Quantitative Alterations of Capillary Diameter Have a Predictive Value for Development of the Capillaroscopic Systemic Sclerosis Pattern

Amelia Chiara Trombetta, Vanessa Smith, Carmen Pizzorni, Marianna Meroni, Sabrina Paolino, Caterina Cariti, Barbara Ruaro, Alberto Sulli, and Maurizio Cutolo

ABSTRACT. ***Objective.*** To quantify earlier capillary diameter abnormalities, observed by nailfold videocapillaroscopy (NVC), in primary Raynaud phenomenon (PRP) subjects compared with RP subjects later evolved to systemic sclerosis (SSc)-associated secondary Raynaud phenomenon (SRP).

Methods. There were 6112 NVC images of 191 subjects analyzed at baseline and after a mean followup of 42.77 ± 35.80 months. We selected 48 patients affected by SRP and 143 matched controls confirmed with PRP. The diameter of the most dilated limbs (arterial, venous, and apical) was measured in 16 images per subject. Statistical analysis was performed using nonparametric tests. The threshold values for capillary diameters associated with the development of SSc-associated SRP were determined through receiver-operating characteristic curves.

Results. Mean capillary diameter values were significantly different for arterial, venous, and average diameters (mean value of arterial, venous, and apical) between patients with PRP and SRP ($p < 0.0001$). These alterations were found to be independent predictors for disease development ($p = 0.015$). Threshold values of 30 μm (area under the curve = 0.802, sensitivity/specificity = 0.85/0.63) to 31 μm were identified for average, arterial, and venous diameters, with a shortening effect on time to disease development.

Conclusion. The study showed that capillary diameter is an independent predictor for development of SSc-associated SRP. Progression to SRP is unlikely for subjects affected by RP when average capillary diameter is under 30 μm. Subsequently, the execution of the qualitative/quantitative integrated analysis should be part of the NVC followup of RP subjects. (J Rheumatol First Release February 1 2016; doi:10.3899/jrheum.150900)

Key Indexing Terms:
RAYNAUD PHENOMENON SYSTEMIC SCLEROSIS EARLY SCLERODERMA PATTERN
NAILFOLD VIDEOCAPILLAROSCOPY MICROVASCULAR DISEASE EARLY DIAGNOSIS

Raynaud phenomenon (RP) is elicited by an abnormal microvascular response to temperature changes or emotional factors, most often localized in the body extremities[1]. RP is classified as primary (PRP) or secondary (SRP) when associated to another clinical condition, i.e., a connective tissue disease (CTD)[2].

Nailfold videocapillaroscopy (NVC) is a reliable, safe, and inexpensive method to qualitatively differentiate PRP from SRP, allowing early diagnosis, at least in systemic sclerosis (SSc)[3]. NVC alterations are also recognized by capillaroscopic analysis in other CTD (dermatomyositis, mixed CTD, systemic lupus erythematosus)[4,5,6].

Distinct morphological patterns on NVC and a gradual increase in severity of microvascular abnormalities are

From the Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, Genoa, Italy; Department of Rheumatology, Ghent University Hospital, Ghent University, Ghent, Belgium.

V. Smith is supported by a research grant of the Research Foundation – Flanders (Belgium; FWO; grant number 1.5.217.13N) and is Senior Clinical Investigator of the FWO.

A.C. Trombetta, MD, PhD, Trainee in Clinical and Experimental Immunology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; V. Smith, MD, Associate Professor of Rheumatology, Department of Rheumatology, Ghent University Hospital, Ghent University; C. Pizzorni, MD, PhD, Assistant Professor of Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; M. Meroni, MD, Trainee in Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; S. Paolino, PhD, Assistant Professor of Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; C. Cariti, MD, Trainee in Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; B. Ruaro, MD, Trainee in Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; A. Sulli, MD, Associate Professor of Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa; M. Cutolo, MD, Full Professor of Rheumatology, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa.

Address correspondence to Dr. M. Cutolo, Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genoa, Viale Benedetto XV, no. 6, 16132 Genoa, Italy. E-mail: mcutolo@unige.it

Accepted for publication November 25, 2015.

observed during clinical progression of SSc and seem to reflect the evolution of the disease process[7,8,9]. The American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) introduced capillaroscopic findings among the SSc classification criteria, resulting in improved sensitivity/specificity when compared with the 1980 ACR criteria[10].

Patients with RP, before transition to SRP and development of NVC scleroderma pattern, may exhibit either a normal capillaroscopic pattern, or minimal or nonsignificant microvascular changes[11]. On the other hand, tortuous and crossed capillaries may occur in high prevalence within a normal population[4].

The aim of the research was to evaluate differences in larger capillary diameter frequencies and average measures at baseline NVC evaluation in PRP subjects compared with RP subjects later evolved to SSc-associated SRP. To this purpose, we defined patients with RP who later developed clinical features of definite SSc as cases (among whom there was always RP and an NVC SSc pattern). This population was compared with the baseline larger vessel evaluation of a control group of patients with PRP who did not progress to SRP during the followup.

Further, we studied the probability of evolution for RP subjects, based on larger vessel measures at baseline, searching for capillary diameter threshold values predictive for development of SSc signs and symptoms[12].

MATERIALS AND METHODS

Study design. We performed a retrospective case-control study, evaluating a dataset of 6112 NVC images of subjects presenting with RP from baseline. From this group we selected as cases, patients later diagnosed with SRP, developing clinical signs of a definite SSc, among which an NVC SSc pattern. A greater number of patients confirmed with isolated RP was chosen as controls. Therefore, we selected 191 subjects initially referred to our outpatient unit for NVC analysis (baseline): 48 patients with SRP and 143 patients with PRP. Each patient with SRP was matched with 3 PRP for sex, age, RP duration, comorbidities, and drug assumption.

Cases and controls were evaluated semiannually for an average followup of 42.77 ± 35.80 months. This was the average elapsed time from baseline until reaching the study endpoint, i.e., fulfillment of criteria for SSc diagnosis and development of NVC SSc pattern (SRP), or until the end of the study in February 2015 (PRP). RP diagnosis was established in presence of typical clinical symptoms, including discoloration of skin of fingers and/or toes induced by cold or stressful conditions.

Patients diagnosed with SSc met the 2001 LeRoy or 2013 ACR/EULAR classification criteria[10]. Moreover, we selected patients presenting at baseline with PRP, i.e., without signs and symptoms of CTD and no abnormal NVC. Being an NVC-based study, we included as cases only those patients who developed NVC alterations among other criteria for definite SSc. We excluded patients who developed CTD other than SSc or overlap syndromes (Table 1).

Informed consent was obtained from all subjects attending the Scleroderma Clinic during ordinary evaluations, approved by the ethical committee (University of Genoa).

Laboratory evaluation included measurement of the most common antibodies found in SSc: total antinuclear antibodies (ANA), as well as the antitopoisomerase I (anti-Scl70), and anticentromere antibodies (ACA). ANA were detected by indirect immunofluorescence using HEp-2 cells as substrate (Euroimmun). Anti-Scl70 antibodies and ACA were evaluated using an ELISA (Euroimmun). The same serologic methods were applied during the entire followup period.

Process of NVC. Each subject was inside the building for a minimum of 15 min at room temperature of 20–22°C before the NVC. Nailfolds of the second, third, fourth, and fifth fingers of both hands were examined in each patient. A single operator (PC) performed all NVC examinations using an optical probe with magnification 200× contact lens connected to image analysis and storage software (VideoCap 8.14, DS MediGroup). The operator had no prior information of a patient's clinical conditions. Images were then semiquantitatively scored for each patient at baseline and at the end of followup. Results were separately controlled by 2 experts, such as described in previous studies (SA, CM) with an interrater and intrarater proportion of agreement of 90% and 96%, respectively[12].

The following qualitative capillaroscopic variables were considered according to previous observations: presence of enlarged and giant capillaries, bleedings, capillary loss, disorganization of microvascular array, and abnormally shaped capillaries[13,14].

Capillaroscopic variables were defined as follows: (1) capillary dilation: increased capillary diameter (homogeneous or irregular) > 20 μm, (2) giant capillary: homogeneously enlarged loops with normal shape and diameter > 50 μm, (3) microhemorrhage: dark mass attributable to hemosiderin deposit, and (4) capillary loss: reduction of capillary number below normal range (normal range was adopted from our confirmed previous studies: average of 9 capillaries per linear millimeter, counted at distal row)[4,5,11,14,15].

Quantitative evaluation of dilations. A single observer (TAC) semiautomatically measured, in every image, the diameter of the most dilated loop limb (arterial, venous, and apical), analyzing at least a total of 16 nailfold images per patient. The rater marked the diameter manually and the NVC software calculated the length (VideoCap 8.14, DS MediGroup). The average value for each site-specific diameter abnormality (arterial, venous, apical) and the total average diameter value of all observed larger dilations were calculated in each subject.

With capillary diameter, we meant the functional vascular lumen, i.e., the real space where red blood cells flow. Therefore, the quantitative evaluation of capillary dilations by NVC is conventionally done by measuring the space between the 2 parallel layers of the vessel wall, regardless of the thickness of the wall itself.

Statistics. Statistical analysis was performed using IBM SPSS Statistics 20 (IBM Corp.). Descriptive statistic was used to determine the basic population features. The chi-square test was used to analyze the statistical differences between categorical variables. The Mann-Whitney U test was used to calculate differences between patients and controls in continuous variables. Univariate logistic regression was used to identify each predictor for disease development. Multiple logistic regression was done by inclusion of each variable with a $p < 0.1$. Time to event was calculated by Kaplan-Meier curves and expressed elapsed time to SSc diagnosis (from first observation or from RP onset) in patients with SRP. Receiver-operating characteristic (ROC) curves were used to determine cutoff values for average, apical, arterial, and venous capillary diameters associated with disease development. Kaplan-Meier curves for patients with SRP were split into 2 groups: below or above the threshold value. A p value < 0.05 was considered statistically significant.

RESULTS

We studied a population of 191 subjects: 143 PRP and 48 SRP.

Both groups were uniform in sex distribution, mean age, RP duration, and comorbidities, as a consequence of matched selection (Table 2). Comorbidities mainly included hypertension (26 PRP and 15 SRP), hypothyroidism (22 SRP and 8 PRP), osteoporosis (16 PRP and 8 SRP), dyspepsia (12 PRP

Table 1. Inclusion criteria for PRP and SRP subjects.

Baseline Patient Characteristics + Confirmed PRP Patient Characteristics	SRP, Characteristics at End of Study
Raynaud Phenomenon	
Negative NVC	NVC SSc pattern
No clinical signs of SSc or other CTD	Any other clinical sign of SSc, no clinical sign of other CTD
Negative ANA	Negative or positive ANA
Negative ENA	Negative or positive ENA

RP: Raynaud phenomenon; PRP: primary RP; SRP: secondary RP; NVC: nailfold videocapillaroscopy; SSc: systemic sclerosis; CTD: connective tissue disease; ANA: antinuclear antibodies; ENA: extractable nuclear antigens.

Table 2. Clinical characteristics of all RP subjects at baseline and in patients with PRP and SRP. Values are n (%) and mean ± SD unless otherwise specified.

Clinical Variables	Total Population at Baseline, n = 191	RP Groups		p
		PRP, n = 143	SRP, n = 48	
Sex	Male, 4 (2) Female, 187 (98)	Male, 3 (2) Female, 140 (98)	Male, 1 (2) Female, 47 (98)	NS
Age, yrs	49.97 ± 6.90	50.00 ± 16.14	49.70 ± 17.52	0.94
RP duration at baseline, yrs	7.70 ± 6.98	7.68 ± 7.04	7.75 ± 6.87	0.98
ANA	27 (14)	0	27 (56.2)	< 0.0001
ENA	20 (10.4)	0	ACA = 15 (55.5% of ANA+) TOPO-I = 5 (18.5% of ANA+)	< 0.0001
Comorbidities	121 (63)	87 (60)	34 (70)	0.13
Any treatment*	131 (68)	93 (65)	38 (79)	0.13

* Detailed list of treatments not shown (available). RP: Raynaud phenomenon; PRP: primary RP; SRP: secondary RP; ANA: antinuclear antibodies; ENA: extractable nuclear antigens; ACA: anticentromere antibodies; TOPO-I: antitopoisomerase I; NS: not significant.

and 4 SRP), dyslipidemia (13 PRP and 3 SRP), and osteoarthritis (5 PRP and 2 SRP). No subject had any other comorbidity or none were taking any treatment recognized to interfere with microvascular morphology (Table 2).

Almost all patients with SRP were diagnosed with SSc before 2013 (only 2 after), so the vast majority of patients were diagnosed according to the LeRoy 2001 criteria. All had negative or aspecific baseline NVC findings. The second time (NVC-based selection method), they evolved to an SSc pattern. In the vast majority (41/48 = 85.4%), the first pattern identified was the "early" SSc pattern, and a smaller percentage developed an "active" SSc pattern (7/48 = 14.6%). There were 27 patients (56.2% of cases) who were ANA-positive. In particular, 55.5% (15 patients) of ANA-positive patients had ACA and 18.5% (5 patients) had antitopoisomerase I (TOPO-I or Scl-70) antibodies positivity.

There was no significant difference in frequency of apical dilations (29% in PRP vs 25% in SRP, p = 0.43) and in apical dilation diameter values (31 ± 6 μm in PRP vs 32 ± 6 μm in SRP, p = 0.07) between patients with PRP and SRP (Table 3). On the contrary, a statistically significant difference was observed regarding arterial branch dilation frequency (77.6% in PRP vs 93.7% in SRP, p < 0.0001; Table 3). A significant difference was also detected for arterial branches diameter values (27 ± 5 μm in PRP vs 35 ± 8 μm in SRP, p < 0.0001; Table 3).

Similarly, regarding venous branch dilation frequency, a significant difference was detected (77.6% in PRP vs 87% in

Table 3. Apical, venous, and arterial branches capillary dilation frequencies and values in PRP and SRP subjects. Values are n (%) and mean ± SD unless otherwise specified.

Site-specific Dilations	Frequency in Total Population	Frequency in RP Groups and Total Population		p	Diameter in Total Population, μm, n = 191	Diameter in RP Groups, μm		p
		PRP, n = 143	SRP, n = 48			PRP, n = 143	SRP, n = 48	
Apical dilations	54/191 (28.3)	42/143 (29)	12/48 (25)	0.43	31 ± 6	31 ± 6	32 ± 6	0.07
Arterial dilations	156/191 (81.6)	111/143 (77.6)	45/48 (93.7)	< 0.0001	29 ± 7	27 ± 5	35 ± 8	< 0.0001
Venous dilations	153/191 (80)	111/143 (77.6)	42/48 (87)	< 0.0001	31 ± 6	28 ± 4	37 ± 6	< 0.0001
Total dilations		153/191 (80)		NA	30 ± 5	29 ± 4	35 ± 6	< 0.0001

RP: Raynaud phenomenon; PRP: primary RP; SRP: secondary RP; NA: not applicable.

SRP, p < 0.0001; Table 3). Moreover, diameter values for venous branch dilations were significantly different (28 ± 4 μm in PRP vs 37 ± 6 μm in SRP, p < 0.0001; Table 3).

The average diameter value of the site-specific dilations together (apical, arterial, and venous) was found to have a statistically significant difference between the 2 groups (29 ± 4 μm in PRP vs 35 ± 6 μm in SRP, p < 0.0001, Figure 1).

A predictive effect of average (p < 0.0001, OR 1.242, 95% CI 1.14–1.34), arterial (p < 0.0001, OR 1.27, 95% CI 1.16–1.4), and venous diameters (p < 0.0001, OR 1.3, 95% CI 1.19–1.44) was observed on disease development by univariate logistic regression. No predictive effect was observed for other variables tested: apical diameter (p = 0.12), age (p = 0.98), sex (p = 0.99), RP duration (p = 0.95), hemosiderin deposits (p = 0.085), tortuosities (p = 0.26), edema (p = 0.22), venous plexus visibility (p = 0.14), comorbidity (p = 0.99), aspirin (p = 0.59), and calcium antagonists (p = 0.98). Consequently, we performed multiple logistic regression, introducing the only variable with a p < 0.1, i.e., presence of hemosiderin deposits, and the matching variables as covariates (age, RP duration, disease duration, comorbidity, and use of aspirin and/or calcium channel blockers). Average capillary diameter showed to be an independent predictor in the equation (p = 0.01, OR 1.2, 95% CI 1.13–1.42).

Patients with RP who reached the endpoint of our study (diagnosed with SSc, having also an NVC SSc pattern of microangiopathy) did it in an average time from baseline of 29.19 ± 26.94 months (2.4 yrs), with a maximum of 123 months (10 yrs; Figure 2.1). The RP duration until reaching the endpoint of the study was on average 127 ± 89 months (10 yrs) with a maximum time of 372 months (31 yrs; Figure 2.2).

ROC curve showed that the average diameter threshold value predictive for evolution toward SSc was 30.08 μm [area under the curve (AUC) = 0.802 (95% CI 0.73–0.87)]; sensitivity and specificity for the cutoff value were 85% and 63%, respectively (Figure 3). Negative predictive value (NPV) was 0.92 whereas the positive predictive value (PPV) was 0.44. ROC curves were also performed for apical, arterial, and venous diameters. The AUC for apical diameter ROC curve was low (AUC = 0.59, 95% CI 0.49–0.69). For the arterial branch diameter, we found a threshold value of 30.73 μm, with a sensitivity/specificity of 79.07% and 73.68%, respectively. The AUC was 0.806 (95% CI 0.72–0.88). For the venous branch dilation, we found a threshold value of 31.2 μm, with a sensitivity of 81% and a specificity of 72% (AUC = 0.86, 95% CI 0.78–0.94; Figure 3).

Kaplan-Meier analysis performed according to the threshold of 30 μm showed a median time to transition of 17 months for patients with SRP with capillary diameter > 30 μm and 24 months for those < 30 μm (p = 0.058, 95% CI 0.9631–1.860; Figure 2.3).

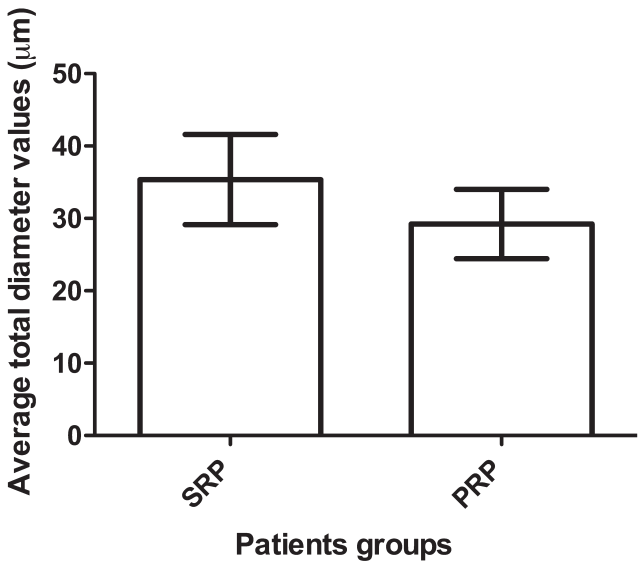

Figure 1. Average diameter values of capillary dilations in PRP and SRP subjects. The mean diameter values of the site-specific dilations together (apical, arterial, and venous branches) were found to have a high statistical difference between PRP and SRP groups (29 ± 4 μm in PRP vs 35 ± 6 μm in SRP, p < 0.0001). PRP: primary Raynaud phenomenon; SRP: secondary Raynaud phenomenon.

DISCUSSION

To date, RP may be present in 5% of healthy subjects, with a prevalence of women, whereas about 90% of patients with SSc and 85% of patients with mixed CTD present RP as an early symptom[15,16].

Various authors have stated that NVC abnormalities could be observed in healthy individuals as well as in PRP subjects[17,18]. A prospective study of 3029 consecutive patients with RP showed that nonspecific and SSc-type capillary changes have a predictive value during followup in patients who developed SSc-spectrum diseases[19]. Ingegnoli, *et al*[20] developed the first prognostic index for identifying, by NVC, patients with RP who were at a higher risk for development of SSc-spectrum disorders. The study demonstrated significant prognostic values for giant loops, microhemorrhages, and capillary number < 7, but not for enlarged loops, branching loops, or capillary disorganization.

Our group did a previous prospective study of 412 patients followed up for 5 years that determined the percentage of patients initially diagnosed with PRP who later developed SRP. Moreover, we showed the percentage of progression from the "early" to other patterns of microangiopathy. In the preliminary study, we gave only a hint of the increased probability of transition in patients with RP presenting at baseline with capillary dilations and slight reduction of capillary number compared with those who did not present such alterations, while not analyzing the data quantitatively[21].

In our present study, patients with SRP were all SSc-related and almost all diagnosed according to the LeRoy criteria (only 2 diagnosed after 2013). There were 85.4% of

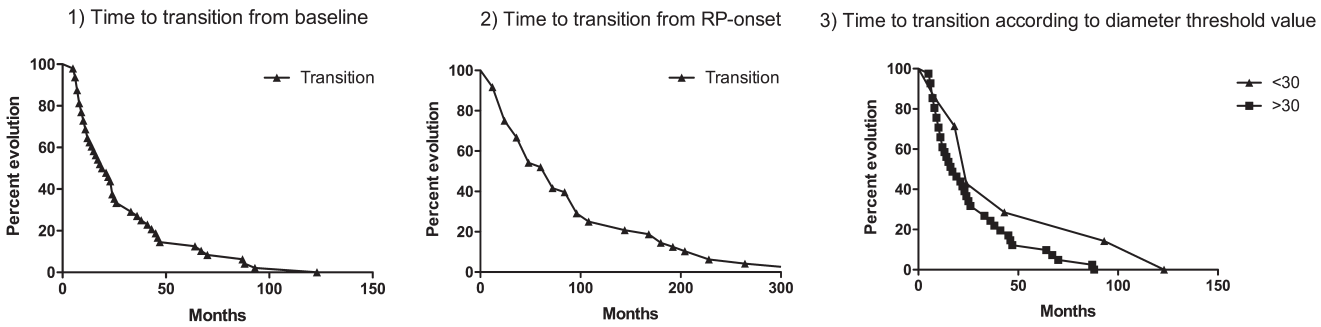

Figure 2. (2.1) Kaplan-Meier curve for time to transition from baseline (first capillaroscopic evaluation) to SSc diagnosis. All our patients with SRP reached the endpoint of the study (SSc diagnosis) in 123 months (10 yrs) from the first NVC (baseline). The average time to transition from baseline to the endpoint was 29.19 ± 26.94 months (2.4 yrs). (2.2) Kaplan-Meier curve for time to transition from RP onset to SSc diagnosis. RP duration until reaching the endpoint of the study in patients with SRP: 127 ± 89 months (10 yrs) on average with a maximum time of 372 months (31 yrs). (2.3) Kaplan-Meier curve for patients with SRP split into 2 groups: below and above the threshold value of 30 μm. Median time to transition of 17 months for patients with SRP with capillary diameter > 30 μm and 24 months for those < 30 μm (p = 0.058, 95% CI 0.9631–1.860). SSc: systemic sclerosis; RP: Raynaud phenomenon; SRP: secondary RP; NVC: nailfold videocapillaroscopy.

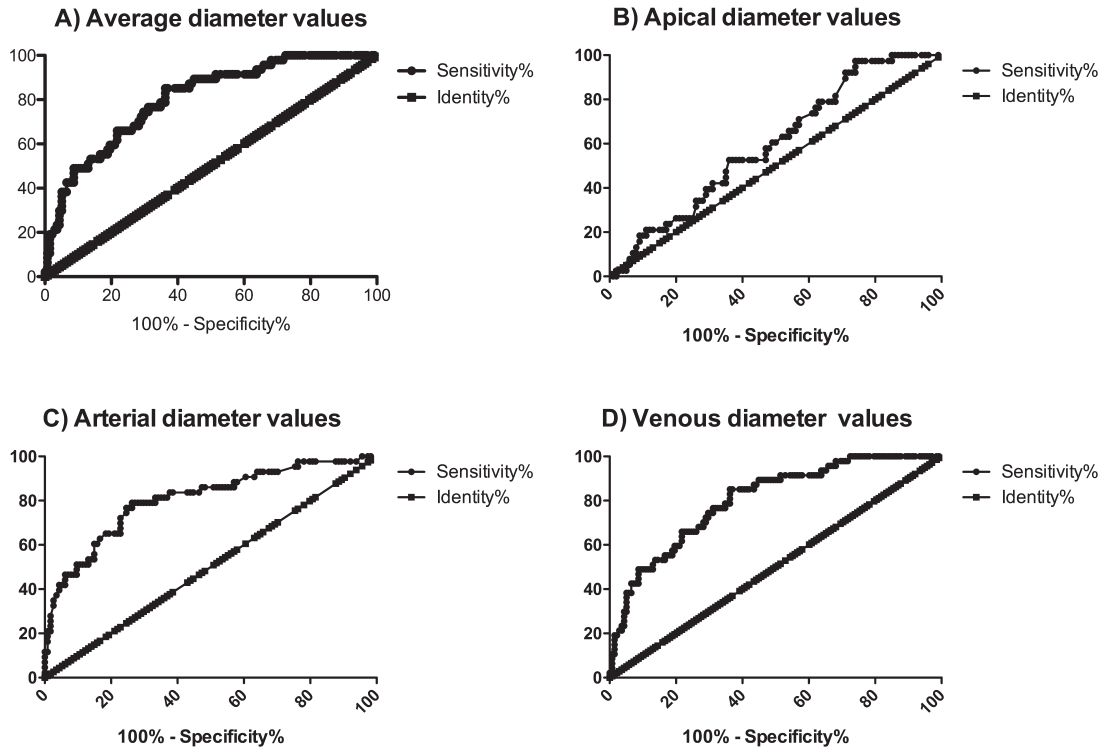

Figure 3. ROC curves were used to determine the predictive cutoff values for (A) average, (B) apical, (C) arterial, and (D) venous capillary diameters associated with the development of the disease and their sensitivity and specificity. The first curve (A) shows that the threshold value for average capillary diameter associated to the evolution toward SSc is 30 μm, with an AUC of 0.802. This value has the maximum sensitivity and specificity among all diameter values observed in the 2 patient populations: 0.85 and 0.63, respectively. The negative predictive value was 0.92, whereas the positive predictive value was 0.44. The AUC for apical diameter ROC curve (A) was low (0.59) with sensitivity of 0.57% and a specificity of 0.53%. For arterial branch diameter (C), we found a threshold value of 30.73 with a sensitivity/specificity of 79.07% and 73.68%, respectively. The AUC was 0.806. For venous branch diameter (D), we found a threshold value of 31.2, with a sensitivity of 81.4% and a specificity of 72.3%. In the last case, the AUC was 0.86. ROC: receiver-operating characteristic; SSc: systemic sclerosis; AUC: area under the curve.

patients identified at NVC analysis in the "early" SSc pattern stage, probably because of the frequent NVC followup of patients with RP in our study.

Our findings show no significant diameter difference at the level of capillary apex between PRP subjects and patients later evolved to SRP. Instead, we found a significant

increased baseline frequency of average, arterial, and venous capillary dilations in patients with SRP.

Moreover, and for the first time, to our knowledge, NVC analysis of diameters allowed the determination of a threshold value with high NPV for reassurance of patients who are probably not going to develop SSc.

Apical dilations could have different pathogenesis but similar values between patients with PRP and SRP. Studies made in healthy subjects have shown that apical capillary pressure results from a balance between precapillary resistance adjustments in response to postcapillary pressure increase[22,23]. In PRP and in the early phases of SRP, functional alterations in physiological upstream/downstream regulatory mechanisms (wall vessel compliance in the venous compartment and vasoconstriction/vasodilation in the arterial one) could cause an increase in capillary pressure and a consequent dilation, located mainly in the apical area for anatomical reasons (higher friction attributable to the shape of the vessel wall). Likely, in subsequent phases leading to established SSc vasculopathy, there is a structural destruction of the vessel wall. This alteration could be typical of the vessels of patients evolving to SSc and related to different immune-pathogenetic factors. The progression of structural damage could also cause, only in those patients evolving to SRP, diameter abnormalities in other areas of the loop (arterial and venous branches).

NVC enlarges 200 times our vision of capillaries, but does not give the possibility to look inside the capillary or to see the structure of capillary walls. The enlargement in the apical area could be due to a greater width of the lumen, but the subsequent arterial and venous wall destruction seems to be characterized by endothelial cell alteration, cellular infiltration, and production of extracellular matrix proteins that could contribute to the higher capillary diameters observed in SSc.

The very early diameter threshold value (over 30 μm) observed here, might represent the structural alteration preceding the uniform capillary loop dilation that underlies the formation of the giant capillaries (over 50 μm), pathognomonic for the "early" scleroderma pattern[12].

We also had threshold values for arterial and venous branches diameters, but we decided to use the average value. In fact, arterial and venous branches threshold values were highly significant but were very similar to each other. In our population, apical diameter also has a tendency to be different between the 2 groups, so for practical reasons it can be included without affecting the significance of the study. Moreover, if diameter abnormalities are present, it would be easier to measure all branches than to distinguish between arterial or venous. Finally, to have a different threshold value for arterial or venous branches involves distinguishing a fraction of a micrometer that is not applicable in actual NVC technologies.

NVC abnormalities in a subject with isolated RP are known to predict for evolution to a CTD within 2 years[24]. With a time to transition of 2.4 years from baseline, our observation confirmed this data, indicating NVC as reliable for the evaluation of RP prognosis. In the context of the "very early" SSc diagnosis and in the direction indicated by the ACR/EULAR 2013 diagnostic criteria, NVC evaluation should be provided with new and more precise quantitative microvascular damage markers to predict the evolution toward more severe organ involvement[25,26,27,28,29]. Interestingly, calculating time to transition according to the threshold of 30 μm, we observed an earlier transition in patients above this threshold at the verge of significance. It would be interesting to observe the performance of this diameter threshold value in prospective studies on larger populations.

The limitations of our study include that it is a single-center study consisting of a relatively small population of patients with PRP and SRP attending a referral institution. Koenig, *et al* observed that among patients who developed definite SSc, the median time to SSc diagnosis was 4.56 years after the onset of RP[9]. The longer RP duration as well as the older age of our patient population could be justified by a selection bias. The selection bias could be amplified because of regional demographic differences (mean age 48.3 yrs in 2015). In addition, our controls are ANA-negative RP subjects with no clinical abnormalities. This is not representative of all RP patients. In particular, we would expect quite a high incidence of ANA positivity. It should be considered that the exclusion of subjects from the control group with antibody positivity made the difference in capillary diameters more likely. However, those choices were necessary to exclude from controls patients potentially on the way to developing a CTD and to identify diameter as a risk factor without confounding effect of other variables. Our present study does not identify likelihood for transition in the general RP population because our groups are not representative of general SRP and PRP populations. However, it describes a tool to reassure patients about the reduced possibility of developing SSc. Also, similar to the general medical literature, our study is not to be taken as a "true" case-control study, but rather a frequency-matched control population study[30,31].

Quantitative analysis of NVC was demonstrated to be of great value for early detection of capillary diameter abnormalities and our present paper quantified that, defining the significance of differences between PRP and SRP[32]. In addition, we measured not only 1 capillary diameter, but differentiated the anatomical site of dilations, observing the differences among apical, arterial, and venous branches to investigate whether an alteration in 1 was more predictive for disease development.

Based on the already assessed relationship between NVC patterns and disease complications, the early detection of microvascular damage together with RP and autoimmune

biomarkers (i.e., autoantibodies) might be used to stratify patients for the risk of development of organ involvement and subsequently to start early therapies[33,34].

For particular anomalies of capillary loops, such as the evaluation of microvascular damage through the measurement of capillary diameters, the integrated quantitative approach seems to give significant results.

Interestingly, our cutoff value has a high NPV but a low PPV so that we might have a relatively high rate of both false-positive and real-negative results. To improve the PPV of the test, it would be useful to integrate it with other tests (qualitative and semiquantitative capillaroscopy, clinical, and laboratory diagnostic examinations).

Early NVC findings might be used as a screening tool for different purposes. In particular, the results of our study showing a high NPV (0.92) of threshold capillary diameter might help to identify patients with RP who will not develop SSc, at least not in the next 4–5 years from baseline.

Immunological data for patients with SRP (serum autoantibodies) seem to represent another reliable tool to monitor the evolution toward SSc[35]. To this effect, ANA, ACA, and TOPO-I reports were consistent in our clinical records with previous literature regarding early SSc stages[13,29].

Capillary diameter can be considered a very early independent predictor for disease development, at least in patients with normal capillaroscopic picture/aspecific findings at baseline.

Moreover, it is less likely for RP subjects with capillary diameters below the cutoff value of 30 μm to develop SSc and an NVC SSc pattern, at least in an average time of 4 years. Conversely, capillary diameter over 30 μm seems to be an independent risk factor for the evolution toward SSc[12].

We therefore suggest qualitative/quantitative integrated analysis during the NVC followup of a subject affected by RP, especially for those patients who present with immunological markers indicative of an SSc diagnosis.

REFERENCES

1. Flavahan NA. A vascular mechanistic approach to understanding Raynaud phenomenon. Nat Rev Rheumatol 2015;11:146-58.
2. LeRoy EC, Medsger TA Jr. Raynaud's phenomenon: a proposal for classification. Clin Exp Rheumatol 1992;10:485-8.
3. Cutolo M, Sulli A, Smith V. Assessing microvascular changes in systemic sclerosis diagnosis and management. Nat Rev Rheumatol 2010;6:578-87.
4. Cutolo M, Sulli A, Smith V. How to perform and interpret capillaroscopy. Best Pract Res Clin Rheumatol 2013;27:237-48.
5. Cutolo M, Smith V. State of the art on nailfold capillaroscopy: a reliable diagnostic tool and putative biomarker in rheumatology? Rheumatology 2013;52:1933-40.
6. Schlager O, Kiener HP, Stein L, Hofkirchner J, Zehetmayer S, Ristl R, et al. Associations of nailfold capillary abnormalities and immunological markers in early Raynaud's phenomenon. Scan J Rheumatol 2014;43:226-33.
7. Sulli A, Ruaro B, Alessandri E, Pizzorni C, Cimmino MA, Zampogna G, et al. Correlations between nailfold microangiopathy severity, finger dermal thickness and fingertip blood perfusion in systemic sclerosis patients. Ann Rheum Dis 2014;73:247-51.
8. Smith V, Riccieri V, Pizzorni C, Decuman S, Deschepper E, Bonroy C, et al. Nailfold capillaroscopy for prediction of a novel future severe organ involvement in systemic sclerosis. J Rheumatol 2013;40:2023-8.
9. Koenig M, Joyal F, Fritzler MJ, Roussin A, Abrahamowicz M, Boire G, et al. 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. Arthritis Rheum 2008;58:3902-12.
10. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum 2013; 65:2737-47.
11. Cutolo M, Pizzorni C, Sulli A. Identification of transition from primary Raynaud's phenomenon to secondary Raynaud's phenomenon by nailfold videocapillaroscopy: comment on the article by Hirschl et al. Arthritis Rheum 2007;56:2102-3.
12. Cutolo M, Sulli A, Pizzorni C, Accardo S. Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. J Rheumatol 2000;27:155-60.
13. Cutolo M, Pizzorni C, Tuccio M, Burroni A, Craviotto C, Basso M, et al. Nailfold videocapillaroscopic patterns and serum autoantibodies in systemic sclerosis. Rheumatology 2004; 43:719-26.
14. Grassi W, Medico PD, Izzo F, Cervini C. Microvascular involvement in systemic sclerosis: capillaroscopic findings. Semin Arthritis Rheum 2001;30:397-402.
15. Heidrich H. Functional vascular diseases: Raynaud's syndrome, acrocyanosis and erythromelalgia. VASA 2010;39:33-41.
16. Kallenberg CG. Early detection of connective tissue disease in patients with Raynaud's phenomenon. Rheum Dis Clin North Am 1990;16:11-30.
17. Ingegnoli F, Gualtierotti R, Lubatti C, Bertolazzi C, Gutierrez M, Boracchi P, et al. Nailfold capillary patterns in healthy subjects: a real issue in capillaroscopy. Microvasc Res 2013;90:90-5.
18. Anderson ME, Allen PD, Moore T, Hillier V, Taylor CJ, Herrick AL. Computerized nailfold video capillaroscopy—a new tool for assessment of Raynaud's phenomenon. J Rheumatol 2005;32:841-8.
19. Pavlov-Dolijanovic S, Damjanov NS, Stojanovic RM, Vujasinovic Stupar NZ, Stanisavljevic DM. Scleroderma pattern of nailfold capillary changes as predictive value for the development of a connective tissue disease: a follow-up study of 3,029 patients with primary Raynaud's phenomenon. Rheumatol Int 2012;32:3039-45.
20. Ingegnoli F, Boracchi P, Gualtierotti R, Lubatti C, Meani L, Zahalkova L, et al. Prognostic model based on nailfold capillaroscopy for identifying Raynaud's phenomenon patients at high risk for the development of a scleroderma spectrum disorder: PRINCE (prognostic index for nailfold capillaroscopic examination). Arthritis Rheum 2008;58:2174-82.
21. Bernero E, Sulli A, Ferrari G, Ravera F, Pizzorni C, Ruaro B, et al. Prospective capillaroscopy-based study on transition from primary to secondary Raynaud's phenomenon: preliminary results. Reumatismo 2013;65:186-91.
22. Mahy IR, Tooke JE, Shore AC. Capillary pressure during and after incremental venous pressure elevation in man. J Physiol 1995;485:213-9.
23. Ubbink DT, Reneman RS, Jacobs MJ. Effects of venous pressure and posture on skin capillary perfusion. Eur J Clin Invest 1999;29:737-43.
24. Wigley FM. Clinical practice. Raynaud's phenomenon. N Engl J Med 2002;347:1001-8.
25. Avouac J, Fransen J, Walker UA, Riccieri V, Smith V, Muller C, et al; EUSTAR Group. Preliminary criteria for the very early diagnosis

of systemic sclerosis: results of a Delphi Consensus study from EULAR Scleroderma Trials and Research Group. Ann Rheum Dis 2011;70:476-81.
26. Smith V, Pizzorni C, De Keyser F, Decuman S, Van Praet JT, Deschepper E, et al. Reliability of the qualitative and semiquantitative nailfold videocapillaroscopy assessment in a systemic sclerosis cohort: a two-centre study. Ann Rheum Dis 2010;69:1092-6.
27. Sebastiani M, Manfredi A, Cassone G, Giuggioli D, Ghizzoni C, Ferri C. Measuring microangiopathy abnormalities in systemic sclerosis patients: the role of capillaroscopy-based scoring models. Am J Med Sci 2014;348:331-6.
28. Maricq HR, Harper FE, Khan MM, Tan EM, LeRoy EC. Microvascular abnormalities as possible predictors of disease subsets in Raynaud phenomenon and early connective tissue disease. Clin Exp Rheumatol 1983;1:195-205.
29. Minier T, Guiducci S, Bellando-Randone S, Bruni C, Lepri G, Czirjàk L, et al. Preliminary analysis of the very early diagnosis of systemic sclerosis (VEDOSS) EUSTAR multicentre study: evidence for puffy fingers as a pivotal sign for suspicion of systemic sclerosis. Ann Rheum Dis 2014;73:2087-93.
30. Niven DJ, Berthiaume LR, Fick GH, Laupland KB. Matched case-control studies: a review of reported statistical methodology. Clin Epidemiol 2012;4:99-110.
31. Conway A, Rolley JX, Fulbrook P, Page K, Thompson DR. Improving statistical analysis of matched case-control studies. Res Nurs Health 2013;36:320-4.
32. Cutolo M, Sulli A, Smith V. Evaluating microangiopathy in systemic sclerosis: what have we learnt and what is left to discover? Expert Rev Clin Immunol 2011;7:395-7.
33. Herrick AL. The pathogenesis, diagnosis and treatment of Raynaud phenomenon. Nat Rev Rheumatol 2012;8:469-79.
34. Ingegnoli F, Ardoino I, Boracchi P, Cutolo M; EUSTAR co-authors. Nailfold capillaroscopy in systemic sclerosis: data from the EULAR scleroderma trials and research (EUSTAR) database. Microvasc Res 2013;89:122-8.
35. Cutolo M, Sulli A. Therapy: Optimized treatment algorithms for digital vasculopathy in SSc. Nat Rev Rheumatol 2015;11:569-71.