Nailfold Capillary Microscopy Can Suggest Pulmonary Disease Activity in Systemic Sclerosis

MARKUS BREDEMEIER, RICARDO MACHADO XAVIER, KARINA GATZ CAPOBIANCO, VICENTE GREGÓRIO RESTELLI, LUIS EDUARDO PAIM ROHDE, ANTÔNIO FERNANDO FURLAN PINOTTI, EDUARDO HENNEMANN PITREZ, MARCELO VASCONCELOS VIEIRA, MARIA ÂNGELA FONTOURA, DOLORES HELOÍSA de CAMPOS LUDWIG, and JOÃO CARLOS TAVARES BRENOL

ABSTRACT. Objectives. To evaluate the association of capillaroscopic alterations with pulmonary disease activity in systemic sclerosis (SSc).

Methods. Ninety-one patients with SSc were studied by means of interview, physical examination, nailfold capillary microscopy (NCM), serology, pulmonary function tests, esophageal transit scintigraphy, Doppler echocardiography, and pulmonary high resolution computed tomography (HRCT). Pulmonary disease activity was diagnosed by the observation of ground-glass opacities on pulmonary HRCT. Capillary loss on NCM was evaluated using the avascular score: patients with mean score ≥ 1 or mean number of megacapillaries per finger ≥ 1 were considered to have severe capillaroscopic alterations.

Results. Patients with higher skin scores, longer disease duration, signs of peripheral ischemia, esophageal dysfunction, antitopoisomerase I antibodies, and ground-glass opacities had higher mean avascular scores (p ≤ 0.05 in all tests). The association between ground-glass opacities and higher avascular scores was particularly strong in patients with disease duration ≤ 5 years. Among these patients, ground-glass opacities were present in 14 of 19 patients with severe NCM alterations, but were absent in all patients (n = 8) with mild or no NCM alterations (p < 0.001). ROC curves confirmed the ability of NCM to discriminate between patients with and without ground-glass opacities among those with disease duration ≤ 5 years. However, NCM could not predict the presence of reduced pulmonary diffusing capacity.

Conclusion. The severity of NCM abnormalities is associated with lung disease activity in SSc, particularly when the disease duration is relatively short. (J Rheumatol 2004;31:286–94)

Key Indexing Terms:
SYSTEMIC SCLEROSIS
PULMONARY HYPERTENSION
CAPILLAROSCOPY
FIBROSING ALVEOLITIS
COMPUTED TOMOGRAPHY

Systemic sclerosis (SSc) is a disease characterized by a variable degree of vascular dysfunction, skin and visceral fibrosis, and circulating autoantibodies. The associated lung disease (interstitial fibrosis or vascular disease) is currently the most important cause of mortality in SSc1.

Among the different tests to evaluate vascular dysfunc-

From the Divisions of Rheumatology, Cardiology, Radiology, Pneumology, and Nuclear Medicine, Hospital de Clínicas de Porto Alegre, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil.

M. Bredemeier, MD, MSc; R.M. Xavier, MD, PhD, Associate Professor of Rheumatology; K.G. Capobianco, MD, MSc, Division of Rheumatology; V.G. Restelli, Medical Student; L.E.P. Rohde, MD, PhD; A.F.F. Pinotti, MD, MSc, Division of Cardiology; E.H. Pitrez, MD; M.V. Vieira, MD, PhD, Division of Radiology; M.A. Fontoura, MD, Division of Pneumology; D.H.C. Ludwig, MD, Division of Nuclear Medicine; J.C.T. Brenol, MD, PhD, Associate Professor of Rheumatology, Head, Division of Rheumatology.

Supported in part by grants from Fundo de Incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre (FIFE/HCPA).

Address reprint requests to Dr. M. Bredemeier, Serviço de Reumatologia do Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350/sala 645, Porto Alegre, RS, 90035-003, Brazil. E-mail: markbred@terra.com.br

duration seems to be an important determinant for the severity of capillaroscopic abnormalities in SSc\(^\text{17,23-25}\), and to diminish the influence of the accumulation of vascular damage in the interpretation of the alterations observed in NCM, some analyses were additionally performed in a subgroup of patients with disease duration \(\leq 5\) years.

**MATERIALS AND METHODS**

**Patients.** We prospectively studied 98 patients with definite or strongly suspected SSc (by evaluation of experienced rheumatologists). All patients were Brazilian and the great majority inhabitants of the urban area of Porto Alegre/RS. To be included, the patient was required to meet the American College of Rheumatology (ACR) criteria for SSc\(^\text{24}\) or the criteria suggested by LeRoy and Medsger\(^\text{25}\) for diagnosis of early forms of SSc: i.e., objective evidence of Raynaud’s phenomenon (RP) plus SD pattern on NCM or SSc selective autoantibodies; alternatively, subjective evidence of RP plus SD pattern on NCM and SSc selective autoantibodies. Patients with overlapping syndromes, acute chronic or acute infections, insulin dependent diabetes mellitus, or with long-standing diabetes (> 5 years since diagnosis) were excluded. However, patients with definite diagnosis of SSc (ACR criteria) who developed inflammatory myopathy were not excluded from the analysis. All patients signed written informed consent. The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre.

**Clinical evaluation.** All patients were interviewed and examined by the same researcher (MB), according to an extensive questionnaire directed to the evaluation of end-organ damage. The severity and extent of cutaneous disease was quantified using the modified Rodman skin score\(^\text{26}\). The severity of sclerodactyly was assessed according to the following scale: 0, normal skin; 1, mild skin thickening; 2, skin thickened and unable to be pinched; 3, skin thickened and unable to be moved\(^\text{26}\). Dyspnea was graded according to the New York Heart Association scale. Disease subtype was classified as follows: diffuse cutaneous SSc (truncal and acral skin tautness); limited cutaneous SSc (skin tautness restricted to extremities and/or face); and limited SSc (absence of skin tautness)\(^\text{25,27}\). Proximal sclerodermia was defined as skin thickening affecting face or regions proximal to metacarpophalangeal or metatarsophalangeal joints. Duration of the disease was defined as the time interval since the onset of either RP or skin symptoms (whatever came first) until the day of clinical evaluation. The presence of digital pitting scars (depressed areas at the tips of digits as a result of ischemia rather than trauma or exogenous causes), telangiectases (visible macular dilations of superficial blood vessels that collapse under pressure and fill slowly when pressure is released), and calcinosis (subcutaneous calcifications detected by inspection, palpation, radiograph) was observed and recorded.

**Nailfold capillary microscopy.** NCM was performed 14 days (on average) after the initial evaluation, under standard conditions (after a 20-min rest or longer, with room temperature 20–25°C). Blood samples were collected immediately after NCM for complete blood count, serology, erythrocyte sedimentation rate (ESR), and biochemistry. Anemia was defined as a hemoglobin concentration \(\leq 12\) g/dl for women and \(\leq 13.5\) g/dl for men. An ESR greater than the age divided by 2 for men, or age divided by 2 plus 10 for women, was considered elevated.

Digits 2, 3, 4, and 5 of both hands were examined with a stereoscopic Stemi-2000 C microscope (Zeiss) with \(6.5–65\times\) magnification. Digits on which the capillary bed was not well visualized were excluded from the analysis. Patients had at least 4 fingers examined (median 8 fingers, percentiles 25–75 = 7–8), with the exception of 2 patients who had one and 2 fingers each examined due to severe hand contractures and/or finger amputations. Incident lighting at \(45°\) was provided by a cold light source and a fiber optic illuminator. Cedar oil was deposited over the skin to allow visualization of the capillary bed\(^\text{28}\). At least 2 digits of each patient were photographed using a Contax 167MT camera attached to the microscope.

All capillaroscopic examinations were performed by an experienced observer (KGC), who was blinded to the results of other tests and clinical data. The evaluation followed a protocol based on the model proposed by Andrade, et al\(^\text{29}\). The severity of capillary loss was evaluated on each digit according to the score described by Lee, et al (0, no avascular areas; 1, one or 2 discrete areas of vascular deletion; 2, > 2 discrete areas of vascular deletion; 3, large confluent avascular areas)\(^\text{17}\). A discrete avascular area is defined by the loss of > 2 consecutive capillary loops in the distal capillary bed. The mean avascular score was calculated by dividing the sum of the scores by the number of digits examined. The number of megacapillaries (giant capillary loops, about 10 or more times the diameter of normal loops), ectatic loops (capillaries about 4 or more times the diameter of normal loops), and capillary hemorrhages counted in each digit were individually summed and divided by the number of digits examined, providing the mean number of each of these abnormalities per finger\(^\text{17,25}\). The presence of the SD pattern was defined qualitatively by the observation of avascular areas and/or dilated capillaries according to the description of Maricq, et al\(^\text{7}\).

Considering that the NCM abnormalities in SSc are represented by variable degrees of avascularity and capillary dilatation, and that avascular areas seem to originate mostly from the rupture and obliteration of capillaries that developed progressive enlargement\(^\text{19}\), capillary deletion and dilatation were incorporated into a combined severity score. This score was determined by the largest numerical value among the following 2 variables: mean avascular score or mean number of megacapillaries per finger (3.0 being the maximal value). For analytical purposes, patients with a combined severity score \(\geq 1\) were considered to have severe capillaroscopic alterations.

**Intraobserver agreement for the capillaroscopic variables was tested using the data of 7 patients (50 fingers) who had repeated NCM within 6 months (mean interval 106 days, range 44–177 days) and had had no major clinical events during that period.** There was good agreement between the 2 observations for the avascular score (Fleiss weighted kappa = 0.66) and moderate agreement for the number of megacapillaries (Fleiss weighted kappa = 0.53). Interobserver agreement for the capillaroscopic variables was tested (blindly for identification and clinical status) using the pictures of 56 patients and 11 controls (one finger per individual). The scores obtained by direct observation were compared to the scores obtained through the inspection of the pictures by another observer (MB), also showing good agreement between observations for the avascular score (Fleiss weighted kappa = 0.65) and moderate agreement for the number of megacapillaries (Fleiss weighted kappa = 0.55).

**Serology.** The presence of antinuclear antibodies (ANA) and antitopoiso- merase I antibodies was tested by the indirect immunefluorescence method (HEP-2 cells as substrate) and enzyme immunoassay (Quanta lite\(^\text{30}\) Scl-70 ELISA, Inova Diagnostics, San Diego, CA, USA), respectively. ANA was considered positive in titers \(\geq 1:80\). The presence of antitopomerase antibodies was determined based on the typical fluorescence pattern on HEP-2 cells.

**Pulmonary HRCT.** The scans were obtained with a Somatom Plus 4 (Siemens) scanner with no use of intravenous contrast, during breathing after deep inspiration in supine position. The interval between sections (varying between 10 and 30 mm) was determined by the radiologist who executed the examination, using a 1.0 mm collimation. The scans were viewed at window level appropriate for pulmonary parenchyma and pleura. All HRCT scans were assessed for the presence and extent of interstitial disease (ground-glass opacities, reticular pattern, and honeycombing) by 2 radiologists. They estimated, in consensus, the percentage (to the nearest 5%) of parenchyma affected by interstitial disease in each lung, according to the method proposed by Staples, et al\(^\text{31}\). The overall percentage of interstitial disease represents the mean value of both lungs.

**Pulmonary function tests.** Patients underwent spirometry and carbon monoxide diffusing capacity test (DLCO), Forced vital capacity (FVC)
maneuvers were performed as recommended by the American Thoracic Society, with the best FVC obtained in 3 efforts being used for analysis. DLCO measurement was performed by the single-breath technique, with values corrected for hemoglobin concentration. Data were expressed as percentages of the predicted values for healthy adults of the same age and body surface area. FVC and DLCO were considered reduced when < 80% and < 75% of the predicted values, respectively.

Doppler echocardiography. The pulmonary systolic arterial pressure was calculated by adding the transtricuspid pressure gradient to the estimated inferior vena cava pressure. Patients with pulmonary systolic arterial pressure ≥ 40 mm Hg were considered to have pulmonary arterial hypertension.

Esophageal transit scintigraphy. This examination was performed with patients in the supine position under a large field of view gamma camera. Patients received 6 ml of water labeled with 1 mCi 99mTc-phytate. Computer acquisition at the rate of 1 second per frame was initiated and the patients were asked to ingest the bolus by a single deglutition. Residual activity ≥ 20% (in relation to peak activity) at 15 s after beginning of the swallow was considered abnormal and indicative of esophageal dysfunction.

All clinical and capillaroscopic evaluations were performed between April 2000 and November 2001. The radiological, echocardiographic, scintigraphic, and pulmonary function tests were performed within 6 months of NCM. No observer received information on the patients’ clinical details or other results.

Statistical analysis. Data were analyzed using Epi-Info version 6, SPSS for Windows version 6, and Medcalc version 6. The association between categorical variables was tested using Yates’ corrected chi-square or Fisher’s exact tests. Quantitative variables were analyzed graphically (with normal probability plots) and statistically (with Kolmogorov-Smirnov goodness-of-fit test) for the normality of its distribution. The variable mean avascular score (MAS), which showed positive skewness, was submitted to logarithmic transformation (log10[MAS + 1]) to allow the use of parametric tests (Student t test and analysis of variance), presented as geometric mean and 95% confidence interval. When heterogeneity of variances was detected (by Levene’s test) or variables presented non-normal distributions, nonparametric tests were applied (Mann-Whitney and Spearman correlation coefficient, rs). Continuous variables with non-normal distributions were presented as median and interquartile range (IQR). Analysis of covariance was used to control for confounding variables. Subgroup analyses were performed in patients with disease duration ≤ 5 years. A p value ≤ 0.05 was considered statistically significant. All p values are 2-tailed.

Receiver operating characteristic (ROC) curves were used to test the ability of NCM to differentiate patients with and without ground-glass opacities on HRCT. The area under the ROC curve is a suitable measure to summarize the discrimination power of a diagnostic model (representing the accuracy of the model) and can range from 0.5 (no discrimination) to 1.0 (perfect discrimination). Ninety-five percent confidence intervals for the areas under the curves were also calculated.

Models of multiple linear regression were designed to test the simultaneous association of several variables with the mean avascular score (after logarithmic transformation). The selection of independent variables of the model was based on the following criteria: clinical significance of the variable (capacity of the variable in representing the severity of a specific aspect of the disease), strength of the bivariate association with the dependent variable; improvement ≥ 3% of the coefficient of determination after inclusion of the variable in the model; and keeping a statistically significant association with the dependent variable in the model. The adequacy of assumptions of multiple linear regression models (normal distribution of the dependent variable, linear association of the independent variables with the dependent variable, homogeneity of variances of the dependent variable, normal distribution of residual values, and absence of interactions) was tested and met.

RESULTS

Four out of the 98 patients initially evaluated were excluded from the study for not fulfilling the entry criteria or for meeting exclusion criteria. Three other patients did not undergo NCM (one unable to perform the examination because of finger contractures; one died a few days after the initial evaluation; and the third patient failed to return for NCM). A description of the 91 patients analyzed (classified according to fulfillment of ACR criteria for SSc and disease subtype) is given in Table 1. Thirty patients had a disease duration ≤ 5 years; of these, 24 were positive for the ACR criteria, 9 had diffuse cutaneous disease, and 6 presented antitopoisomerase I antibodies.

Comparison of mean avascular scores (MAS) according to clinical features is given in Table 2. There was a significant correlation of MAS with the total skin score (rs = 0.59, p < 0.001), severity of sclerodactyly (rs = 0.68, p < 0.001), time since onset of RP (rs = 0.35, p = 0.001), and age (rs = 0.35, p = 0.001).

The association between the severity of avascularity and alterations in complementary examinations is shown in Table 3. Given that the time since onset of RP (duration of RP) is a variable correlated to the mean avascular score and to end-organ damage, representing a possible confounding factor in the association of disease severity with capillaroscopic abnormalities, the p values in Table 3 were presented with and without adjustment for the duration of RP. Antitopoisomerase I antibodies and pulmonary ground-glass opacities on HRCT (representing areas of active pulmonary disease) were positively associated with the mean avascular score, particularly after adjustment for duration of RP, suggesting more severe avascularity in these patients. The observed tendency for association of anticientromere antibodies with greater avascular scores disappeared after adjustment for the duration of RP.

The mean avascular score tended to be correlated with the extent of interstitial disease in the entire patient sample (rs = 0.19, p = 0.083, n = 86). Among patients with disease duration ≤ 5 years, there was a marginally significant correlation between the mean avascular score and the extent of interstitial disease (rs = 0.40, p = 0.040, n = 27).

Table 4 shows a multiple linear regression model having as dependent variable the mean avascular score (MAS), after logarithmic transformation. According to this model, total skin score, duration of RP, presence of digital pitting scars or finger amputations, pulmonary ground-glass opacities on HRCT, and age ≥ 50 years were independently associated with the MAS. The variable severity of sclerodactyly lost its statistical significance and relevance in the model after adjustment for the influence of other variables. The model suggests that about 60% of the MAS variance can be predicted by this group of variables. The application of this model restricted to patients with disease duration ≤ 5 years shows that the presence of pulmonary ground-glass
Similar analyses were also performed making use of the mean number of megacapillaries, ectatic loops, and hemorrhages per digit as dependent variables. Patients with anticientromere antibodies presented a higher number of megacapillaries per finger in comparison to other patients (median 0.9, IQR 0.3–2.2, n = 36 vs median 0.4, IQR 0–0.7, p < 0.001).

Table 1. Clinical and demographic characteristics of patients according to ACR criteria for SSc (patients designated ACR positive or negative). Data are presented as number (%) of patients, except when indicated otherwise.

<table>
<thead>
<tr>
<th>Clinical Abnormalities</th>
<th>ACR Positive, n = 22</th>
<th>ACR Negative, n = 54*</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs, mean ± SD</td>
<td>48.8 ± 11.1</td>
<td>52.5 ± 12.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>7 (2.0–13.5)</td>
<td>10 (4.7–23.5)</td>
<td>0.09</td>
</tr>
<tr>
<td>Sclerodactyly</td>
<td>22 (100)</td>
<td>49 (90.7)</td>
<td>0.00</td>
</tr>
<tr>
<td>Digital pitting scars or loss of digital pad tissue</td>
<td>20 (90.9)</td>
<td>42 (77.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>Proximal scleroderma</td>
<td>22 (100)</td>
<td>50 (92.6)</td>
<td>0.00</td>
</tr>
<tr>
<td>Calcinosis</td>
<td>5 (22.7)</td>
<td>20 (37.0)</td>
<td>0.00</td>
</tr>
<tr>
<td>Telangiectases</td>
<td>13 (59.1)</td>
<td>30 (55.6)</td>
<td>0.05</td>
</tr>
<tr>
<td>Finger amputations</td>
<td>3 (13.6)</td>
<td>8 (14.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>Puffy hands</td>
<td>2 (9.1)</td>
<td>7 (13.0)</td>
<td>0.00</td>
</tr>
<tr>
<td>SD pattern on NCM</td>
<td>20 (90.9)</td>
<td>53 (98.1)</td>
<td>0.00</td>
</tr>
<tr>
<td>ANA ≥ 1:80</td>
<td>19 (86.4)</td>
<td>48 (88.9)</td>
<td>0.00</td>
</tr>
<tr>
<td>Anticientromere antibodies***</td>
<td>4/21 (19.0)</td>
<td>29/52 (55.8)</td>
<td>0.00</td>
</tr>
<tr>
<td>Anti-Scl-70 antibodies***</td>
<td>6/21 (28.6)</td>
<td>10/52 (19.2)</td>
<td>0.00</td>
</tr>
<tr>
<td>Ground-glass opacities on HRCT***</td>
<td>13/20 (65.0)</td>
<td>24/51 (47.1)</td>
<td>0.00</td>
</tr>
<tr>
<td>Reticular pattern or honeycombing on HRCT***</td>
<td>9/20 (45.0)</td>
<td>20/51 (39.2)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* 53 patients with limited cutaneous SSc and one with limited SSc. ** 2 patients with limited cutaneous SSc and 13 with limited SSc. *** Data not available for all patients; values represent number of patients with the indicated abnormalities over the number of patients that had the respective complementary examination, with percentages given in parentheses. IQR: interquartile range, ANA: antinuclear antibody, NCM: nailfold capillary microscopy, HRCT: high resolution computed tomography, FVC: forced vital capacity.

* Student t test, applied after logarithmic transformation of the MAS (log10 [MAS + 1]). † Mann-Whitney test. RP: Raynaud’s phenomenon.

Table 2. Comparison of the mean avascular scores (MAS) according to the presence of clinical abnormalities. Mean (95% CI) represents the geometric mean of the MAS and 95% confidence interval.

<table>
<thead>
<tr>
<th>Clinical Abnormalities</th>
<th>Present, mean (95% CI)</th>
<th>Absent, mean (95% CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 50 yrs</td>
<td>1.63 (1.42–1.86)</td>
<td>1.10 (0.92–1.29)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Digital pitting scars</td>
<td>1.68 (1.48–1.89)</td>
<td>1.03 (0.86–1.22)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Loss of digital pad tissue</td>
<td>1.51 (1.30–1.75)</td>
<td>1.08 (0.92–1.24)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Finger amputations</td>
<td>2.09 (1.63–2.62)</td>
<td>1.25 (1.02–1.70)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calcinosis</td>
<td>1.72 (1.38–2.10)</td>
<td>1.21 (1.05–1.37)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Telangiectases</td>
<td>1.60 (1.37–1.84)</td>
<td>1.09 (0.91–1.27)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Sclerodactyly</td>
<td>1.46 (1.29–1.65)</td>
<td>0.89 (0.68–1.11)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Proximal scleroderma</td>
<td>1.49 (1.32–1.68)</td>
<td>0.83 (0.65–1.02)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Skin score ≥ 16</td>
<td>1.56 (1.33–1.81)</td>
<td>1.16 (0.98–1.36)</td>
<td>0.009</td>
</tr>
<tr>
<td>Duration of RP ≥ 10 yrs</td>
<td>1.43 (1.30–1.63)</td>
<td>0.80 (0.59–1.04)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Student t test, applied after logarithmic transformation of the MAS (log10 [MAS + 1]). † Mann-Whitney test. RP: Raynaud’s phenomenon.
Table 3. Comparison of the mean avascular scores (MAS) according to the presence of abnormalities in complementary examinations. Mean (95% CI) represents the geometric mean of the MAS and 95% confidence interval.

<table>
<thead>
<tr>
<th>Abnormalities</th>
<th>Present, mean (95% CI)</th>
<th>Absent, mean (95% CI)</th>
<th>p*</th>
<th>Adjusted p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>1.60 (1.21–2.05)</td>
<td>1.26 (1.11–1.43)</td>
<td>0.073</td>
<td>0.107</td>
</tr>
<tr>
<td>Elevated ESR</td>
<td>1.31 (0.97–1.70)</td>
<td>1.35 (1.19–1.52)</td>
<td>0.801</td>
<td></td>
</tr>
<tr>
<td>ANA ≥ 1:80</td>
<td>1.43 (1.28–1.59)</td>
<td>0.88 (0.51–1.33)</td>
<td>0.004</td>
<td>0.007</td>
</tr>
<tr>
<td>Anticentromere antibodies</td>
<td>1.49 (1.27–1.73)</td>
<td>1.21 (1.01–1.42)</td>
<td>0.076</td>
<td>0.479</td>
</tr>
<tr>
<td>Antitopoisomerase I antibodies</td>
<td>1.73 (1.39–2.11)</td>
<td>1.23 (1.07–1.40)</td>
<td>0.016</td>
<td>0.003</td>
</tr>
<tr>
<td>Reduced FVC</td>
<td>1.75 (1.25–1.67)</td>
<td>1.26 (1.06–1.48)</td>
<td>0.224</td>
<td></td>
</tr>
<tr>
<td>Reduced DLCO</td>
<td>1.37 (1.21–1.55)</td>
<td>1.10 (0.74–1.54)</td>
<td>0.164</td>
<td></td>
</tr>
<tr>
<td>Esophageal dysfunction</td>
<td>1.42 (1.26–1.59)</td>
<td>1.03 (0.71–1.42)</td>
<td>0.026</td>
<td>0.038</td>
</tr>
<tr>
<td>Ground-glass opacities</td>
<td>1.53 (1.32–1.75)</td>
<td>1.20 (0.99–1.44)</td>
<td>0.040</td>
<td>0.002</td>
</tr>
<tr>
<td>Reticular pattern or honeycombing</td>
<td>1.50 (1.26–1.77)</td>
<td>1.28 (1.09–1.49)</td>
<td>0.191</td>
<td></td>
</tr>
<tr>
<td>Pulmonary arterial hypertension</td>
<td>1.62 (1.24–2.05)</td>
<td>1.29 (1.13–1.46)</td>
<td>0.162</td>
<td></td>
</tr>
</tbody>
</table>

* Student t test, applied after logarithmic transformation of the MAS (log_{10} [MAS + 1]). ** Analysis of covariance, p value adjusted for Raynaud’s phenomenon duration (applied only when some statistical trend was found in bivariate analysis).

Comparing the prevalence of active pulmonary disease according to the severity of capillaroscopic abnormalities (definition based on a categorization of the combined severity score), 41 out of 73 patients with severe capillaroscopic alterations had ground-glass opacities, in comparison with 2 of 13 patients with mild or no abnormalities (p = 0.016). The 2 patients with ground-glass opacities and mild or no capillaroscopic abnormalities had disease duration > 10 years and had < 5% of lung parenchyma affected by interstitial lung disease on HRCT. Pulmonary arterial hypertension was present in patients with severe NCM abnormalities (12 of 74 patients), but was absent in 16 patients with mild or no capillaroscopic abnormalities (p = 0.114).

Among patients with disease duration ≤ 5 years, 14 out of 19 patients with severe capillaroscopic abnormalities had ground-glass opacities, compared with none of 8 patients.
with mild or no capillaroscopic alterations (p < 0.001). In this subgroup, the sensitivity of severe NCM alterations for ground-glass opacities was 100% (95% CI 74.7–100) with a specificity of 61.5% (95% CI 32.3–84.9). Only 2 patients in the subgroup with disease duration ≤ 5 years presented pulmonary arterial hypertension, and both had severe capillaroscopic abnormalities.

Considering that the selection of cutoff points was rather arbitrary, additional tests on the discriminatory ability of the combined severity score were performed using ROC curves. Studying the entire sample of patients, this score showed a weak discriminatory capacity (area under the curve = 0.61, 95% CI 0.50–0.71) for the presence of ground-glass opacities. However, in patients with disease duration ≤ 5 years, the area under the ROC curve was 0.83 (95% CI 0.63–0.94) for ground-glass opacities, suggesting a good discriminatory capacity of the combined severity score (Figure 1A). In this same subgroup of patients, the mean avascular score also showed good discriminatory ability for the presence of ground-glass opacities, with area under the ROC curve = 0.81 (95% CI 0.61–0.93) (Figure 1B). However, the MAS showed less sensitivity (but more specificity) than the combined severity score, although there was no statistical difference between the curves.

The discriminatory ability of other clinical variables (total skin score, fulfillment of ACR criteria, diffuse form of disease, presence of proximal scleroderma, presence and severity of dyspnea and pulmonary crepitations, pulmonary diffusing capacity, and antitopoisomerase I antibodies) was also tested in the subgroup with disease duration ≤ 5 years. None of these variables showed discriminatory capacity for ground-glass opacities on HRCT.

**DISCUSSION**

The results show a relatively strong association between capillaroscopic alterations, especially avascularity, and the extent of cutaneous lesions in SSc, as previously observed. The duration of RP was also independently associated with the severity of avascularity. This finding, corroborated by some studies, suggests that the prolonged and continuous vascular damage associated with SSc is an important determinant of avascularity on NCM. Since age has also been found to have an independent association with the avascularity score, it is possible that the sensitivity to vascular damage increases with aging.

Findings related to the presence of autoantibodies indicate that these may be important factors associated with qualitative and quantitative capillaroscopic alterations. Studies have associated the presence of anticentromere antibodies to a capillaroscopic pattern with predominance of capillary dilatation, sometimes called the “slow” SD pattern because of the limited modification of the nailfold capillary bed in serial examinations. These findings are supported by our results, showing a greater number of megacapillaries in patients with anticentromere antibodies. However, a study of serial photographic analysis by Wong, et al has suggested that avascular areas are usually derived from the rupture, extravasation, and obliteration of capillaries that have previously suffered progressive dilatation. Considered together, these observations indicate that the evolution of the vascular damage occurs in a slow manner in patients with anticentromere antibodies, with megacapillaries having a longer lifetime before obstruction, or even persisting indefinitely without obliteration, thus providing the observation of a greater number of these abnormalities. The pres-

![Figure 1. ROC curves of the combined severity score (A) and mean avascular score (B) for the presence of ground-glass opacities in patients with disease duration ≤ 5 years.](www.jrheum.org.)
ence of antitopoisomerase I was significantly associated with the avascular score, reproducing results obtained by Schmidt and Mensing[5]. There are no clear explanations for this phenomenon, but the association of antitopoisomerase I with more severe skin and visceral damage may represent more accentuated and early vascular involvement.

Although some studies suggest an association of severity of capillary dilatation or avascularity on NCM with visceral damage in SSc[4,8,16], others failed to reproduce these findings[17,18,22]. These variable results are probably related to different patient characteristics and different quantification methods for clinical and capillaroscopic abnormalities. As far as we are aware, only one study attempted to find an association, in a secondary analysis, of measures of pulmonary disease activity and capillaroscopic alterations. Silver, et al[40] performed bronchoalveolar lavage (BAL) in patients with SSc, and observed that patients with inflammatory BAL tended to have a greater prevalence of the SD pattern on NCM than patients with noninflammatory BAL (8/8 vs 4/7 patients, respectively; p = 0.077). However, the number of patients was too small to permit conclusive results.

In our study, the use of pulmonary HRCT allowed evaluation of the presence, extent, and activity of interstitial lung disease in a relatively large sample of patients with SSc. On HRCT, ground-glass opacity areas represent regions of active inflammatory infiltrate on lung biopsy[41] that may evolve to a reticular pattern or honeycombing that almost always represents irreversible lung fibrosis[20]. Through multivariate analysis, an association between active lung disease and higher mean avascular scores was observed, even after adjustment for several potential confounding variables (skin score, duration of RP, signs of peripheral ischemia, and age). The results also suggest that the severity of nailfold capillaroscopic alterations may have discriminatory capacity in evaluation of the activity of pulmonary disease in patients with relatively short duration SSc. No other single variable (derived from clinical examination or complementary examinations) was comparable to nailfold capillary microscopy in this context.

The association of NCM abnormalities with the severity of skin disease and activity of pulmonary disease may be explained by the pathogenesis of SSc. Systemic microvascular damage, along with abnormal immune system activation, seems to be an important factor subjacent to fibrosis in skin and internal organs. The primary sites of vascular dysfunction are the endothelial cells, which show evidence of injury and activation. Activated endothelial cells release vasoconstrictor substances and activate platelets, which in turn secrete substances such as platelet derived growth factor and transforming growth factor-β that stimulate fibroblasts and promote collagen production, leading to tissue fibrosis[42]. Considering that NCM permits direct visualization of alterations that reflect systemic microvascular damage, an association between disease activity and capillaroscopic abnormalities seems to be possible and plausible.

One might ask why NCM could have good discriminatory ability for pulmonary disease activity only in patients with short disease duration. As shown by this and other studies, the disease duration is an independent predictor of capillary loss, probably meaning that even patients with mild or slowly-evolving (but long-standing) forms of SSc may develop severe avascularity after many years of disease. On the other hand, an elevated number of giant capillaries in patients with short disease duration may represent a fast evolution of capillary loss (and more aggressive disease) among these patients, considering that avascular areas seem to originate mainly from the obliteration of capillaries that became progressively enlarged. However, in patients with long-standing disease, an elevated number of giant capillaries may be related to a slow evolution of capillaroscopic and clinical abnormalities. These factors can be associated with a reduction in the specificity of NCM for pulmonary disease activity in patients with long-standing disease.

Nailfold capillary microscopy abnormalities were not significantly associated with pulmonary function measure. The presence of a restrictive pattern and a reduced diffusing capacity may represent early forms of pulmonary interstitial and/or vascular disease, and can be present in patients with mild or no alterations on HRCT scans and with pulmonary arterial pressure within the normal range. Keeping in mind that HRCT findings may sometimes be normal in patients with active fibrosing alveolitis detected by other methods (especially bronchoalveolar lavage)[43,44], it is possible that some patients with active pulmonary disease have been misclassified.

The lack of associations of the mean avascular score with abnormalities on lung function tests and with the presence of honeycombing, but positive association with ground-glass opacities, may also represent the absence (or reduction) of ongoing vascular damage in patients with inactive pulmonary interstitial disease. This raises the possibility that once the disease becomes less active, there might be an improvement in capillaroscopic abnormalities. This matter should be addressed by a specific study, although there is already some evidence in this direction. Filaci, et al[45] observed an improvement in capillary loss and disorganization parallel to skin and esophageal disease improvement after treatment with cyclosporine and intravenous iloprost. Normalization of capillaroscopy (even after the observation of extensive avascular areas) has been observed in patients with dermatomyositis that have had complete remission of disease[46].

Considering the results in our study and others, it can be concluded that the extent of nailfold capillary microscopy abnormalities, besides being associated with the severity, duration, and activity of systemic sclerosis, may indicate the
The presence of active pulmonary disease in patients with relatively short disease duration. Also considering that the presence of active pulmonary disease may predict a good response to immunosuppressive treatment, the results suggest that NCM can help in the selection of patients that need more extensive evaluation and more aggressive therapeutic interventions.

ACKNOWLEDGMENT

We acknowledge Drs. Flávio Dani Fuchs, Lenita Wannmacher, Sérgio Saldanha Menna Barreto, Raquel Facconieri, Marcus Franck, Tatiana Freitas Tourinho, Marcelo Maltchick, Paulo Sérgio Thys, Patrícia Minuzzi da Motta, Max Brenner, Tamara Mucenic, Adriano Barbiero, Tatiana Karenini Müller, Carmen Both Schenatto, Charles Lubianca Kohem, Lilian Scussel-Lonzenetti, Claiton Viegas Brenol, Sandra Helena Machado, and Ilôite Scheibel on their valuable support. We also thank Eliane Regina Moreira Pereira, Janie Pires dos Santos, Leila Krammer, Lorena Koglin, Denilson dos Santos Marques, Rossimar Rocha, Andréia da Silva Ramiro, Leticia Souza Bisotto, Marcello Baquero, and Juliana Bredemeier.

REFERENCES