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Autoantibodies and Microvascular Damage Are Independent Predictive Factors for the Progression of Raynaud's Phenomenon to Systemic Sclerosis

A Twenty-Year Prospective Study of 586 Patients, With Validation of Proposed Criteria for Early Systemic Sclerosis

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Objective. **To identify in patients with Raynaud's phenomenon (RP) independent markers that predict progression to definite systemic sclerosis (SSc) and to determine in patients with progression to SSc the type and sequence of microvascular damage and its relationship to SSc-specific autoantibodies.**

Methods. **Consecutive patients referred for evaluation of RP who had no definite connective tissue disease were evaluated for microvascular damage by nailfold capillary microscopy (NCM) and for anticentromere (anti–CENP-B), anti-Th/To, anti–topoisomerase I, and anti–RNA polymerase III (anti–RNAP III) autoantibodies by specific assays. Patients were studied prospectively.**

Results. **Of the 586 patients who were followed up for 3,197 person-years, 74 (12.6%) developed definite SSc. A characteristic sequence of microvascular damage was identified, starting with enlarged capillaries, followed by capillary loss, and then by capillary telangiectases. Definite SSc was diagnosed in close temporal relationship to capillary loss. Enlarged capillaries, capillary loss, and SSc-specific autoantibodies independently predicted definite SSc. Anti–CENP-B and anti-Th/To antibodies predicted enlarged capillaries; these autoantibodies and anti–RNAP III predicted capillary loss. Each autoantibody was associated with a distinct time course of microvascular damage. At followup, 79.5% of patients with 1 of these autoantibodies and abnormal findings on NCM at baseline had developed definite SSc. Patients with both baseline predictors were 60 times more likely to develop definite SSc. The data validated the proposed criteria for early SSc.**

Conclusion. **In RP evolving to definite SSc, microvascular damage is dynamic and sequential, while SSc-specific autoantibodies are associated with the course and type of capillary abnormalities. Abnormal findings on NCM at baseline together with an SSc-specific autoantibody indicate a very high probability of devel-**

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oping definite SSc, whereas their absence rules out this outcome.

Raynaud's phenomenon (RP) is the clinical reflection of diffuse microvascular damage in systemic sclerosis (SSc) (1–4). Occurring in more than 90% of patients, RP often precedes skin and visceral fibrosis by years or decades (2). Hence, at initial presentation of RP, there is a need for novel predictors to help physicians in identifying the patients who are at risk of developing SSc or another connective tissue disease (CTD) (5–22).

Thirty years ago, Maricq et al (23) reported that nailfold capillary microscopy (NCM) was a useful noninvasive method of visualizing microangiopathic events in SSc (23). An abnormal NCM profile specific for SSc, consisting of enlarged capillaries and capillary loss, was identified (24). The presence of this profile in patients with isolated RP predicted the future evolution to SSc (3,14). We have also shown that the inclusion of severe NCM abnormalities markedly increased the sensitivity of the American College of Rheumatology (ACR) classification criteria for limited cutaneous SSc (25). However, it is still unclear whether enlarged capillaries and capillary loss are independent predictors of SSc in patients with isolated RP. Moreover, there are no prospective NCM data from patients with isolated RP progressing to SSc, and therefore, the pathophysiologic sequence of microvascular damage is unknown.

SSc is also characterized by serum autoantibodies, including anticentromere (anti–CENP-B), anti-Th/To, anti–topoisomerase I (anti–topo I), and anti-RNA polymerase I/III (anti–RNAP III) (26). Together, these markers account for ~85% of autoantibodies specific for SSc (26). Anti–CENP-B and anti–topo I are known predictors of progression from isolated RP to SSc (14). However, previous studies were limited by their small sample sizes, incorrect classification of patients with CTD manifestations as having primary RP, use of varying definitions of patient subsets, lack of standardized methods for determining antinuclear antibodies (ANAs), omission of tests for anti-Th/To and anti–RNAP III antibodies, and absence of multivariable analyses. Furthermore, whether SSc autoantibodies are linked to the course and type of microvascular damage by NCM has not been determined prospectively.

LeRoy and Medsger (21) proposed that patients with RP who had abnormal findings on NCM and an SSc-specific autoantibody be classified as having early SSc. However, that set of criteria has not yet been validated (21).

Hence, over a period of 20 years, we prospectively studied a large cohort of patients who were referred to a single center for evaluation of RP. Our objectives were to identify the strongest independent predictors of progression to definite SSc, to determine the type and time course of microvascular damage by NCM and its relationship to major SSc autoantibodies, and to validate the criteria for early SSc.

PATIENTS AND METHODS

Patients. All consecutive adult patients (n = 784) referred by their treating physicians to the Vascular Medicine and Connective Tissue Diseases Clinics, Centre Hospitalier de l'Université de Montréal (CHUM), between April 7, 1984 and September 22, 1999, for evaluation of RP were included. Primary care physicians accounted for 93.4% of referrals. Participants were studied prospectively until February 2005. RP was defined as a history of at least 2 of 3 phases of color change (white, blue, red), usually induced by cold exposure, and involving at least 1 finger of each hand (21,27–29). Using this definition, 356 patients had triphasic RP, and 428 had biphasic RP, with 92% of the latter group having a white phase. In all patients, RP was induced by exposure to cold temperatures.

Patients were excluded if they had an identifiable cause of RP at baseline (i.e., first evaluation by us), including definite SSc, dermatomyositis, Sjögren's syndrome, or another definite CTD (see below). None of the patients had ever received disease-modifying drugs. Recruited patients were invited for a return visit at 6 months and an annual visit thereafter. The project was approved by the CHUM Research and Ethics Committees.

Evaluation at baseline and followup visits. A medical history was obtained and a physical examination was performed according to a standard protocol. A modified Rodnan skin score based on the degree of skin binding/tethering (scored on a scale of 0–4) was determined (29,30). Objective clinical signs included puffy fingers, digital ulcers, pitting scars, loss of distal finger pad, clinically visible capillary telangiectases, calcinosis, and arthritis. Visceral evaluation was done as clinically indicated.

Nailfold capillary microscopy. Semiquantitative NCM was performed at the baseline and followup visits using the validated method of Maricq (14,24,25,29,31). All digits of both hands were examined with an SR stereomicroscope (Carl Zeiss, Montreal, Quebec, Canada) at 8–50× magnification, using a cool source of illumination and an eyepiece incorporating a 19.7-μm microscale that allowed reproducible measurements of capillary diameter (25,29). The following data were recorded: degree of capillary enlargement (graded 0–4, where 0 = normal; 1 = borderline, <2 times the normal diameter; 2 = definitely enlarged, ≥2 times, but ≤4 times, the normal diameter; 3 = extremely enlarged, >4 times the normal diameter), and capillary loss (graded A–D, where A = no capillary loss; B = rare capillary loss; C = moderate capillary loss; and D = extensive capillary loss).

All digits of both hands were examined by 2 observers (FJ and AR), and patients were seen on followup by the same observers, both of whom are internists specialized in vascular medicine, whose training in NCM was validated by Dr. Maricq

in 1984, allowing high consistency throughout this study. Intraobserver variability was low. Interobserver agreement was high ($\kappa = 0.95$) (29). At all visits, the observers were blinded to the diagnosis, disease duration, and autoantibody results in the individual patients. Abnormal findings on NCM were the presence of an SSc pattern, which was defined as definitely enlarged capillaries (i.e., grade 2 or 3 capillary enlargement) and/or capillary loss of grades C or D and/or capillary telangiectases. The specificity of grade 2 or 3 capillary enlargement and of grade C or D capillary loss for SSc is >95%, as determined in control patients with primary RP and in patients with CTDs and RP (25).

Immunoassays. Serum samples obtained at baseline were coded and stored at −80°C. All sera were tested blindly. ANAs were determined by indirect immunofluorescence (IIF) on HEp-2 cells (Antibodies Inc., Davis, CA) (performed by J-LS) (32). SSc-specific autoantibodies were determined at the end of the study as follows. Anti–CENP-B antibodies were identified by enzyme-linked immunosorbent assay (ELISA) and anti–CENP by IIF (performed by J-LS) (33). Anti-Th/To antibodies were determined in sera displaying a nucleolar pattern of ANA, using protein A–assisted immunoprecipitation for nucleic acid analysis from nonradiolabeled HeLa cells (performed by MK and GB at the University of Sherbrooke) (34). Anti–topo I antibodies were determined by addressable laser bead immunoassay (ALBIA) and ELISA (35–37). Only sera that showed positive results on both assays were counted as positive. For ALBIA, microspheres embedded with laser-reactive dyes (Luminex, Austin, TX) coupled with native antigen, which were part of a commercial kit (QuantaPlex SLE Profile 8; Inova Diagnostics, San Diego, CA), were used (performed by MK and MJF at the University of Calgary) (35). Anti–topo I (37) and anti–RNAP III antibodies were detected by ELISA using a kit from MBL (Nagoya, Japan) (performed by MK and J-LS) (35,38).

Classification of RP patients at the first visit. Based on clinical, laboratory, capillaroscopic, and overall autoantibody findings at baseline, but without knowledge of the clinical diagnosis at the last followup, each patient was classified into 1 of 3 groups: primary RP, pre-CTD, or early SSc. Patients with primary RP had no objective clinical signs of CTD or another cause of RP, normal findings on laboratory tests, absence of an SSc pattern on NCM, and negative findings on autoantibody assays (21). Patients with pre-CTD had objective clinical signs of CTD (e.g., puffy fingers) and/or abnormal findings on a laboratory test(s) and/or positive findings on an autoantibody assay(s), but not any of the 4 SSc-specific autoantibodies, and absence of an SSc pattern on NCM (39). Patients with early SSc had any of the 4 SSc specific-autoantibodies and/or an SSc pattern on NCM (21,25,29). These patients with early SSc did not display clinical manifestations of SSc or another CTD, such as sclerodactyly, digital ulcers, or pitting scars, loss of distal finger pad, clinically visible capillary telangiectases, or calcinosis.

Classification of patients at followup. At each followup visit, clinical, laboratory, and NCM data were recorded according to the same protocol used at baseline, and patients were assessed for progression to definite SSc or another definite CTD. Definite SSc was defined as fulfillment of the major ACR criterion for SSc or 2 of the 3 minor criteria (40); or, if the ACR criteria were not fulfilled, definite SSc was defined by the presence of RP plus sclerodactyly plus at least a third manifestation of CREST syndrome (calcinosis, RP, esophageal dysmotility, sclerodactyly, telangiectases) (25); or, in the absence of sclerodermatous skin involvement, definite SSc was defined by the presence of RP plus at least 1 of the following manifestations of SSc: peripheral vascular manifestations (at least 2 clinically visible capillary telangiectases on the hands, mucosal membranes, and/or face; clinically visible nailfold capillaries; digital tip ulcers or pitting scars; loss of distal finger pad) and/or SSc visceral manifestations as described elsewhere (25,41,42).

Statistical analysis. Data were collected for a protocol encompassing 215 variables. Frequencies were compared using a 2-tailed Fisher's exact test. Differences between continuous variables were analyzed by *t*-test or Mann-Whitney U test and analysis of variance or Kruskal-Wallis test. To investigate the risk of definite SSc, we relied on time-to-event analyses. Time to event was the time elapsed between the first evaluation and the first visit at which definite SSc was observed. Subjects who did not develop definite SSc until their last followup assessment were censored at that time. First, Kaplan-Meier curves and univariate log-rank tests were used to compare the cumulative rates of the development of definite SSc. Next, univariate and multivariable Cox proportional hazards models were used to identify baseline predictors of faster progression to definite SSc. Forward stepwise selection with the criteria $P > 0.05$ and $P > 0.10$ for entry and removal, respectively, was used to select variables into the multivariable model. Final results were presented as adjusted hazard ratios (HRs), with 95% confidence intervals (95% CIs) and *P* values determined by the 2-tailed Wald test.

RESULTS

Diagnostic classification of 784 RP patients at first evaluation. The diagnoses of primary RP, pre-CTD, and early SSc were attributed at the first evaluation to 38.1% (n = 299), 40.4% (n = 317), and 21.4% (n = 168) of the 784 patients, respectively (Figure 1). Abnormal findings on NCM were present overall at the first evaluation in 88 of the RP patients (11.2%). Definite or extreme capillary enlargement was the most common NCM abnormality (n = 83 [94%]). Moderate or severe capillary loss was less common (n = 16 [18%]), and capillary telangiectases alone were rare (n = 1 [1.1%]). Among the 696 RP patients with normal findings on NCM, SSc autoantibodies were present in 80 of them (11.5%), with anti–CENP-B in 34 of the 80 patients (42.5%), anti-Th/To in 12 (15%), anti–topo I in 14 (17.5%), and anti–RNAP III in 25 (31.3%). More than one of these autoantibodies was present in 5 of the 80 patients (6.3%). Taken together, NCM abnormalities and/or SSc autoantibodies were present at baseline in 21.5% of the patients (n = 168), all of whom were categorized as having early SSc (Figure 1).

Among the 616 remaining patients, the most common abnormality was ANA positivity, as determined by IIF (n = 180 [29.2%]). Among the 436 patients

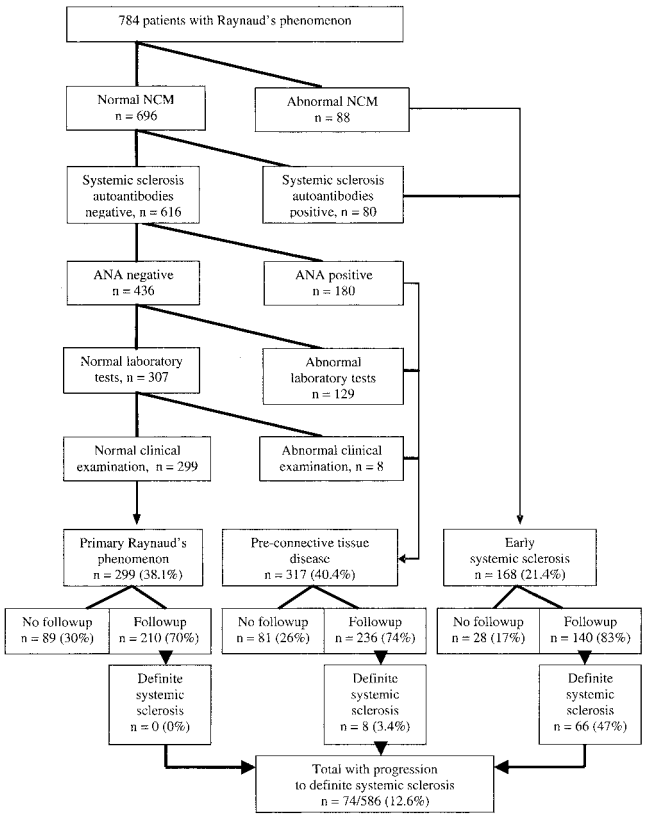

Figure 1. Regression tree showing the diagnostic classification at the first evaluation in 784 patients with Raynaud's phenomenon (RP). Patients were classified as having primary RP, pre–connective tissue disease, or early systemic sclerosis (SSc). Abnormal findings on nailfold capillary microscopy (NCM) consisted of an SSc capillary pattern, which was defined as the presence of either capillary enlargement of grade 2 or 3 and/or capillary loss of grade C or D and/or capillary telangiectases (see Patients and Methods for details). SSc-specific autoantibodies consisted of anti–CENP-B, anti-Th/To, anti–topoisomerase I, and anti–RNA polymerase III autoantibodies. Also shown is the proportion of patients with each diagnosis who had at least 1 followup assessment and experienced progression to definite SSc. Abnormal findings on laboratory tests included leukopenia ($<4{,}000/mm^3$), lymphopenia ($<1{,}500/mm^3$), an elevated erythrocyte sedimentation rate (>25 mm/hour), thrombocytopenia ($<150{,}000/mm^3$), and a decreased hemoglobin value (<12.5 gm/dl). ANAs = antinuclear antibodies.

without ANAs, abnormal findings on laboratory tests were the most common (n = 129 [29.6%]). The most frequent abnormal tests were lymphopenia (n = 86 [67%]) and an ESR >25 mm/hour (n = 31 [24%]). Among the remaining 307 patients without ANAs and with normal results on laboratory tests, objective clinical signs were present in only 8 (2.6%). Taken together, ANA positivity, abnormal findings on laboratory tests, and/or abnormal findings on physical examination were present in 317 of the 616 patients, all of whom were classified as having pre-CTD (Figure 1).

Demographics of primary RP, pre-CTD, and early SSc patients. The overall ratio of women to men was 4.8:1 (Table 1). The mean ± SD age at onset of RP was 34.3 ± 13 years, and the mean ± SD age at first evaluation was 39.6 ± 13 years. Patients with early SSc were significantly older at the onset of RP and at the first evaluation than the patients with pre-CTD as well as the patients with primary RP ($P < 0.0001$). The median duration of RP in the entire cohort at baseline was 3 years (interquartile range [IQR] 1–7 years), and similar durations were observed in each of the 3 groups ($P > 0.05$).

Duration of followup. A total of 586 patients (74.7%) had ≥1 followup assessments (Figure 1). The median followup period in these patients was 4 years (Table 1), and the total duration of followup was 3,197 person-years. The median duration of followup in the primary RP, pre-CTD, and early SSc patients was 4 years, 4.2 years, and 4.6 years, respectively (Table 1). We suspected that "lost to followup" status (n = 198 [25.3%]) would be more common among the patients with primary RP who had normal findings at the first evaluation, since they would therefore be less motivated to come for followup. Indeed, losses to followup were significantly more frequent among patients with primary RP (30% [89 of 299 patients]) than among those with pre-CTD (26% [81 of 317 patients]) or early SSc (17% [28 of 168 patients]) ($\chi^2 = 9.8$, 3 df, $P = 0.007$) (Figure 1).

Frequency and incidence of definite SSc outcome. Among the 586 RP patients who were followed up, 12.6% of the patients (n = 74) progressed to definite SSc (Figure 1). Definite SSc occurred in 47% of the patients with early SSc (66 of 140 patients), 3.4% of the patients with pre-CTD (8 of 236 patients), and none of the patients with primary RP ($P < 0.0001$). The median duration of followup was significantly shorter in the 74 patients with early SSc who did not develop definite SSc as compared with the 66 patients who did develop definite SSc (median ± SD 2.5 ± 3.9 years versus 6.2 ± 5.5 years; $P < 0.001$ by Mann-Whitney U test).

None of the patients with primary RP had developed pre-CTD at followup (Figure 1). The incidence of definite SSc in the RP cohort overall, in pre-CTD patients, and in early SSc patients was 2.3 per 100 person-years, 0.6 per 100 person-years, and 7.9 per 100 person-years, respectively (Table 1). Among those who developed definite SSc, the median time to this diagnosis was 4.56 years after the onset of RP (Table 1). Evolution to other CTDs occurred in another 6 of the

Table 1. Demographic features of 784 consecutive adult patients with RP and progression to definite SSc*

	Primary RP (n = 299)	Pre-CTD (n = 317)	Early SSc (n = 168)	Total cohort (n = 784)	*P*
Ratio of females to males	4:1	4.75:1	7.4:1	4.8:1	0.08
Caucasian, no. (%)	296 (98)	312 (98)	166 (98)	699 (98)	NS
Age at onset of RP, mean ± SD years	31.6 ± 12	34.8 ± 13	38.4 ± 15	34.3 ± 13	<0.0001
Age at first evaluation, mean ± SD years	37.1 ± 11	40.3 ± 13	42.8 ± 14	39.6 ± 13	<0.0001
RP duration at first evaluation, median (IQR) years	3 (1.1–7.5)	2.7 (1–7)	2.3 (1–5)	3 (1–7)	NS
Proportion of patients followed up, no. (%)	210 (70)	236 (74)	140 (83)	586 (75)	0.007
Duration of followup					
Median (IQR) years†	4 (1.1–8.1)	4.2 (1.2–8.5)	4.6 (1.9–8.3)	4 (1.1–8.3)	NS
No. of person-years‡	1,064	1,300	832	3,197	–
Frequency of progression to definite SSc, no. (%)†‡	0 (0)	8 (3.4)	66 (47)	74 (12.6)	<0.0001
Incidence of definite SSc, per 100 person-years†	0	0.6	7.9	2.3	–
Time to definite SSc after first evaluation, median (IQR) years	NA	2.07 (1.61–4.55)	1.75 (0.56–4.77)	1.95 (0.59–4.7)	NS
Time to definite SSc after onset of RP, median (IQR) years	NA	5.67 (2.95–15.5)	4.56 (2.5–8.94)	4.56 (2.5–9.5)	NS
Estimate of progression to SSc, %§					
After 5 years	0	4	47	–	
After 10 years	0	5	69	–	<0.001¶
After 15 years	0	11	79	–	

* *P* values are for the comparison of primary Raynaud's phenomenon (RP) patients versus pre–connective tissue disease (pre-CTD) patients versus early systemic sclerosis (SSc) patients, by Pearson's chi-square test (significant at $P < 0.05$). NS = not significant; IQR = interquartile range; NA = not applicable.

† Determined in 586 patients (210 with primary RP, 236 with pre-CTD, and 140 with early SSc) who had at least 1 followup assessment (see Results and Figure 1 for details). Evolution to other connective tissue diseases occurred in another 6 (1%) of the 586 patients (rheumatoid arthritis in 3, systemic lupus erythematosus in 2, and autoimmune myositis in 1).

‡ The median ± SD duration of followup was significantly longer in the 66 patients with early SSc who developed definite SSc (6.2 ± 5.5 years) as compared with the 74 patients with early SSc who did not develop definite SSc (2.5 ± 3.9 years) ($P < 0.001$ by Mann-Whitney U test).

§ Determined in 586 patients who had at least 1 followup assessment and who were followed up for a mean of 5.46 years.

¶ Patients with early SSc were 17 times more likely to develop definite SSc than were patients with pre-CTD (hazard ratio 17 [95% confidence interval 8.8–23.8], $P < 0.0001$ by log-rank test).

586 patients (1%), with rheumatoid arthritis occurring in 3, systemic lupus erythematosus in 2, and autoimmune myositis in 1. Overall, progression to any CTD occurred in 13.6% of patients.

Progression to definite SSc outcome according to diagnosis at baseline. Kaplan-Meier analyses were limited to the 586 subjects with ≥1 followup assessment; the other 198 RP patients were censored at time 0. At 5, 10, and 15 years of followup, respectively, 47%, 69%, and 79% of the early SSc group had developed definite SSc, whereas 4%, 5%, and 11% of the pre-CTD group and none of the primary RP group had developed definite SSc ($P < 0.001$) (Table 1). Patients with early SSc were 17 times more likely to experience progression to definite SSc than were patients with pre-CTD (HR 17, $P < 0.0001$).

Clinical manifestations warranting classification as definite SSc. The most common change was the appearance of skin thickening in 66 of the 74 patients with progression to definite SSc (89%). Most patients (50 of 66 [76%]) had sclerodactyly only, 11 had sclerodermatous skin of the forearms and/or legs and/or face, 3 had involvement proximal to the elbows or knees, and 2 had trunk involvement. In the remaining 8 patients, the diagnosis of definite SSc was based on clinically visible capillary telangiectases (n = 5), digital ulcers (n = 2), and loss of distal finger pad (n = 1). Thus, most patients developed limited SSc, and only 5 of the 74 patients (6.8%) developed diffuse SSc.

ACR criteria for SSc in patients with a definite SSc outcome. Only 24 of the 74 patients (32%) fulfilled the ACR criteria for SSc, which is consistent with the low sensitivity of these criteria for identifying limited SSc (21,25). Sclerodermatous skin proximal to the metacarpophalangeal joints occurred in 16 patients (21%), whereas 2 of the ACR minor criteria were present in 8 patients (11%), all of whom had sclerodactyly plus digital ulcers (n = 7) or pulmonary fibrosis (n = 1).

Frequency of NCM abnormalities and SSc autoantibodies at baseline and progression to definite SSc. Abnormal findings on NCM were present at baseline in 58% of the patients who experienced progression to definite SSc (43 of 74). SSc autoantibodies were present in 78.4% of the patients (58 of 74): anti–CENP-B (n = 33 [44.6%]) and anti-Th (n = 13 [17.6%]) were the most frequent, whereas anti–topo I (n = 6 [8.1%]) and anti–RNAP III (n = 9 [12.2%]) were the least frequent. All of these patients were also positive for ANAs. Of the 16 patients without an SSc autoantibody, most (n = 13 [81.3%]) were positive for ANAs by IIF, with specifici-

Table 2. Identification of predictors of progression to definite SSc at the first evaluation in 586 patients with RP, by univariate and multivariable Cox proportional hazards models*

Predictor variable	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Demographic variables				
Age at onset of RP	1.04 (1.02–1.06)	<0.001	1.02 (1.01–1.04)	0.006
Female sex	1.59 (0.76–3.31)	0.217	NA	–
Duration of RP	0.95 (0.91–0.99)	0.045	NS	–
Clinical and capillaroscopic variables				
Objective clinical signs†	5.68 (3.57–9.3)	<0.001	1.98 (1.21–3.25)	0.007
Puffy fingers	8.30 (5–13.7)	<0.001	1.89 (1.12–3.26)	0.017
SSc pattern on NCM†	14.05 (8.7–22.6)	<0.001	4.5 (2.7–7.5)	<0.001
Capillary enlargement of grade 2 or 3	11.61 (7.26–18.58)	<0.001	7.2 (4.2–12.4)	<0.001
Capillary loss of grade C or D	16.85 (8.94–31.75)	<0.001	2.5 (1.2–4.9)	<0.001
Laboratory variables				
ANAs by indirect immunofluorescence	23.17 (8.45–63.5)	<0.001	5.67 (1.87–17.1)	0.002
SSc-specific autoantibodies†	18.3 (10.5–31.97)	<0.001	4.7 (2.48–8.9)	<0.001
Anti–topoisomerase I	2.84 (1.23–6.6)	<0.001	3.8 (1.49–9.7)	0.005
Anti-Th/To	5.9 (3.2–10.98)	<0.001	3.56 (1.5–5.3)	0.002
Anti–CENP-B	9.34 (5.85–14.9)	<0.001	2.8 (1.59–7.9)	<0.001
Anti–RNA polymerase III	4.2 (2.1–8.5)	<0.001	2.44 (1.19–5)	0.015
Any other abnormal laboratory finding	1.56 (0.98–2.48)	0.058	NA	–

* Forward stepwise analysis was performed after identification of predictive variables by univariate analysis, using $P > 0.05$ for entry and $P > 0.10$ for removal (see Patients and Methods for details). Variables were adjusted for age at onset of RP (significant at $P < 0.05$). SSc = systemic sclerosis; RP = Raynaud's phenomenon; 95% CI = 95% confidence interval; NA = not assessed; NS = not significant; ANAs = antinuclear antibodies.
† Component variables were first analyzed together and were then analyzed individually. Objective clinical signs consisted of puffy fingers, digital ulcers, pitting scars, loss of distal finger pad, clinically visible capillary telangiectases, calcinosis, and arthritis. See Patients and Methods for an explanation of the nailfold capillary microscopy (NCM) scoring system.

ties being anti–U1 RNP (n = 2), anti-Ro (n = 1), and undefined (n = 10, including 5 with a nucleolar pattern who were anti–PM-Scl negative). Thus, 71 of the 74 patients (96%) who progressed to definite SSc were positive for ANAs by IIF.

Predictors of definite SSc. To identify the baseline predictors of definite SSc, analyses were limited to the 586 RP patients who had ≥1 followup assessment. First, we used a univariate Cox regression model to test various factors that were present at baseline for their ability to predict progression to definite SSc (Table 2). For every 1-year increase in age at the onset of RP, there was a 4% increase in the risk of progression. Therefore, all other factors at baseline were adjusted for age at RP onset. In separate Cox models, the strongest predictors of definite SSc were positive ANAs, positive SSc-specific autoantibodies, and an SSc pattern on NCM, while both enlarged capillaries and capillary loss, as well as each SSc autoantibody were also highly predictive ($P < 0.001$ for each variable, adjusted only for age) (Table 2).

On Cox proportional hazards multivariable analysis, the strongest independent predictors of definite SSc were positive ANAs (adjusted HR 5.67), SSc autoantibodies (HR 4.7) and an SSc pattern on NCM (HR 4.5), especially capillary enlargement (HR 7.2) ($P \leq 0.002$ for each variable) (Table 2). Capillary loss (HR 2.5) was also an independent predictor ($P < 0.001$), as well as each of the 4 SSc autoantibodies (HR 2.44–3.8, $P \leq 0.015$) (Table 2).

Time course of microvascular damage. Although an SSc pattern on NCM was present at baseline in 43 of the 74 patients who experienced progression to definite SSc (58%), the frequency rose to 65 of the 74 patients (88%) at the last followup, indicating that 22 additional patients had developed abnormal findings on NCM. Specifically, enlarged capillaries, which were the most frequent finding at baseline (39 of the 74 patients [52.7%]), occurred at the last NCM in 57 patients (77%). Similarly, capillary loss and capillary telangiectases, which were observed at baseline in 12 patients (16.2%) and 1 patient (1.4%), respectively, were present at the last NCM in 49 patients (66.2%) and 26 patients (35.1%), respectively. This allowed us to determine whether NCM abnormalities evolved synchronously or sequentially and to identify the earliest pathophysiologic microvascular event.

Thus, we focused on the 65 patients who had abnormal findings on NCM at the initial visit and/or

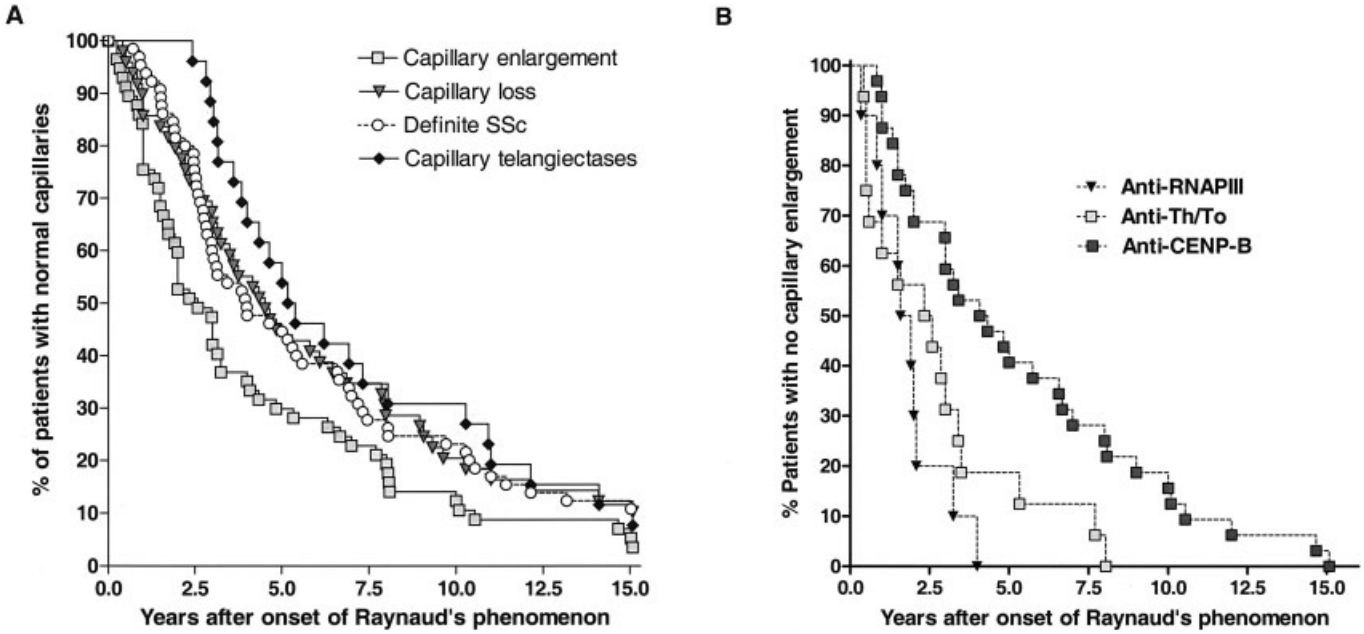

Figure 2. Sequential time course of microvascular damage and relationship to systemic sclerosis (SSc)–specific autoantibodies, as shown by Kaplan-Meier curves of the time to development of nailfold capillary microscopy (NCM) abnormalities. The time to event was calculated as the time elapsed between the onset of Raynaud's phenomenon (RP) and the first visit at which capillary abnormalities were observed. Analysis was based on the 65 of the 74 patients who experienced progression to definite SSc and had abnormal findings on NCM at the initial visit and/or during followup. **A,** Capillary enlargement of grade C or D occurred first, followed by capillary loss of grade 2 or 3, and then by telangiectases. Definite SSc occurred in close temporal relationship to the appearance of avascular areas, as shown by the overlapping curves ($P = 0.8$). The median time from the onset of RP to the identification of enlarged capillaries was 2.58 years (interquartile range [IQR] 1.16–6.83), to the identification of capillary loss was 4.51 years (IQR 2.28–9.19), to the development of definite SSc was 3.99 years (IQR 2.5–8.87), and to the development of capillary telangiectases was 5.28 years (IQR 3.38–10.96) ($P = 0.022$ for all 4 curves, by log-rank test). **B,** Enlarged capillaries occurred earliest with anti–RNA polymerase III (anti–RNAP III) autoantibodies and latest with anti–CENP-B autoantibodies, whereas the time of occurrence was intermediate with anti-Th/To autoantibodies ($P = 0.002$ for all 3 curves, by log-rank test). No curve is shown for anti–topoisomerase I autoantibodies because of their low frequency in the study population.

developed abnormalities at followup. As shown in Figure 2A, a characteristic sequence of microvascular changes was noted. Capillary enlargement was the earliest event, occurring at a median of 2.58 years after the onset of RP, whereas capillary loss was noted later (4.51 years) and telangiectases even later (5.28 years) ($P = 0.022$). The diagnosis of definite SSc occurred in close temporal relationship to capillary loss (Figure 2A).

SSc autoantibodies as independent predictors of microvascular damage. We used Cox proportional hazards multivariable analysis to determine whether SSc-specific autoantibodies were independent predictors of microvascular damage, as detected by NCM. First, SSc autoantibodies were strong independent predictors of enlarged capillaries (HR 6.64) (Table 3). Separate analyses revealed that the presence of anti-Th/To and anti–CENP-B autoantibodies independently predicted this microvascular change (Table 3). Second, capillary loss was most strongly predicted by the presence of prior capillary enlargement (HR 11.33), as were SSc autoantibodies (HR 2.62). Anti–RNAP III, anti-Th/To, and anti–CENP-B autoantibodies were also independently linked to capillary loss. Third, the strongest predictors of telangiectases were prior capillary loss identified on NCM (HR 6.9) and SSc autoantibodies (HR 5.57), notably, anti–CENP-B (Table 3).

Association of each SSc-specific autoantibody with a distinct rate of microvascular damage. Given the independent correlation between SSc-specific autoantibodies and microvascular damage, we determined whether the time course of capillary damage after the onset of RP varied according to the SSc autoantibody specificity. The temporal occurrence of definitely or extremely enlarged capillaries was indeed correlated with the autoantibody present (Figure 2B). Specifically, capillary enlargement occurred earliest with anti–RNAP III autoantibodies and latest with anti–CENP-B autoantibodies, whereas the time of occurrence was intermediate with anti-Th/To autoantibodies ($P = 0.002$). A similar temporal development occurred for capillary loss

Table 3. Identification of independent factors predictive of progression to microvascular damage as seen on NCM at the first evaluation in 586 patients with RP, by multivariable Cox proportional hazards models*

Microvascular damage, predictor variable	Hazard ratio (95% CI)	*P*
Capillary enlargement of grade 2 or 3		
SSc-specific autoantibodies†	6.64 (4.24–10.45)	<0.001
Anti-Th/To	9.39 (5.43–16.25)	<0.001
Anti–CENP-B	5.48 (3.45–8.71)	<0.001
ANAs	2.59 (1.58–4.26)	<0.001
Puffy fingers	2.51 (1.60–3.95)	<0.001
Capillary loss of grade C or D		
Presence of prior capillary enlargement	11.33 (5.91–21.71)	<0.001
Puffy fingers	2.9 (1.61–5.25)	<0.001
SSc-specific autoantibodies†	2.62 (1.51–4.70)	0.001
Anti–RNA polymerase III	3.05 (1.37–6.8)	0.006
Anti–CENP-B	2.46 (1.39–4.35)	0.002
Anti-Th/To	2.4 (1.14–5.06)	0.022
Capillary telangiectases		
Presence of prior capillary loss	6.9 (2.60–18.39)	<0.001
SSc-specific autoantibodies†	5.57 (1.90–16.31)	0.002
Anti–CENP-B	3.1 (1.27–7.59)	0.012

* Forward stepwise analysis was performed after identification of predictive variables by univariate analysis, using $P > 0.05$ for entry and $P > 0.10$ for removal (see Patients and Methods for details). NCM = nailfold capillary microscopy; RP = Raynaud's phenomenon; 95% CI = 95% confidence interval; SSc = systemic sclerosis; ANAs = antinuclear antibodies.
† Component variables were first analyzed together and were then analyzed individually.

in relation to these 3 autoantibodies ($P = 0.021$) (data not shown).

Evaluation of proposed criteria for early SSc. The presence of both an SSc pattern on NCM and SSc-specific autoantibodies had been proposed as a criterion for early SSc in patients with RP (21). We therefore examined the impact of these predictors at baseline on an outcome of definite SSc. First, we found that definite SSc rarely occurred in patients without these predictors (8 of 446 patients [1.8%]) (Table 4). Second, with either an SSc pattern on NCM or an SSc-specific autoantibody, progression occurred in 25.8% (8 of 31) and 35.4% (23 of 65) of the patients, respectively. However, the highest frequency of progression occurred when both variables were present at baseline, rising from 65.9% (29 of 44) at 5 years to 79.5% (35 of 44) of the patients at the last followup (Table 4).

Third, the concurrent presence of abnormal findings on NCM and SSc-specific autoantibodies was strongly associated with a definite SSc outcome (odds ratio [OR] 50, $P < 0.0001$, positive predictive value [PPV] 79%, and negative predictive value [NPV] 93%) (Table 4). Moreover, the presence of abnormal findings on NCM increased by 5-fold the risk of a definite SSc outcome (adjusted HR 5.03) above the risk conferred by the presence of SSc-specific autoantibodies, whereas the presence of SSc-specific autoantibodies was associated

Table 4. Definite SSc outcome in 586 patients with RP, classified according to NCM profiles and SSc-specific autoantibodies at the first evaluation*

Predictors at first evaluation	No. of patients	Definite SSc outcome		
		At 5 years of followup	At 10 years of followup	At last followup
Normal findings on NCM and negative SSc-specific autoantibodies	446	6 (1.3)	7 (1.6)	8 (1.8)
SSc pattern on NCM and negative SSc-specific autoantibodies	31	7 (22.6)	7 (22.6)	8 (25.8)
Normal findings on NCM and positive SSc-specific autoantibodies	65	14 (21.5)	21 (32.3)	23 (35.4)
SSc pattern on NCM and positive SSc-specific autoantibodies†‡§	44	29 (65.9)	32 (72.7)	35 (79.5)
Total	586	56 (9.5)	67 (11.4)	74 (12.6)
P	–	<0.001	<0.001	<0.001

* A systemic sclerosis (SSc) pattern on nailfold capillary microscopy (NCM) *and* positive SSc-specific autoantibodies corresponds to LeRoy and Medsger's proposed classification criterion for early SSc in patients with subjective Raynaud's phenomenon (RP) (21). An SSc pattern on NCM *and/or* positive SSc-specific autoantibodies corresponds to the definition of early SSc used in this study. Values are the number (%) of patients.
† Definite SSc developed in 35 of the 44 patients with *both* an SSc pattern on NCM and positive SSc-specific autoantibodies, as compared with 39 of the remaining 542 patients without this pair of predictors: odds ratio (OR) 50 (95% confidence interval [95% CI] 22–111), $P < 0.0001$, with a sensitivity of 47%, a specificity of 98%, a positive predictive value (PPV) of 79%, a negative predictive value (NPV) of 93%, a positive likelihood ratio (LR) of 26.9, and a negative LR of 54.
‡ Definite SSc developed in 66 of the 140 patients with an SSc pattern on NCM *and/or* positive SSc-specific autoantibodies, as compared with 8 of the remaining 446 patients with none of these predictors: OR 20 (95% CI 11–37), $P < 0.0001$, with a sensitivity of 89%, a specificity of 85%, a PPV of 47%, a NPV of 98%, a positive LR of 6.7, and a negative LR of 12.9.
§ Abnormal findings on NCM increased by 5-fold the risk of a definite SSc outcome (adjusted hazard ratio [HR] 5.03 [95% CI 3.0–8.45]) above the risk conferred by the presence of SSc-specific autoantibodies, whereas the presence of SSc-specific autoantibodies was associated with an 8-fold increased risk, even after adjusting for abnormal findings on NCM (adjusted HR 8.5 [95% CI 4.57–15.8]), as determined by Cox proportional hazards analysis. Patients with both predictors at baseline were 60 times more likely to experience progression to definite SSc than were patients without these predictors (adjusted HR 60.08 [95% CI 27.11–133.14]).

with an 8-fold increased risk, even after adjusting for abnormal findings on NCM (adjusted HR 8.5). As a consequence, patients who had both risk factors at baseline were 60 times more likely to experience progression to SSc than were patients without these predictors (adjusted HR 60.08). However, the sensitivity of this pair of predictors of a definite SSc outcome was only 47%. In contrast, the presence of an abnormal NCM *and/or* SSc autoantibodies at baseline was associated with a sensitivity of 89% and a near-perfect NPV (98%) (OR 20, $P < 0.0001$) (Table 4).

DISCUSSION

This is the first prospective, large, single-center study of patients with rigorously defined isolated RP progressing to definite SSc, in which the major SSc-specific autoantibodies were determined at the first evaluation, NCM was performed serially, and the results were correlated by multivariable analysis. Several conclusions stem from our data.

First, the incidence of progression from isolated RP to definite SSc was 12.6%, and the incidence of progression to any CTD was 13.6%. The latter incidence is similar to that reported in a recent meta-analysis (12.6%) (43).

Second, progression to definite SSc occurred mostly in patients who were classified as having early SSc at baseline. The estimates for progression in the group with early SSc were 47% at 5 years, 69% at 10 years, and 79% at 15 years. Patients with early SSc were 17 times more likely to progress to definite SSc than were patients with pre-CTD. These data suggest that, given enough time, most early SSc patients will develop definite SSc.

Third, SSc-specific autoantibodies and abnormal findings on NCM were present at baseline in 78.4% and 58%, respectively, of patients who experienced progression to definite SSc (Table 4). Both abnormalities were present in 47.3% of patients, and at least 1 of them was present in 89.2% of patients (Table 4). As expected, anti–CENP-B autoantibody was the most common (44.6%) and anti–topo I less common (8.1%) (12,14,18). Hitherto unreported anti-Th/To and anti–RNAP III specificities were common as well (30%).

Fourth, the strongest independent predictors of progression to definite SSc were positive ANAs, positive SSc autoantibodies, and an SSc pattern on NCM. Capillary enlargement, capillary loss, and either of the 4 SSc autoantibodies were also independent predictors. The frequency of progression was higher with both an SSc autoantibody and an SSc pattern on NCM at baseline (79.5%) than with only 1 of these predictors (32.3%) (Table 4). Therefore, patients with RP who present to a specialized center should be evaluated both for nailfold capillary abnormalities by NCM and for SSc-specific autoantibodies, including anti-Th/To and anti–RNAP III (44).

Fifth, in RP evolving to definite SSc, microvascular damage is characteristically sequential, starting with enlarged capillaries, followed by capillary loss, and then by capillary telangiectases. Moreover, the strongest predictor of capillary loss was prior capillary enlargement, and in turn, capillary loss was the strongest predictor of the development of telangiectases. Definite SSc occurred in close temporal relationship to the appearance of capillary loss. Taken together, these data suggest that a sustained and cumulative burden of microvascular damage has already occurred when definite SSc is diagnosed. Since new therapeutic agents are being evaluated in patients with SSc (45,46), awareness of this sequence of microvascular damage has potential implications for future trials. For example, in trials of novel angiogenic, vasculogenic, or fibrosis-modulating agents, it would appear logical to select patients at a uniform stage of microvascular damage and with similar SSc-specific autoantibodies and to evaluate such damage longitudinally by NCM to assess response to therapy.

Sixth, SSc-specific autoantibodies were strong predictors of microvascular damage. Anti–CENP-B and anti-Th/To predicted enlarged capillaries, whereas capillary loss was predicted by these autoantibodies and anti–RNAP III. Moreover, each SSc autoantibody was associated with a distinct rate of microvascular damage. Previous studies were of cross-sectional design and were restricted to assessments of anti–CENP-B and anti–topo I autoantibodies (47–49).

Last, this study is the first to validate the criteria for early SSc that were proposed by LeRoy and Medsger, but were not validated (21). According to these criteria, when the presence of RP is subjective only (i.e., by patient report only), as in the present study, early SSc may be diagnosed when *both* an SSc pattern on NCM and SSc-specific autoantibodies are observed (21). In our cohort, patients in whom both predictors were present at baseline were 60 times more likely to develop definite SSc than were patients without these predictors. The positive likelihood ratio for these criteria was 26.9, the NPV was 93%, and the PPV was 79%, suggesting that such criteria could be used at baseline to accurately predict a definite SSc outcome, although with a low sensitivity (47%). Interestingly, using a modification of the early SSc criteria defined by the presence of any of the 4 SSc-specific autoantibodies *and/or* an SSc pattern

on NCM (25,29), we found a much higher sensitivity (89%), with an almost perfect NPV (98%), but a low PPV (47%). Taken together, these data suggest that the concurrent presence at baseline of both criteria indicates a very high probability that a patient will develop definite SSc, whereas their absence rules out this outcome.

The statistical aspects of our study warrant some comments. This study was performed at a tertiary health center; however, this should not limit the generalizability of our results, for 2 reasons. First, 93.4% of referring physicians were primary care physicians. Second, referral bias (i.e., might the patients who were referred to our specialized center be more likely to have an underlying disease process) is unlikely, since these physicians refer almost all their RP patients for evaluation at this institution anyway. Also, 25% of the RP patients initially identified effectively did not contribute to our analyses because they failed to return for followup assessment. These 198 patients were unlikely to experience progression, since normal findings on the initial evaluation were significantly more frequent in this group than in the group of 586 patients who were studied in followup. Therefore, while their exclusion might have overestimated the overall progression rate, it is unlikely to have overestimated the observed, statistically significant associations between NCM abnormalities, SSc-specific autoantibodies, and progression to definite SSc. Finally, while our study population was mostly French Canadian, ethnogeographic and immunogenetic factors are known to modulate the frequency of SSc autoantibodies (29). Indeed, whereas the large sample size allowed us to demonstrate statistical significance for each of the 4 SSc-specific autoantibodies in terms of progression to definite SSc, the study still did not have sufficient power to demonstrate the effect of anti–topo I as an independent predictive factor for microvascular damage. Anti–topo I is known to be less prevalent in French Canadian SSc patients (29) and was present in only 8% of the patients with progression to definite SSc in our study. Therefore, our findings should be replicated in a multicenter study with larger ethnic and geographic variations.

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AUTHOR CONTRIBUTIONS

Drs. Koenig and Senécal had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Koenig, Joyal, Roussin, Senécal.

Acquisition of data. Koenig, Joyal, Fritzler, Roussin, Boire, Goulet, Rich, Grodzicky, Senécal.

Analysis and interpretation of data. Koenig, Joyal, Roussin, Abrahamowicz, Raymond, Senécal.

Manuscript preparation. Koenig, Joyal, Fritzler, Roussin, Boire, Raymond, Senécal.

Statistical analysis. Koenig, Abrahamowicz, Senécal.

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