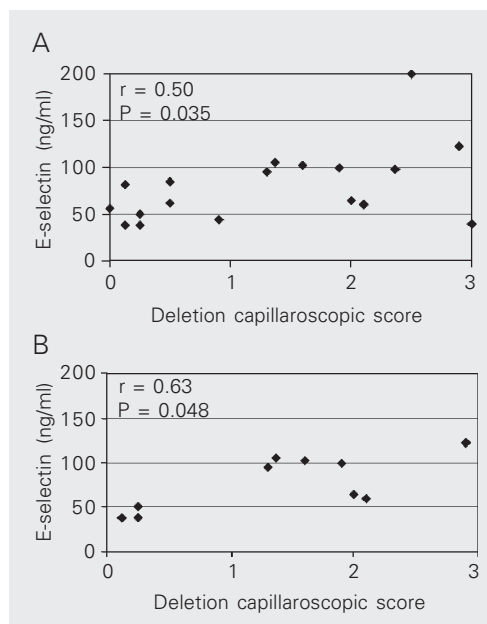


Figure 1. Correlation between E-selectin and the deletion capillaroscopic score in all patients (A) and in those within the first 48 months of diagnosis (B) (Spearman correlation).

Table 1. E-selectin and capillaroscopic parameters (ectasia and deletion score) between disease subset and disease duration.

	Limited (N = 9)	Diffuse (N = 9)	<i>P</i> value	≤48 months (N = 10)	>48 months (N = 8)	<i>P</i> value	All (N = 18)
Ectasia score	4.21 ± 2.99	4.64 ± 4.09	0.80	5.43 ± 3.61	3.15 ± 3.07	0.18	4.43 ± 3.48
Deletion score	0.93 ± 0.83	1.70 ± 1.08	0.11	1.38 ± 0.92	1.24 ± 1.19	0.78	1.31 ± 1.02
E-selectin (ng/dl)	63.56 ± 21.77	96.44 ± 48.04	0.08	112.33 ± 44.95	60 ± 22.73	0.06	80 ± 39.94

N = number of patients. Data are reported as means ± SD (Student *t*-test).

early (<48 months) and late disease (>48 months) (Table 1).

## Discussion

In this study we have determined the levels of E-selectin in serum samples from patients with systemic sclerosis to investigate a possible correlation with disease subset and with capillaroscopic abnormalities. Capillaroscopy has been reported to be useful not only for early diagnosis, but also for activity evaluation (18). Patients with diffuse disease tended to present higher serum E-selectin levels than those with limited disease, a fact that was more evident in patients within the first 48 months of disease. This result agrees with reports suggesting that this adhesion molecule could reflect disease severity in systemic sclerosis (4). This observation also agrees with the view that diffuse disease may be associated with extensive endothelial activation as compared to a restricted panorama in limited disease. If this is correct, the study of a larger number of patients would be expected to statistically demonstrate our observed trend in serum E-selectin levels between diffuse and limited systemic sclerosis.

The most specific and prevalent capillaroscopic abnormalities of the disease are ectasia and deletion. The deletion score was correlated to E-selectin levels, but ectasia was not, suggesting that E-selectin is associated with more severe disease.

An interesting observation was that the correlation between deletion capillaroscopic score and serum E-selectin levels increased from 50 to 63% when considering all patients and only patients within the first 4 years of diagnosis, respectively. This observation probably reflects the fact that elevated serum levels of soluble adhesion molecules are predominantly observed in the early inflammatory stages of systemic sclerosis (19,20). Since the capillaroscopic abnormalities of systemic sclerosis are structural, and therefore irreversible, in an advanced disease stage, patients with long-standing disease deletion might not present current endothelial inflammatory disease and may be considered to be merely a sequel of the disease. Thus, it seems reasonable that the capillaroscopic abnormalities observed in the first years of disease should be more closely correlated with current disease activity. In view of the variability of the data, it is very likely that a study with more patients would show statistical significance in the levels of selectin between the diffuse and limited forms of the disease. However, the present data are relevant because this is the first study showing a correlation between soluble serum E-selectin levels and alterations in capillaroscopy.

The stronger correlation with deletion score in capillaroscopy in early disease suggests that serum E-selectin levels might be a useful biochemical marker of disease activity in systemic sclerosis.

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