Capillary loss reflects disease activity and prognosis in patients with systemic sclerosis

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Abstract. Capillary density on nailfold capillaroscopy (NFC) is considered a promising instrument for assessing disease characteristics in patients with systemic sclerosis (SSc), however, there is no agreement yet over how to analyze and interpret the results. The objective of this study was to investigate the possible associations of the mean number of capillaries with disease characteristics, disease activity [measured by the European Scleroderma Study Group (EScSG) disease activity score] and survival in a single-center cohort of patients with SSc. Sixty-eight patients were included; 54 had follow-up at 6 months. Thirty-two images per patient were assessed independently by two raters, scoring the mean number of capillaries in all fingers (N), in the 3rd finger of the dominant hand (dN_3) and in the 4th finger of the non-dominant hand (ndN_4) for each patient. NFC 'early', 'active' and 'late' patterns were also assessed. Two thousand and seventy-six images were scored at baseline, 1,728 at follow-up. Baseline N was median (IQR) 5.1 (2.7) for rater 1, and 4.9 (1.7) for rater 2, respectively. N was significantly lower in patients with a history of digital ulcers (DUs), vs. those who never had DUs 4.8 (1.4) vs. 6.4 (3.1), P=0.016. A lower N was associated with higher disease activity at baseline and follow-up (linear regression adjusted for age, sex and history of DUs). A lower ndN_4 was associated with increased mortality (logistic regression adjusted for age and sex). In conclusion, in patients with SSc, a lower mean number of capillaries assessed by NFC was associated with higher disease activity after 6 months of follow-up and with shorter survival.

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Introduction

Nailfold capillaroscopy (NFC) is a valuable tool for the early diagnosis of systemic sclerosis (SSc) (1,2) and is included in the 2013 ACR/EULAR classification criteria for SSc (3). The SSc-specific changes observed by NFC are markedly dilated capillaries (giant capillaries) and capillary loss (4). Cutolo *et al* (5) have described three NFC patterns (early, active and late), in which capillary loss is absent, slight and considerable, respectively, while giant capillaries vary from few in the early pattern, to many in the active pattern and to, again, few, or even absent, in the late pattern.

The NFC patterns are currently the mostly used NFC method for assessing the SSc-associated vasculopathy, but recently semi-quantitative and quantitative methods have been gaining more recognition (6-9), especially in patients with already established disease. In these, prospective studies are the most valuable, as they may identify prognostic factors and therefore offer treatment decision aids.

Several prospective studies have shown that the 'late' NFC pattern is an independent predictor for digital ulcers (DUs) (10). Assessments such as the one developed by Smith *et al* (7), based on a semiquantitative mean score of capillary loss over 8 fingers, on a scale from 0 to 3 and using one NFC image per finger, have shown that capillary loss is associated with the presence of severe digital ischemia at 6-12 months follow-up. More recently, in the large, multicentric, prospective CAP study (9), a quantitative method counting all microhemorrhages, all capillaries and all giant capillaries in the distal row has been used. The CAP study identified the mean number of capillaries per millimeter in the middle finger of the dominant hand, together with the number of DUs at enrollment and the presence of critical digital ischemia at enrollment, as the most important risk factors for the development of new DUs over a follow-up time of 6 months (9). Capillary density is an attractive candidate for a prognostic factor (11), also because it showed superior intra- and inter-observer reliability when compared with other quantitatively assessed NFC abnormalities (such as giant capillary counts), as well as to NFC patterns (12).

The aim of this study was to investigate the associations of the mean number of capillaries on NFC, assessed in three

different ways, with disease characteristics such as vascular involvement (DUs or history of DUs), disease activity (measured by the European Scleroderma Study Group (EScSG) disease activity score (13) and survival in a single-center cohort of patients with SSc.

Patients and methods

Sixty-eight patients with SSc, fulfilling the ACR/EULAR 2013 classification criteria (3), were prospectively enrolled from May to December 2016. NFC and extensive assessment per the recommendations of EUSTAR were performed in all patients. Fifty-four patients had a follow-up at 6 months. Moreover, survival status was investigated in all patients at up to 12 months after the end of study. The study was approved by the Cantacuzino Clinical Hospital Ethics Committee (Bucharest, Romania). All patients provided written informed consent for their participation in the study and the publication of the data.

Eight digits were examined (II to V of both hands) by NFC and four images for each finger were saved. All NFC examinations were performed by the same rater (rater 1). Image quality was assessed as good, moderate, poor or very poor.

The NFC images were assessed by two raters (one highly experienced, rater 1 and the other moderately experienced, rater 2) independently, each rater scoring the mean number of capillaries: in all fingers (N), in the 3rd finger of the dominant hand (dN_3) and in the 4th finger of the non-dominant hand (ndN_4) for each patient. dN_3 and ndN_4 were chosen based on previous literature reports suggesting they have the best chances of predicting new DUs, differentiating between primary and SSc associated Raynaud's phenomenon, or detecting NFC abnormalities (9,14,15). Qualitative NFC assessment was also performed, classifying patients into NFC patterns (early, active, late, normal NFC, or unclassifiable in any pattern). Previously published data from our group suggests intra- and inter-rater concordance is good to excellent for semiquantitative scores (with ICC coefficients between 0.745 and 0.923), whereas for qualitative scores it is weaker (Cohen's κ coefficient <0.7) (16).

Disease activity was assessed by the ESScG score 2003 (13), a composite disease activity index based on the modified Rodnan skin score (mRSS), presence of DUs and of arthritis, recent worsening of skin and of peripheral vasculopathy, decreased diffusion capacity of carbon monoxide (DLCO), increased ESR and hypocomplementemia.

IBM SPSS v.20 was used for the statistical analysis. Because of a relatively low number of patients and moderate skewness, values are presented as median (IQR) for continuous variables; categoric variables are presented as number (%). For assessing correlation between the all the mean numbers of capillaries, Spearman's rank correlation coefficient was computed. NFC pattern was considered an ordinal variable (with values from 0 'normal', to 3 'late pattern'), so for correlations with the mean number of capillaries, Spearman's rho and also one-way ANOVA (with the disadvantage that ANOVA does not yield an effect size) were used. Differences of all mean scores of capillaries between patients with and without DUs were assessed by Mann-Whitney U test. Differences in NFC patterns between patients with and without DUs were assessed by Pearson's Chi-square test. Linear or logistic regression was used to test the associations between the mean number of capillaries and NFC patterns at baseline and disease activity or individual disease characteristics at baseline and at 6 months follow-up, while logistic regression was used for capillaroscopy risk factors for survival status at 12 months follow-up. $P \leq 0.05$ was considered statistically significant.

Results

A total of 68 patients were selected for the study, including 7 men and 61 women, with a median (IQR) age of 54.5 (17.0) years and disease duration since first non-Raynaud's symptom 7 (9.0) years; 69.1% had a history of DUs. In total, 2,176 capillaroscopy images were scored at baseline and 1,728 at follow-up (Table I). Visibility at baseline varied from good and moderate in 1244 images (57.2%) to low and very low in 932 images (42.8%). Lack of images was due to flexion contractures of the fingers, or, in very few cases, to digital amputations, while decreased visibility was due to edema or fibrosis.

Baseline N ranged between 3.4-9.1, with a median (IQR) of 5.1 (2.7) for rater 1, respectively 3.3-8.9, 4.9 (1.7) for rater 2; scores for dN_3 and ndN_4 had similar values, and were similar between the 2 raters (Table I).

Analysis of cross-sectional data

Association between qualitative and quantitative NVC assessment, intra- and inter-rater. Intra-rater correlations of N with dN_3 and, respectively, ndN_4 ranged from fair to very strong for both raters, with intra-rater Spearman correlation indices between dN_3 and ndN_4 were 0.620 for rater 1 and 0.423 for rater 2, both $P<0.005$ (Table II).

Inter-rater correlations were good to very good, with inter-rater Spearman's correlation coefficient between 0.68 and 0.94, both $P<0.001$ (Table II). NFC patterns correlated moderately with N, dN_3 and ndN_4 for rater 1 and were poor for rater 2 (Table II).

DUs. At baseline, patients with a history of DUs had significantly lower mean scores of capillaries by rater 1, vs. patients who never experienced DUs (Table III). Thus, there was a trend for significance in the mean number of capillaries in all fingers, $P=0.09$, however, this difference was not found by rater 2. No difference was found for either rater between NFC patterns in patients with and without history of DUs, although there was a trend for statistical significance, $P=0.07$, for rater 1 (by Chi-square, data not shown).

Disease activity. A lower number of capillaries at baseline was associated with a higher disease activity at baseline: in linear regression adjusted for age, sex and history of DUs, dN_3 and ndN_4 for rater 1, respectively N and ndN_4 for rater 2 were inversely associated with the ESScG score at baseline, with also a trend for significance for dN_3 for rater 2 (Table IV).

Significant associations at baseline were also found between the mean number of capillaries and forced vital capacity (percentage of predicted value) (FVC%), modified Rodnan skin score (mRSS) (only for rater 2) and erythrocyte sedimentation rate (ESR) (only for rater 2), but these were not

Table I. Baseline characteristics of the patients.

Variable (n=68)	Median (IQR) or n (%)
Age, years	54.5 (17.0)
Male sex	7 (10.3%)
Disease duration, years	7 (9.0)
dcSSc	22 (34.2%)
DUs history	47 (69.1%)
Telangiectasia	53 (77.9%)
Smokers	9 (13.2%)
EScSG score	2.3 (1.0)
Rater 1	
Early pattern	10 (14.7%)
Active pattern	4 (5.9%)
Late pattern	47 (69.1%)
Normal/unclassifiable in any	2 (2.9%)/5 (7.4%)
NFC pattern	
N	5.1 (2.7)
dN_3	4.6 (2.9)
ndN_4	5.0 (2.0)
Rater 2	
Early pattern	3 (4.4%)
Active pattern	18 (26.5%)
Late pattern	33 (48.5%)
Normal/unclassifiable in any	3 (4.4%)/11 (16.2%)
NFC pattern	
N	4.9 (1.7)
dN_3	5.0 (2.0)
ndN_4	5.0 (1.9)

Values are median (IQR) unless otherwise specified. NFC, nailfold capillaroscopy. N, mean number of capillaries in all fingers; dN_3, mean number of capillaries in the 3rd finger of the dominant hand; ndN_4, mean number of capillaries in the 4th finger of the non-dominant hand; EScSG, European Scleroderma Study Group disease activity score (13).

Table II. Associations between qualitative and quantitative NFC assessment by the same rater, respectively inter-rater.

Item	N rater 1	N rater 2	NFC pattern rater 1[a]
N rater 1	-	0.923	-0.647
dN_3 rater 1	0.848	0.943	-0.644
ndN_4 rater 1	0.894	0.687	-0.516
Item	N rater 2		NFC pattern rater 2[a]
N rater 2	-	-	-0.455
dN_3 rater 2	0.736	-	-0.469
ndN_4 rater 2	0.769	-	-0.265[b]

[a]$P \leq 0.005$; [b]$P \leq 0.01$ as calculated by one-way ANOVA. N, mean number of capillaries in all fingers; dN_3, mean number of capillaries in the 3rd finger of the dominant hand; ndN_4, mean number of capillaries in the 4th finger of the non-dominant hand; NFC, nailfold capillaroscopy.

Table III. Differences in mean number of capillaries in patients with and without history of DUs.

Item	History of DUs range; median (IQR)	Without history of DUs range; median (IQR)	P-value[a]
N rater 1	3.4-8.6; 4.8 (1.4)	3.4-9.1; 6.4 (3.1)	0.016
dN_3 rater 1	2.5-9.8; 4.4 (2.2)	2.3-10.3; 6.3 (3.4)	0.033
ndN_4 rater 1	2.0-9.0; 5.0 (1.8)	2.5-11.5; 5.5 (3.0)	0.017
N rater 2	3.5-7.9; 5.2 (1.8)	3.3-8.9; 6.0 (2.6)	0.090
dN_3 rater 2	2.8-9.3; 4.9 (2.1)	2.8-8.3; 5.8 (3.0)	0.230
ndN_4 rater 2	2.5-9.0; 5.1 (1.6)	3.5-9.3; 5.5 (2.8)	0.450

[a]Mann-Whitney U test. N, mean number of capillaries in all fingers; dN_3, mean number of capillaries in the 3rd finger of the dominant hand; ndN_4, mean number of capillaries in the 4th finger of the non-dominant hand.

significant anymore at the 6-month follow-up (linear regression adjusted for age, sex and history of DUs). No association was found with other disease characteristics at baseline, including (but not limited to) existence of interstitial lung disease (ILD), percentage of diffusing capacity for carbon monoxide (DLCO%), smoking, antibodies, calcinosis cutis (data not shown).

Analysis of follow-up data

DUs. There were 12 patients who had new DUs at the 6-month follow-up visit; all of them also had a history of DUs. No difference was found between mean number of capillaries at baseline and incident of DUs after 6 months of follow-up (data not shown).

Disease activity. A lower number of capillaries at baseline was associated with a higher disease activity also at 6 months: in linear regression adjusted for age, sex and history of DUs, N and dN_3 for rater 2 were inversely associated with the ESScG score at 6-month follow-up (Table IV). In addition, there was a trend for significance for dN_3 for rater 1 and ndN_4 for rater 2. NFC patterns were not significant for either rater. We did not find any association of any baseline mean number of capillaries with separate disease characteristics at follow-up.

Survival. For both raters, ndN_4 was associated with survival status of up to 12 months post end-of-study: in logistic regression adjusted for age and sex, lower ndN_4 was associated with increased mortality, with an odds ratio (95% confidence interval) of 2.6 (1.04, 6.29) for rater 1 and of 3.2 (1.09, 9.60) for rater 2. In fact, the median (IQR) ndN_4 was 5.0 (1.9) capillaries

Table IV. Association between the mean number of capillaries at baseline and EScSG disease activity score at baseline and at follow-up.

	EScSG disease activity score at baseline		EScSG disease activity score at follow-up	
Predictors	B (95% CI)	P-value	B (95% CI)	P-value
N rater 1	-0.22 (-0.50, 0.10)	0.141	-0.03 (-0.40, 0.34)	0.866
dN_3 rater 1	-0.26 (-0.51, -0.02)	0.037	-0.23 (-0.47, 1.0)	0.056
ndN_4 rater 1	-0.26 (-0.48, -0.05)	0.017	-0.11 (-0.34, 0.12)	0.346
N rater 2	-0.51 (-0.82, -0.20)	0.002	-0.45 (-0.834, -0.07)	0.022
dN_3 rater 2	-0.20 (-0.43, 0.02)	0.069	-0.33 (-0.62, -0.03)	0.032
ndN_4 rater 2	-0.35 (-0.62, 0.09)	0.011	-0.27 (-0.57, 0.02)	0.070

Linear regression adjusted for age, sex and history of DUs; dependent variable EScSG disease activity score at baseline and at follow-up; DUs, digital ulcers; N, mean number of capillaries in all fingers; dN_3, mean number of capillaries in the 3rd finger of the dominant hand; ndN_4, mean number of capillaries in the 4th finger of the non-dominant hand; EScSG, European Scleroderma Study Group disease activity score (13).

for survivors vs. 3.3 (2.9) for deceased for rater 1, respectively, 5.5 (1.8) vs. 3.5 (2.4) for rater 2.

NFC patterns by either rater were not associated with survival. Causes of death included interstitial lung disease (2 cases), cardiac arrhythmias (3 cases), mesenteric infarction, intestinal occlusion and esophageal neoplasia (one case each).

Discussion

Our results show that a decreased mean number of capillaries per millimeter is associated with a history of DUs at baseline and with higher disease activity and decreased survival at follow-up.

Unsurprisingly, we found very good correlations between the mean number of capillaries in all fingers with mean number in the middle finger of the dominant hand and the ring finger of the non-dominant hand. There was good to moderate correlation with the NFC pattern.

Significant differences were found in all mean capillary scores between patients with and without history of DUs at baseline, suggesting that they could be used alternatively depending on the context (i.e., the total mean score per patient in a research context and the mean score per finger in a clinical context). We did not find any associations with the development of DUs at 6 months follow-up, in contrast to other studies in the literature such as the much larger previous CAP study (9), which found that a low mean number of capillaries in the middle finger of the dominant hand is associated with development of new DUs at 6-month follow-up. This was expected, however, since our study included a much smaller number of patients than the CAP study (which analyzed 468 patients with SSc). Another potentially important difference is that, in the CAP study, quantitative capillary density was included in a score together with the number of DUs at baseline (the strongest risk factor) and the presence of critical digital ischemia also at baseline, a combination that had the best discriminatory ability (9). However, creating a composite score which includes capillary density was not the aim of our study.

A recent study found in a cross-sectional analysis that capillary loss and avascular areas were associated with the presence and severity of ILD in a cohort of 48 SSc patients (17). An association was also found at baseline of the mean number of capillaries with FVC% (patients with lower number of capillaries had lower FVC%), but not at follow-up. No association was found with other evidence of pulmonary involvement (existence of ILD, value of DLCO%) either at baseline or at follow-up. But we should mention a difference in methodology (they did not use quantitative assessment) that could explain these results. Moreover, any association by linear or logistic regression with other disease characteristics at baseline or at follow-up was scarce (association with mRSS and ESR for rater 2 only at baseline). However, when disease characteristics were included in a composite index such as the EScSG, we found an influence: one of the most interesting results from our study is that the mean number of capillaries was associated with disease activity at baseline and this relationship continued at 6-month follow-up. We consider this result also underlines the importance of using a composite index to measure disease activity in SSc, which has better performance than its separate components, and thus greater power to detect significant associations.

A score based on the total number of microhemorrhages and of giant capillaries observed in a subject (mNEMO) (18), has been shown to substantially associate with disease activity in SSc, assessed by the EScSG (13), as it was also in our study. However, in our cohort we had many patients with NFC 'late' pattern, thus not so many giant capillaries and hemorrhages were present. For these patients, counting capillaries seems more feasible than counting microhemorrhages or giant capillaries.

Finally, we found that a lower mean number of capillaries in the ring finger on the non-dominant hand was associated with increased mortality in a logistic regression analysis, adjusted for age and sex; in other words, patients with less capillary loss had better survival. There are several studies suggesting that characteristics reflecting severe disease, such as diffuse cutaneous subset, dyspnea, elevated systolic pulmonary arterial pressure, proteinuria or history of DUs are the most important

risk factors for decreased survival (19-21). Our results may in fact suggest that vasculopathy (evaluated by capillaroscopy) is a surrogate marker for patients with a more severe disease. It is unclear why the low mean number of capillaries, which generally performed better in our analysis, was not associated with decreased survival, however, better visibility for the ring finger of the non-dominant hand might play a role.

There are several aspects of strength in our study, such as having a real-life cohort of patients, a homogeneous capillaroscopy assessment (all evaluations were performed by rater 1) and a high number of capillaroscopy images that allowed several comparisons.

Limitations of our study include the small number of patients and short follow-up duration. It is possible that 6 months is not sufficient time to detect significant capillaroscopy associations, especially since ours is not an enriched cohort of early onset, rapidly progressing SSc patients. We registered a high number of deaths in a relatively short period of time (up to 12 months post end-of-study); these were in patients with more severe forms of SSc, so another limitation would be that these patients were not excluded from enrollment. Moreover, low visibility (cca. 40% of the baseline images) did not allow for an accurate evaluation of capillaroscopy images in some cases, especially when calculating the mean number of capillaries per patient, but this is a limitation of real-life cohorts. There was also a difference in rater experience, which led sometimes to inconsistent results between the 2 raters, raising the issue of interrater reliability; however, this is in line with other data from literature (22-24). In addition, we have previously published a report (16) where we showed good reliability between these same 2 raters for (semi)quantitative NFC assessment, so we think these results reflect real-life cohorts.

These results do not allow us to draw strong conclusions on the optimal method of assessing the mean number of capillaries. However, we found multiple associations with disease activity and severity markers for each type of assessment, which confirms the importance of counting capillaries. Our results are encouraging, and they should be reproduced in larger cohorts, and with longer follow-up periods.

In conclusion, in a single center cohort, we found that the mean number of capillaries, especially the total mean number per patient, show good performance in discriminating between patients with and without history of DUs, and in evaluating high disease activity and risk of death at follow-up.

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Availability of data and materials

The dataset used and/or analyzed during the current study is available from the corresponding author on reasonable request.

Authors' contributions

All authors participated in the design of the study. AMG, IA, LM, MB and CM collected the data. AMG and RO collected the data and performed the capillaroscopy scoring. AE and OL were partners in the UEFISCDI PN-II-PT-PCCA-2013-4-1589 research grant. AMG and CM conducted the statistical analyses. MB, VS and CM provided expert advice where needed. AMG drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Cantacuzino Clinical Hospital Ethics Committee (Bucharest, Romania). All patients provided written informed consent for their participation in the study and the publication of the data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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