

## Original article

# Improving outcome prediction of systemic sclerosis from isolated Raynaud's phenomenon: role of autoantibodies and nail-fold capillaroscopy

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## Abstract

**Objective.** A simple weighted prognostic algorithm, based on capillaroscopy and autoantibodies, is developed to classify patients at different risk of transition from isolated RP to SSc within 5 years from the screening visit.

**Methods.** Two hundred and eighty-eight of 768 patients with isolated RP who underwent capillaroscopy were recruited. The prognostic contributions of capillaroscopic findings (giant loops, haemorrhages and the number of capillaries) and SSc-associated autoantibodies (ACAs, anti-topo I and ANAs) were assessed in a semi-parametric regression models suitable for competing risks. A prognostic index was built by a bagging technique. A structured tree approach was used to extract simple classificatory rules that can be directly interpreted.

**Results.** Thirty-four transitions from isolated RP to SSc and 42 to other CTDs were observed. All of the chosen variables had a substantial prognostic impact. A complex non-linear prognostic pattern was observed for capillaries, with the risk of developing SSc increasing as the number of loops decreased. The presence of ANAs had a strong impact on prognosis [hazard ratio (HR)=9.70], which was increased by the presence of ACA (HR=3.94;  $P < 0.001$ ). A weighted prognostic classification for the development of SSc was constructed using capillary number, giant loops and ANAs. The prognostic discrimination was satisfactory (Harrell's C-index = 0.86).

**Conclusion.** Our prognostic model is based on easy-to-obtain features (i.e. the number of capillaries, giant loops and ANAs) and could be used to facilitate clinical decision making in the screening phase, and may also have important implications for stratifying patients into risk groups for future clinical assessment.

**Key words:** Capillaroscopy, Raynaud's phenomenon, Systemic sclerosis.

## Introduction

RP is a common disorder that generally occurs in response to cold or emotion. It classically involves the

closure of the digital arteries, pre-capillary arterioles and subcutaneous arteriovenous shunts, leading to the clinical finding of sharply demarcated cutaneous pallor, followed by cyanosis and/or erythema [1, 2].

RP has a worldwide distribution, although its true prevalence in the general population is not precisely known (3–21%), probably because of the episodic nature of the attacks influenced by ambient temperatures, and variations in its severity and expression [3–5].

It is known that primary (uncomplicated or isolated) RP is a functional vascular disorder, but little is known about whether, when and why it becomes a structural disease. The diagnosis of secondary RP includes associations with

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a whole series of vascular and non-vascular diseases, ranging from peripheral arterial and CTDs to paraneoplastic disorders. The reported rates of transition from primary to secondary RP vary widely from 1 to 17% [6–10], and the incidence of transition seems to depend on the origin of the patient sample [8].

Previous studies have identified various clinical and laboratory factors that help to explain the variable outcomes of patients with RP [11–13]. On the basis of these, LeRoy and Medsger [14] proposed clinical criteria to distinguish primary and secondary RP in 1992; they suggested diagnosing primary RP in the presence of a definite history of episodic attacks (without digital ulcerations, pitting or gangrene of the extremities), normal nail-fold capillaries, a negative ANA test and a normal ESR. If these criteria are met, the 2-year follow-up required by the previous criteria of Allen and Brown [15] was considered unnecessary. However, using these criteria to differentiate primary and secondary RP does not reflect a definite diagnosis, but simply describes current findings in an ongoing screening process.

It is important to diagnose secondary RP early because it may be due to a severe underlying disease that can even be associated with reduced life expectancy: it occurs in 90% of the patients with SSc, 85% of patients with MCTD, 10–45% of patients with SLE, 33% of patients with SS and 20% of patients with DM/PM.

RP may present years before a patient develops an overt CTD. SSc is the most frequently involved and there is often a significantly long period between the onset of RP and the next non-Raynaud clinical feature. It is generally very difficult to predict whether and when patients with isolated RP may develop SSc, and therefore a reliable prediction tool of outcome on the first visit is of great clinical relevance.

As there are still no readily applicable and validated models to identify patients with RP who may develop SSc, our aim was to develop a simple but accurate weighted rule-based prognostic system for isolated RP based on capillaroscopic findings (i.e. giant loops, haemorrhages and the number of capillaries) and the autoantibodies classically associated with SSc (i.e. ANAs, ACAs and anti-topo I). Capillaroscopy and antibodies data are easily obtained in the assessment phase of RP with a non-invasive and financially reasonable screening model with high potential of positive impact on patients' survival and quality of life. A large cohort of patients with isolated RP was used to develop and internally validate the model, which allows such patients to be stratified on the basis of their risk of transition from isolated RP to SSc within 5 years.

## Materials and methods

### Study design

We retrospectively analysed the records of the 1429 consecutive adult subjects (aged >18 years) who were referred to the outpatient Rheumatological Department of Istituto Gaetano Pini (Milan, Italy) for a nail-fold capillaroscopy examination between January 1999 and January 2003. The 768 subjects with isolated RP were

considered eligible for the study on the basis of the criteria described below: 288 recruited from our Rheumatology Outpatient Clinic were followed up at our Centre after the examination in order to monitor their health status and the development of any non-Raynaud features of SSc (skin and/or other visceral involvement); for these patients, the last update for follow-up was available in January 2007. The other 480 were seen at other centres and referred to our outpatient clinic only for nail-fold capillaroscopy and so, although eligible, they were not included in the study follow-up (Fig. 1). This study is described in accordance with the STROBE statement [16].

### Subjects

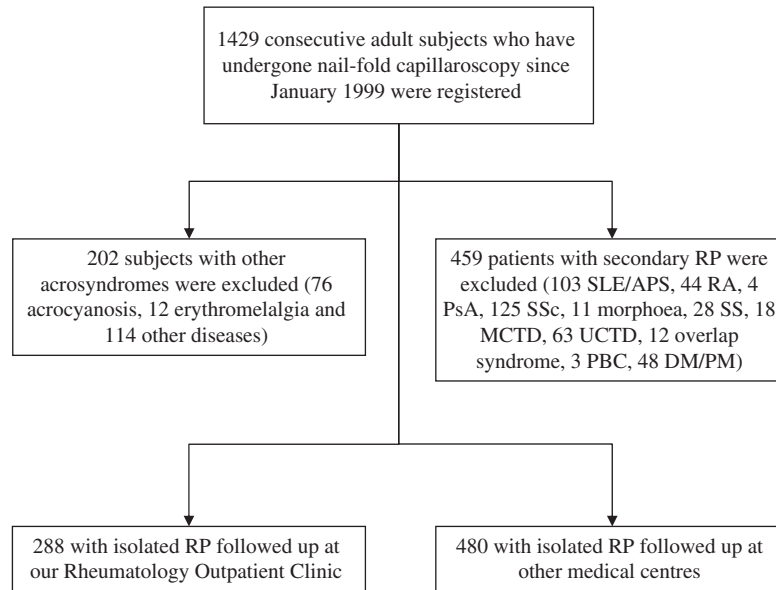
Subjects were considered eligible for inclusion in the study if they had been diagnosed as having isolated RP, which was defined as episodic, reversible vasospastic ischaemia of the digits manifesting upon exposure to the cold and/or in association with emotional stress, and characterized by well-demarcated blanching, possibly leading to cyanosis, followed by post-ischaemic red flushing upon rewarming. The episodes may have involved biphasic or triphasic colour changes, and could have been accompanied by varying degrees of paresthesia, numbness or pain. The disease had to occur in isolation, without any symptoms or signs suggesting a CTD: i.e. skin/mucosal (pitting scars, gangrene, sclerodactyly, photosensitivity, rashes, telangiectasias, orogenital ulcers), ocular (xerophthalmia, episcleritis, scleritis, uveitis), musculoskeletal (arthralgias, arthritis, myalgia, muscle weakness, fatigue), cardiopulmonary (serositis, dyspnoea, pulmonary fibrosis and/or hypertension, heart ischaemia, conduction disturbance), renal (nephrotic syndrome, glomerulonephritis), haematological (leukopenia, anaemia, thrombocytopenia) or gastroenteric manifestations (xerostomia, dysphagia, oesophageal hypomotility) or neuropathy.

Of the 1429 subjects referred to our Rheumatology Clinic for nail-fold capillaroscopy during the study period, 768 satisfied the inclusion criteria: 202 were excluded on the grounds that they were affected by other acrosyndromes (76 acrocyanosis, 12 erythromelalgia and 114 other diseases) and 459 because they were diagnosed as having secondary RP at baseline (103 with SLE/APS, 44 with RA, 4 with PsA, 125 with SSc, 11 with morphoea, 28 with SS, 18 with MCTD, 63 with UCTD, 12 with overlap syndromes, 3 with primary biliary cirrhosis and 48 with DM/PM) (Fig. 1).

### Baseline and follow-up data collection

Data relating to all of the enrolled patients were collected from the standardized procedure used at our centre to assess RP. Namely, at baseline, nail-fold capillaroscopy was performed with the subjects sitting in a comfortable ambient temperature; a drop of immersion oil was applied to the nail-fold in order to maximize the translucency of the keratin layer. All fingers (but not the thumbs) of both hands were examined using a videocapillaroscope equipped with a 200 × optic probe, and the images were

Fig. 1 Enrolment of study participants.



PBC: primary biliary cirrhosis.

captured, coded and stored using Videocap 8.14 software (DS-Medica, Milan, Italy). The images captured all of the nail-fold areas in which capillary visibility was good, and were scanned for three main parameters: i.e. the number of capillaries, giant loops and haemorrhages, defined as reported in the literature [17–19]. Giant loops and haemorrhages were analysed as only two classes (0 = absent; 1 = present), whereas the number of capillaries was defined as the mean number of capillaries in a 1-mm length of each finger, as suggested by the findings of our previous study [19]. Subjects underwent the first clinical follow-up examination after 6 months.

At baseline visit, the serum of the patients followed up at our centre was analysed for the presence of ANAs, ACA and anti-topo I antibodies, and the results were categorized as positive or negative [20]. ANAs were determined by IIF on HEp-2 cells (Antibodies Inc., Davis, CA, USA) and considered positive when a dilution higher than that of 1:80 was obtained.

### Statistical analysis

The endpoint was the time between the first examination and the occurrence of a non-Raynaud clinical feature of SSc, as the first documented event during follow-up or the last follow-up visit (in the case of the patients not developing any sign or symptom suggesting CTDs). The occurrence of UCTD, DM/PM, RA, SLE, MCTD and death were considered as events competing with the end-point of interest. Thus, statistical analysis was performed by methods suitable for the presence of competing risks.

The crude cumulative incidence of SSc was estimated by a non-parametric method [21]. For the following analysis, we decide to truncate the follow-up at 5 years, and the

patients whose follow-up was <5 years were excluded. As a first step, the prognostic effect of each variable (separately and jointly) was estimated by a semi-parametric regression models on sub-distribution hazard ratio (SDHR), i.e. the hazard related to crude cumulative incidence [22]. ANAs, ACA and anti-topo I antibodies were considered a combined variable called 'antibodies'. According to published findings [20], the association of ACA<sup>+</sup> and anti-topo I<sup>+</sup> is very rare and, in line with this, was not observed in any of the patients in our cohort. On the basis of the prognostic severity of each antibody and their possible combinations (as reported in the literature) [20], this variable was scored 0 when ANAs, ACA and anti-topo I were all negative; one when only ANAs were positive; two when ANAs and ACA were positive, and three when ANAs and anti-topo I were positive. Subsequently, in the regression model, the following dummy variables were included:  $d_1 = 1$  when the variable 'antibodies' was  $\geq 1$ ,  $d_2 = 1$  when 'antibodies' was  $\geq 2$  and  $d_3 = 1$  when 'antibodies' was = 3. With regard to the number of capillaries, the presence of a non-linear effect was evaluated using regression restricted cubic splines with three knots. The results relating to the categorical variables are shown in terms of sub-distribution, hazard ratios and Wald's test, and a plot of the shape of the relative sub-distribution hazard function was traced for the number of capillaries.

As a second step, a prognostic index was built starting from the results of the multiple regression. In order to take into account the uncertainty concerning the 'best model' for prediction, we used bootstrap aggregating (bagging). For the bootstrap, 1000 samples were generated, each of which had the same size as the original data set and was obtained by randomly selecting the observation and then

replacing each selected observation before selecting the next [23]. Model selection was applied in each bootstrap sample. A  $P=0.157$  was used, corresponding to Akaike information criterion for backward procedure [24].

As a third step, and with the aim of obtaining a simple prognostic classification rule, the prognostic index >60 months was summarized by means of a regression tree as a function of the prognostic variables (by grouping terminal nodes with a similar prognosis), with the final simplification of three groups with high, intermediate and low incidence of SSc.

To account for censored survival data and the presence of competing risks, the prognostic discrimination ability of the classification was evaluated using a modified version of Harrell's C-index [25], where a weighting procedure for incomplete data was introduced [22]. The index is an extension of the area under receiver operating characteristic curve analysis, which ranges from 0.5 (no discrimination capacity) to 1 (maximum discrimination capacity).

## Results

### Characteristics of the study cohort

All of the following analyses were based on the 288 subjects (255 women and 33 men; median age 49.84 years, range 18.5–79.9) for whom complete data were available. ANAs were negative in 160 subjects (55.5%) and positive in 128 (44.5%); ACA and anti-topo I antibodies were present in, respectively, 44 subjects (15.3%) and 12 subjects (4.2%). Seventy-one patients (24.7%) were only ANA<sup>+</sup>. No clinically relevant differences were observed between this group and the 480 excluded because they were only screened, but followed up at other medical centres (Table 1). The median follow-up of 288 patients was 24 months (range 2–96 months), 34 transitions from isolated RP to SSc were observed, other CTDs developed in 42 patients (11 cases of RA, 25 cases of UCTD, 3 cases of SLE, 2 cases of DM/PM and 1 case of MCTD). The 5-year incidence for all events was 45.8% (95% CI 31, 61) and for SSc was 21% (95% CI 10, 32). It can be shown that most of the SSc events were observed at early follow-up times (Fig. 2).

### Univariate and multivariable analysis

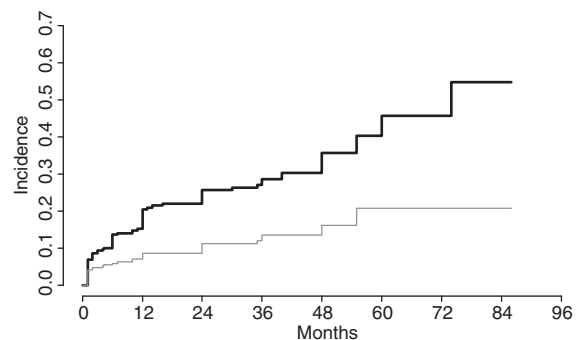
As shown in Table 2, the capillaroscopy variables (i.e. giant loops, haemorrhages and the number of capillaries) showed evidence of prognostic impact ( $P < 0.001$ ). In particular, the number of capillaries had a complex non-linear prognostic relationship. To quantify the prognostic impact, a graph was traced referring to 7 loops/mm as a value used in the published literature [19], and it can be shown that the hazard of transition from isolated RP to SSc increased as the number of capillaries decreased <7 loops/mm (Fig. 3).

In relation to autoantibodies, isolated ANA positivity increased the hazard of an event (SDHR 9.70) in comparison with the absence of all of the considered antibodies, and there was a further increase in comparison with isolated ANA positivity if ACA was also positive (SDHR 3.93).

**TABLE 1** Differences in the characteristics of isolated RP between the subjects for whom complete data were available and those for whom some data were missing

	Isolated RP	
	Subjects followed up at our Rheumatology Outpatient Clinic (n = 288)	Subjects followed up at other medical centres (n = 480)
Age, years		
Median	50	44
Interquartile range	34–60	35–58
Gender		
Female, %	255 (88.54)	414 (86.25)
Capillaroscopy		
Number of capillaries/mm		
Median	7.0	8.0
Interquartile range	6–9	7–9
Giant loops	50/288 (17.36)	53/480 (11.04)
Haemorrhages	57/288 (19.79)	66/480 (13.75)

**Fig. 2** Kaplan–Meier incidence curve of a cohort of 288 patients showing all observed events together with the patients exposed at risk during the follow-up (black line) and the SSc events (grey line).



As anti-topo I positivity did not indicate a further increase in the hazard of SSc (SDHR 1.37), the category ACA<sup>+</sup> was considered together with anti-topo I<sup>+</sup> in the following analysis. The details are given in Table 2.

All of the above variables were then included in the multivariable analysis (Table 3), although the results should be considered cautiously because of the relatively high number of estimated coefficients. In the multivariable analysis, only giant loops, capillary number and ANA positivity retained evidence of a prognostic role.

### Building the prognostic classification

Model selection to each of the 1000 bootstrap samples showed that the number of capillaries, giant loops and ANA<sup>+</sup> had the three highest inclusion frequencies, and

**TABLE 2** Univariate analyses of the parameters used to predict the transition from isolated RP to SSc

Variables	SDHR (95% CI)	$\chi^2$	P
Capillaroscopic			
Number of capillaries (linear, non-linear)		31.09	<0.001
Giant loops (present vs absent)	14.45 (7.02, 29.75)	52.51	<0.001
Haemorrhages (present vs absent)	5.41 (2.79, 10.46)	25.11	<0.001
Autoantibodies		35.80	<0.001
ANA <sup>+</sup> vs all Abs <sup>-</sup>	9.70 (2.11, 44.48)	8.55	0.003
ACA <sup>+</sup> vs only ANA <sup>+</sup>	3.94 (1.74, 8.94)	10.76	0.001
ACA <sup>+</sup> vs anti-topo I <sup>+</sup>	1.37 (0.60, 3.14)	0.56	0.452

Abs: antibodies;  $\chi^2$ : Wald's statistic.

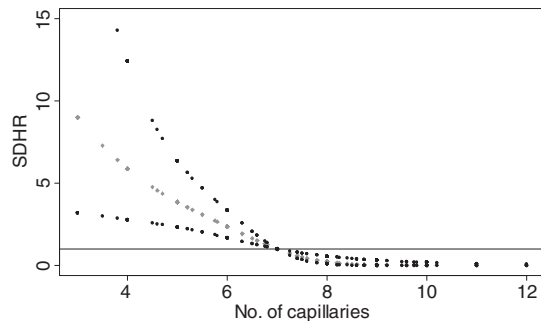
were therefore candidates for being considered relevant prognostic factors: the number of capillaries was included 996 times, the presence of haemorrhages 263 times, the presence of giant loops 947 times and the presence of antibodies 995 times (only ANA<sup>+</sup> vs all antibodies negative 494 times, only ACA<sup>+</sup> or anti-topo I<sup>+</sup> vs only ANA<sup>+</sup> 66 times and both contrasts 435 times).

The three most frequently selected models were: (i) the number of capillaries, the presence of giant loops and ANA<sup>+</sup> vs all antibodies negative (379 times); (ii) the

number of capillaries, the presence of giant loops and isolated ANA<sup>+</sup>, ACA<sup>+</sup> or anti-topo I<sup>+</sup> (274 times); and (iii) the number of capillaries, the presence of haemorrhages, the presence of giant loops and isolated ANA<sup>+</sup> (130 times). No interaction terms were considered in the model because there was no prior hypothesis and the sample size was limited.

Bagging led to a prognostic index that is complex functions of the capillaroscopic and antibody variables. In order to simplify the application of the prognostic index, starting from the original variables, simple rules can be obtained using a regression tree with the prognostic index as the dependent variable and the capillaroscopic and antibody variables as regressors.

As shown in Fig. 4, the variable that better discriminates patients, which differs for prognostic index, is the number of capillaries and, in particular, 8.45 capillaries/mm was the best cut-off chosen by the program algorithm (first tree knot). Each following step is conditioned to this node. Within the group with  $\geq 8.45$  capillaries/mm, the number of capillaries further discriminate the value of prognostic index, while the presence/absence of antibodies is more important with respect to other characteristics within the group with  $< 8.45$  capillaries/mm. In the subgroup with negative antibodies, the number of capillaries needs to be further considered, whereas in the subgroup with positive antibodies, the presence/absence of giant loops are relevant enough and the number of capillaries is not further needed to discriminate patients that

**Fig. 3** SDHR of SSc as a function of capillary number.

The black squares represent the SDHR and the black dots the 95% CIs; the line indicates the reference value of SDHR = 1. The reference value in this graph is 7 capillaries/mm.

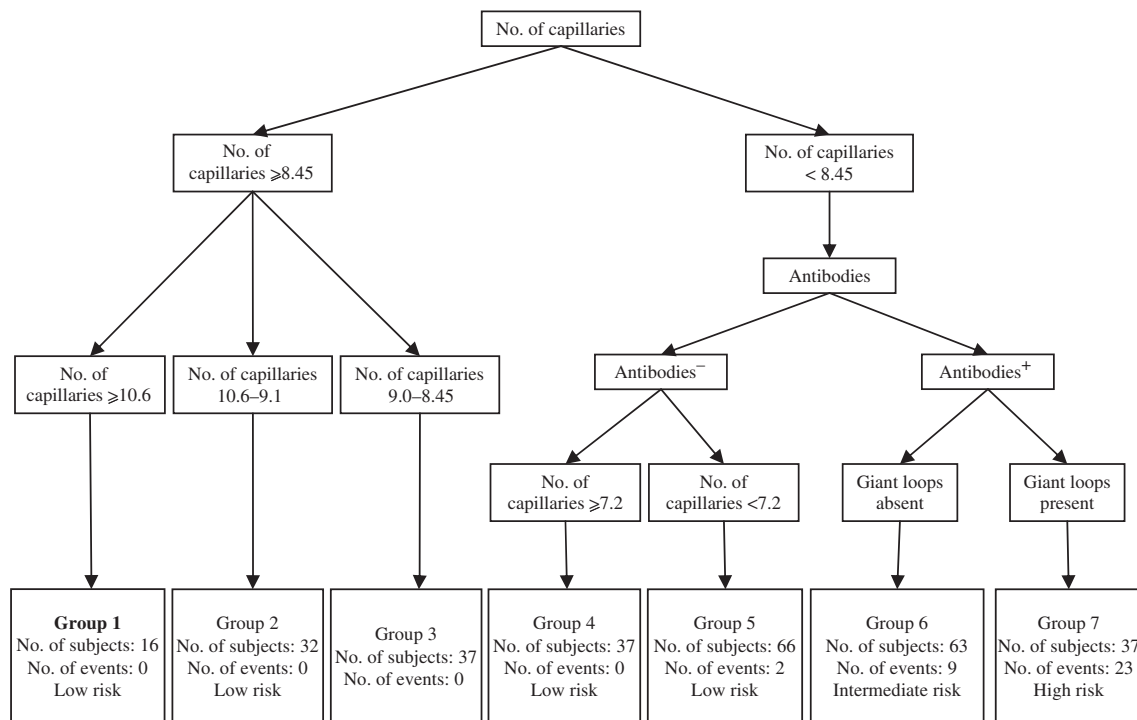
**TABLE 3** Multivariable analyses of the transition from isolated RP to SSc

Variables	SDHR (95% CI)	$\chi^2$ (df)	P
Capillaroscopic			
Number of capillaries (linear, non-linear)		6.50 (2)	0.039
Giant loops (present vs absent)	3.35 (1.38, 8.12)	7.17 (1)	0.001
Haemorrhages (present vs absent)	1.28 (0.64, 2.57)	0.49 (1)	0.49
Autoantibodies		11.75 (2)	0.003
ANA <sup>+</sup> vs all Abs <sup>-</sup>	7.76 (1.63, 36.88)	6.65 (1)	0.01
ACA <sup>+</sup> or anti-topo I <sup>+</sup> vs only ANA <sup>+</sup>	1.79 (0.80, 3.97)	2.03 (1)	0.15

Abs: antibodies;  $\chi^2$ : Wald statistic.



Fig. 4 The regression tree analysis used to build the prognostic classification.



differ for the value of prognostic index. Finally, the regression tree analysis generated seven groups showing the number of events (transition to SSc) in each group.

Then a final classification rule was derived on the basis of the predictive probability to develop SSc within 5 years (Fig. 5): low risk ( $P < 10\%$ ), high risk ( $P > 50\%$ ) and intermediate between 10 and 50%. The prognostic discrimination obtained using this classification rule was very satisfactory: Harrell's C-index = 0.86.

### Discussion

The cornerstone of the various proposed classification criteria of overt SSc is RP [26–29], and the role of a physical examination, nail-fold capillaroscopy and autoimmune serology in classifying SSc subsets is well established [28–30]. Nail-fold capillaroscopy and antibodies are also considered strongly predictive variables of a transition from isolated RP to SSc [7, 10, 13, 27, 31–33].

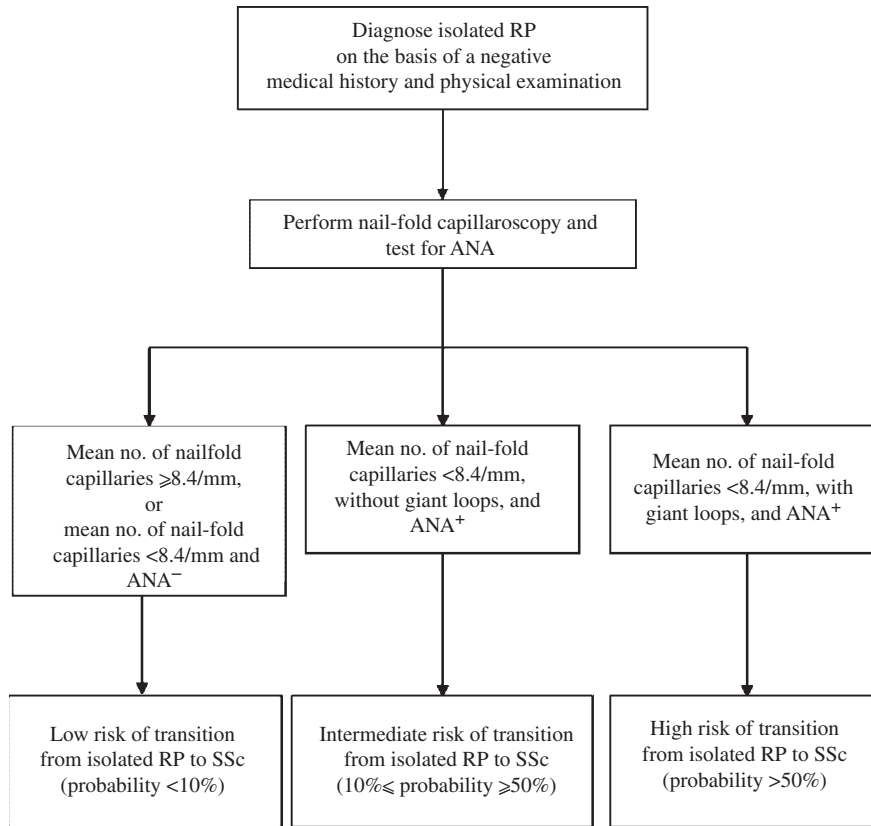
The challenge is to use capillaroscopy and autoantibodies at the time of the first examination in order to identify and stratify subjects with isolated RP who are at risk of transition to SSc. As suggested by previous data [8], we attempted to create a classification rule for staging the 5-year risk of transition to SSc in patients with isolated RP that can be used as a standard for clinical care, and which provides physicians a practical tool for identifying high-risk patients and guiding future monitoring.

Nail-fold capillaroscopy is a widely accepted *in vivo* imaging technique that provides a detailed picture of skin microvasculature. It has been receiving increasingly greater

attention as a potential prognostic tool because it is simple, non-invasive and inexpensive, but the lack of guidelines concerning the interpretation of the most relevant capillaroscopic abnormalities may hinder its widespread use.

In order to overcome the absence of guidelines concerning the recording and interpretation of the results, we chose three easily obtainable capillaroscopic parameters that are widely accepted as being reliable and prognostically useful [19, 34]: the number of capillaries, and the presence/absence of giant loops and haemorrhages. These parameters can be easily and rapidly recorded and interpreted by specialists regardless of their experience, thus overcoming one of the major limitations of capillaroscopy. We have previously used these parameters to develop a prognostic model that accurately predict the development of CTDs [34]. In this study, the multivariable regression analysis showed that the number of capillaries and the presence of giant loops have greater prognostic relevance, whereas the presence of haemorrhages proved to be generally limited and not significant. This is perhaps not surprising because haemorrhages are not an isolated SSc-specific finding, but may be an aspecific expression of the capillary wall weakness that can be observed in various CTDs.

As it seems logical that patients at risk of developing an autoimmune disease are more likely to produce autoantibodies than healthy subjects, we concentrated on the presence of SSc-related antibodies. It is interesting to note that our results are in line with other published findings [7–9, 13, 14], suggesting that ANA positivity can identify a cohort of Raynaud's patients at increased risk of

**Fig. 5** Decision tree for stratifying RP on the basis of the risk of developing SSc.

developing SSc and that, in some cases, their presence predates the onset of clinical disease.

One possible explanation for the much stronger prognostic impact of ANAs in comparison with ACA or anti-topo I antibodies observed in this study is that, although we studied isolated RP, we did not take its duration into account, which may be considered a selection bias. However, our aim was to develop a practical and generalizable algorithm using a limited number of variables because only the more severe cases of RP tend to reach clinical notice, and the diagnostic screening of RP may be handled by physicians with different skills. For these reasons, we studied a highly heterogeneous cohort of subjects who had attended our highly specialized outpatient clinic.

Another possible limitation of the study is that follow-up compliance was sub-optimal. However, the patients who provided complete data (i.e. capillaroscopy, antibodies and follow-up) appeared to be representative of the overall study population in terms of outcomes, and the large sample ( $n = 288$ ) of homogeneous RP patients with sufficient follow-up data allowed an adequate analysis for the purposes of risk staging. Furthermore, we first applied a bootstrap resampling procedure to investigate model selection instability [23], and used this information for model averaging purposes [35].

Finally, we created a model capable of predicting RP outcome on the basis of an empirically weighted combination of three easily obtainable prognostic variables (the number of capillaries, the presence or absence of giant loops obtained by capillaroscopy and the result of an ANA test), and the prognostic discrimination was very satisfactory (Harrell's C-index = 0.86).

The search for predictors of SSc is important in the context of personalized medicine, and our algorithm can help physicians to plan an appropriate clinical strategy with respect to the risk of transition to SSc. If future studies confirm our findings, our algorithm could be used to facilitate clinical decision making in the screening phase, and may also have important implications for stratifying patients into risk groups for future clinical assessment.

#### Rheumatology key messages

- Capillaroscopy and ANAs were used to build a weighted prognostic screening model for RP.
- Our algorithm could help to stratify the risk of transition to SSc within 5 years.
- Clinical decision making in isolated RP could be facilitated by using the prediction model.

*Disclosure statement:* The authors have declared no conflicts of interest.

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