Differential performance of nailfold video capillaroscopic parameters in the diagnosis and prognosis of systemic sclerosis

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Received on February 27, 2019; accepted in revised form on May 6, 2019.

Clin Exp Rheumatol 2020; 38 (Suppl. 125): S29-S39.

Key words: systemic sclerosis, Raynaud's phenomenon, nailfold video capillaroscopy, capillaroscopy pattern, semi-quantitative capillaroscopic scores

Funding. this work was supported by the Hellenic Society for Rheumatology.

Competing interests: V.K. Bournia has received a grant from the Hellenic Society for Rheumatology. The other co-authors have declared no competing interests.

ABSTRACT

Objective. *The contribution of nailfold video capillaroscopy (NVC) in identifying patients with Raynaud's phenomenon (RP) at risk for systemic sclerosis (SSc) is well established. Herein we comparatively assess the performance of different capillaroscopic parameters in diagnosing SSc among patients with RP and evaluate the prognostic capacity of NVC in SSc.*

Methods. *At baseline we clinically and capillaroscopically evaluated 242 consecutive patients referred to our department for NVC (138 with SSc); 175 were reevaluated after 3.38±1.47 years. Sixty-two healthy volunteers served as controls. Capillaroscopy pattern (normal/early/active/late) was qualitatively defined. Capillary loss, dilated, giant or ramified capillaries and micro-haemorrhages were scored semi-quantitatively.*

Results. *Capillary loss score had the highest diagnostic accuracy at discriminating patients with an SSc-spectrum disorder from patients with RP of different etiology and controls, as defined by ROC curve analysis [AUC (95% CI)=0.905 (0.869–0.942)], followed by dilatation score [0.863 (0.818–0.907)] and giant score [0.835 (0.787–0.884)]. By contrast, micro-haemorrhages [0.720 (0.662-0.779)] and ramifications scores [0.604 (0.539-0.670)] performed worse. Multivariate analysis in 94 SSc patients indicated that active (OR=3.305, p=0.043) and late (OR=6.900, p=0.023) baseline capillaroscopy pattern predicted occurrence of a combined adverse disease outcome [forced vital capacity (FVC) deterioration>10% and/or DLCO deterioration>15% and/or mRSS deterioration>3.5 and/or first occurrence of digital ulcers and/or death)] at 3 year follow-up.*

Conclusion. *Dilatation score performs best of all semi-quantitative NVC parameters in diagnosing SSc. In addition, our study confirms earlier reports that worse capillaroscopy pattern at baseline correlates with higher likelihood for adverse prognosis.*

Introduction

Raynaud's phenomenon (RP) is a recurrent vasospastic disorder of fingers and toes, occurring in response to cold or emotional stress. It is characterised by reversible ischaemia of the digits, followed by cyanosis and hyperaemia upon reperfusion. Depending on definition, its prevalence ranges between 3–5% (1). RP can be either primary (pRP) or secondary (sRP), commonly occurring in association with a systemic autoimmune disease. Nailfold video capillaroscopy (NVC) is a non-invasive, inexpensive method that permits direct visualisation of the nail-bed microcirculation in patients with RP and helps differentiate subjects at risk of developing disease of the scleroderma spectrum (2, 3). In fact, capillaroscopic findings were recently added to the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for systemic sclerosis (SSc) (4). Moreover, in patients with an established diagnosis of SSc, a prognostic role for NVC has also been suggested (5). Herein, we aimed to comparatively evaluate the discriminative capacity of different NVC parameters for the diagnosis of SSc-spectrum diseases. Moreover, we longitudinally explored associations of NVC findings with clinical features in SSc patients, in order to assess their consistency over time and attempted to further elucidate the predictive role of NVC in established SSc.

Patients and methods

Study population

Our study enrolled 251 consecutive consenting patients with RP, referred

for basal NVC to our tertiary university rheumatology centre, between January 2011 and January 2016. Nine patients were excluded from the study, due to poor quality of NVC images (bad acquisition). Of the remaining 242 patients, 138 had SSc according to the 2013 ACR/EULAR classification criteria (4) and 12 had very early diagnosis of SSc (VEDOSS) (6). Thirty-six patients with no autoantibodies and no clinical signs or symptoms indicative of connective tissue disease (CTD) formed the primary RP group, while the remaining 56 patients who were either autoantibody positive, or had symptoms or signs indicative of a CTD or a diagnosis of CTD other than SSc, were grouped as non-SSc secondary RP. Sixty-two healthy volunteers served as controls. Written informed consent was obtained from all study participants prior to enrolment. The study was approved by our hospital's Institutional Review Board. After a mean ± SD of 3.38 ± 1.47 years 175 patients were reevaluated prospectively. Table I shows baseline demographic and capillaroscopic characteristics of patients and controls. Table II presents the clinical and laboratory features of patients with SSc and VEDOSS at baseline.

Capillaroscopy

Study participants were acclimatised to a temperature of 22-25°C for 15 minutes prior to examination. NVC was performed by a single operator (VKB), using a DS MEDICA Video Cap 200® device equipped with a 200x lens. Qualitative evaluation of all NVC images taken permitted categorisation of patients to either a normal or an SSc capillaroscopic pattern (early, active or late) (7). Capillary loss, dilated, giant or ramified capillaries and microhaemorrhages were further assessed on the distal row of three consecutive 1mm fields of fingers 2, 3, 4 and 5 of both hands, using a semi-quantitative rating scale (score 0–3) (8). Capillary loss represented the reduction of capillary number below an average of nine per linear mm. Capillaries with a homogeneous or irregular increase in diameter >20μm were defined as dilated. Homogeneously enlarged capillaries with an apical diameter >50μm were defined as giant. Irregularly shaped capillaries (branching, bushy, meandering, coiled) were defined as ramified. Dark-coloured haemosiderin depositions were recognised as microhaemorrhages. Regarding the semi-quantitative scoring system, every capillaroscopic parameter on each field received a score from 0 to 3 as follows: 0=no changes, 1<33% capillary alterations/reduction, 2=33–66% capillary alterations/reduction, 3>66% capillary alterations/reduction. The final score for each parameter was derived as a mean from all eight digits (8). Images were stored and scored by one of two different assessors (VKB, KK).

Clinical and laboratory evaluation

Patients underwent physical examination, both at baseline and at follow-up, recording puffy fingers and current or past digital ulcerations (9). Skin thickening was measured using the modified Rodnan skin score (mRSS). Deterioration in mRSS was considered clinically significant if >3.5 (10). Autoantibody status (antinuclear antibodies >1:160, anti-centromere and anti-Scl70 antibodies) was also noted. Interstitial lung disease (ILD) was documented by high-resolution computed tomography of the chest. Pulmonary function tests (FVC and DLCO), echocardiography derived pulmonary artery systolic pressure (PASP), Health Assessment Questionnaire-Disability Index (HAQ-DI), erythrocyte sedimentation rate (ESR) and patient medications were recorded, both at baseline and follow-up. Deterioration in FVC and DLCO was considered clinically significant if

Table I. Demographic features, autoantibody profile and capillaroscopic findings in 242 patients with systemic sclerosis (SSc), very early diagnosis of SSc (VEDOSS), Raynaud phenomenon secondary to connective tissue disease other than SSc (sRP), primary Raynaud phenomenon (pRP) and 62 healthy controls. Chi-square test and ANOVA with Bonferoni post hoc analysis was used for between group comparisons, as appropriate.

	SSc (n=138)	VEDOSS (n=12)	sRP (n=56)	pRP (n=36)	Controls (n=62)	SSc *vs.* sRP *p*-value	SSc *vs.* pRP *p*-value	SSc *vs.* controls *p*-value
Age (mean±SD)	52.96±14.062	42.17±12.12	45.34± 13.18	36.56±17.35	45.35±15.47	0.010	<0.0001	0.015
Female gender, n (%)	121 (88)	12 (100)	51 (91)	31 (86)	51 (82)	NS	NS	NS
Disease duration in years (mean ± SD)	6.57±6.51	3.87±5.08	7.02±8.65	4.96±9.00	-	NS	NS	NS
ANA n (%)	125/129 (97)	12/12 (100)	33/51 (65)	0	-	<0.0001	-	-
ACA n (%)	22/129 (17)	7/12 (58)	4/51 (8)	0	-	NS	-	-
anti-Scl70 n (%)	70/126 (56)	3/12 (8)	2/35 (6)	0	-	<0 .0001	-	-
smoking n (%)	24 (17)	5 (42)	24 (43)	8 (22)	15 (24)	<0.0001	NS	NS
packyears (mean ± SD)	8.06±15.50	17.60±18.22	15.57±26.11	4.44±9.41	4.05±7.80	NS	NS	NS
Capillaroscopy pattern								
normal n (%)	5 (4)	3 (25)	28 (56)	21 (58)	61 (98)	<0.0001	<0.0001	<0.0001
early n (%)	33 (24)	5 (42)	22 (39)	12 (33)	1 (2)			
active n (%)	70 (51)	4 (33)	6 (11)	3 (8)	0			
late n (%)	30 (22)	0	0	0	0			
Capillary loss score (mean ± SD)	1.58±0.42	1.04±0.38	0.88±0.46	0.80±0.34	0.46±0.26	<0.0001	<0.0001	<0.0001
Giant score (mean ± SD)	0.47±0.49	0.28±0.39	0.11±0.35	0.05±0.17	0.00±0.01	<0.0001	<0.0001	<0.0001
Microhaemorrages score (mean ± SD)	0.39±0.34	0.25±0.26	0.21±0.23	0.21±0.24	0.10±0.12	0.001	0.005	<0.0001
Ramifications score (mean ± SD)	0.27±0.40	0.04±0.07	0.08±0.10	0.11±0.13	0.14±0.16	<0.0001	0.043	0.05
Dilatation score (mean ± SD)	1.73±0.56	1.63±0.66	1.02±0.73	1.01±0.69	0.37±0.40	<0.0001	<0.0001	<0.0001

Table II. Clinical features, lung function tests and treatment of patients in the systemic sclerosis [limited (lcSSc) and diffuse (dcSSc) cutaneous systemic sclerosis subtypes] and very early diagnosis of systemic sclerosis (VEDOSS), at baseline.

	Baseline (n=150)		
Disease subgroup	lcSSc (n=87)	dcSSc (n=51)	VEDOSS (n=12)
mRSS (mean±SD)	4.49±3.62	12.08±8.82	0
Digital ulcers (anytime during disease course) (%)	33 (38)	29 (57)	0
Interstitial lung disease (%)	42/85 (49)	37/48 (77)	-
FVC (mean±SD)	92.77±17.08	79.32±19.21	110.00±8.19
FVC <80% (%)	16/83 (19)	21/50 (42)	0/3
TLC-He (mean±SD)	85.81±17.93	75.92±20.61	116.00
TLC-He<80% (%)	24/68 (35)	22/40 (55)	0/1
DLCO (mean±SD)	70.75 ±20.37	59.22±18.72	86.50±14.85
DLCO <80% (%)	50/72 (69)	38/43 (88)	1/2 (50)
RVSP >45 mmHg (%)	4/80 (5)	3/51 (6)	0/4
ESR (mean±SD)	32.35±21.38	34.63±21.51	13.55±4.80
HAQ-DI score (mean ± SD)	0.32±0.42	0.65±0.78	0.31±0.52
Vasoactive treatment (%)	41 (47)	31 (61)	0
Immunosuppresive treatment (%)	28 (34)	24 (47)	0

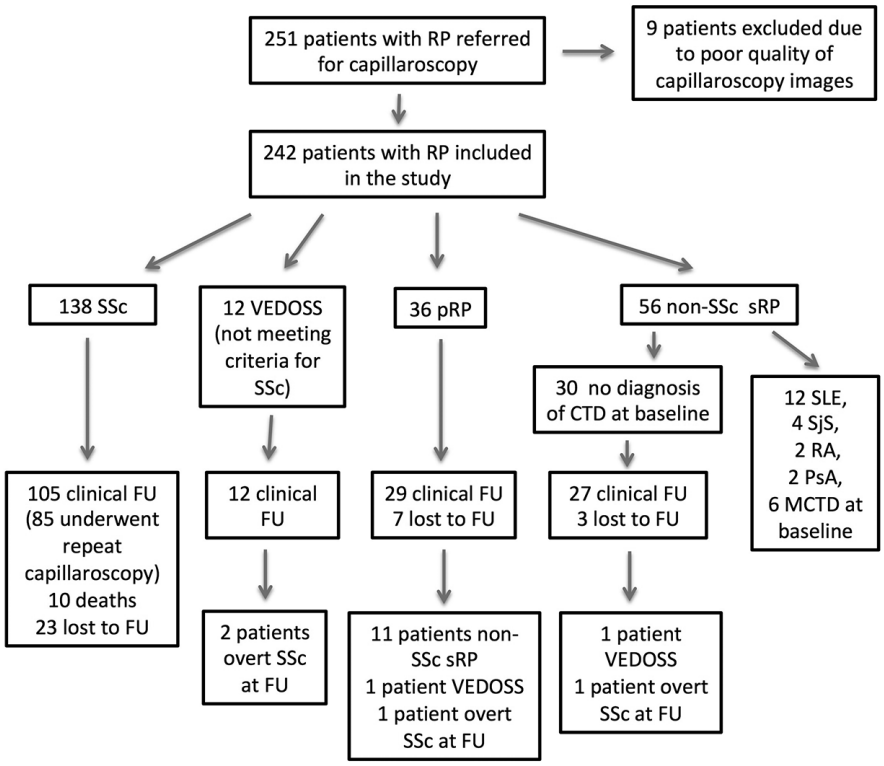


Fig. 1. Patient flowchart showing recruitment, patient categorisation at baseline and at follow-up of patients with Raynaud phenomenon referred to our tertiary university rheumatology clinic for nailfold video capillaroscopy.

>10% and >15%, respectively. Immunosuppressive treatment was defined as prednisolone >10mg or equivalent, methotrexate, mycophenolate, cyclophosphamide, azathioprine, hydroxylchloroquine, rituximab or tocilizumab. Vasoactive treatment included calcium channel blockers, endothelin receptor antagonists, prostanoids or phosphodiesterase-5 inhibitors. The primary composite endpoint for SSc patients in our study was occurrence of at least one of the following: first occurrence of digital ulcers, >10% deterioration of FVC, >15% deterioration of DLCO, >3.5 deterioration of mRSS or death.

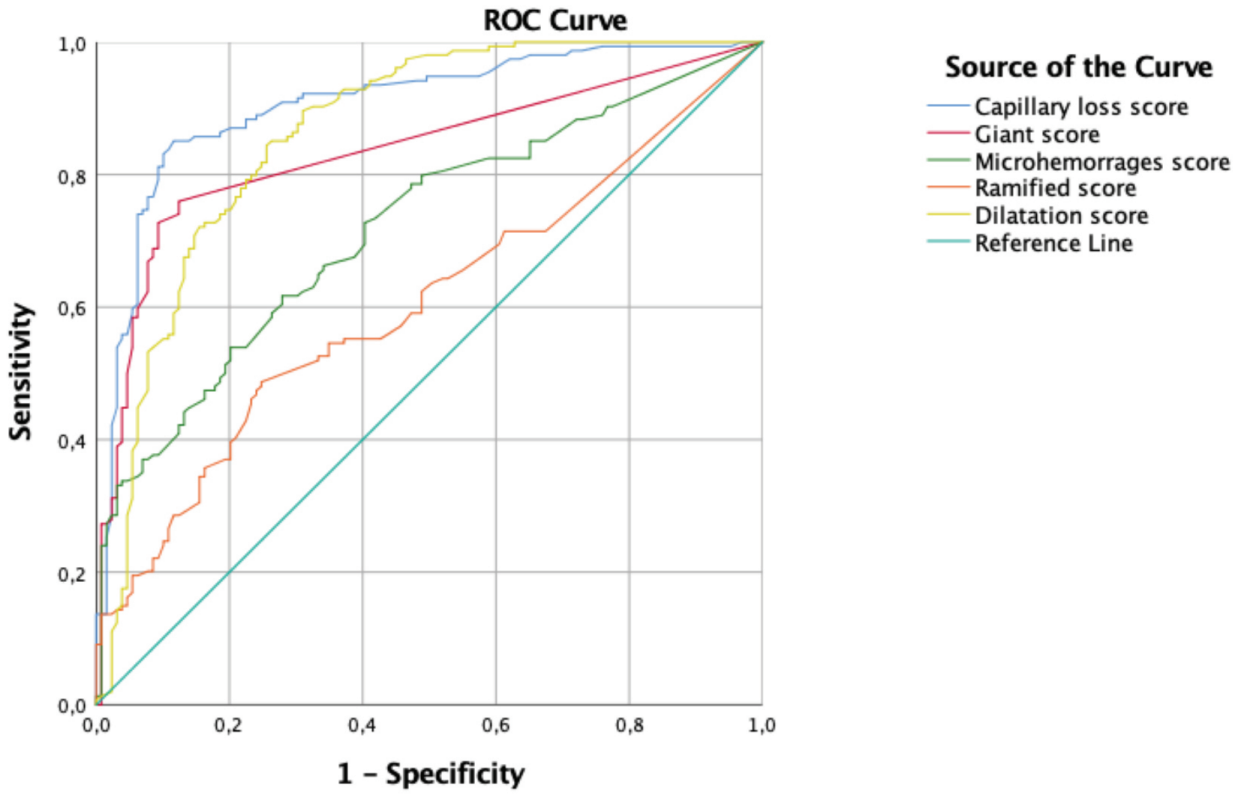
Statistical analysis

Stored NVC images from twenty patients, randomly selected from our cohort, were scored at two different time points by the same assessor and then by the second assessor. Intraclass correlation coefficient (ICC) based on two-way mixed model analyses for intra- and inter-rater variability was calculated for capillary loss score, dilatation score, giant score, microhaemorrhages score and ramification score. The intra- and inter-rater proportion of agreement for the qualitative assessment of a patient as belonging to the normal, or the early, active, or late SSc pattern was also assessed. ROC curve methodology was used to compare the diagnostic performance of different NVC scores. Ordinal and linear logistic regression analyses were used to explore if associations of clinical characteristics with NVC findings in SSc remained stable over two longitudinal assessments. Binary logistic regression analysis was performed to identify NVC parameters able to prognosticate the primary composite adverse outcome, or death in the SSc group, after adjustment for gender, age, disease subtype, disease duration, smoking, vasoactive treatment, FVC and mRSS at baseline. The level of statistical significance was set at p=0.05. The Stata v.12 statistical software package was used for all analyses.

Results

Comparative assessment of the diagnostic accuracy of different capillaroscopic scores

One hundred and five SSc, 12 VEDOSS, 29 pRP and 27 of 30 sRP patients for whom no diagnosis was available at baseline were clinically re-evaluated after a mean±SD of 3.38±1.47 years. Repeat capillaroscopy was available in 85 SSc patients, while in 20 this was either not performed (due to digital ulcers, hand deformity or patient refusal) or resulted in non-evaluable images (Fig. 1). Ten patients in the SSc group were deceased at follow-up and two more died shortly after re-evaluation. Thirteen patients (36%) originally in the pRP group either developed positive autoantibodies and/or signs or symptoms indicative of a CTD, or met criteria for the diagnosis of a CTD, thus being re-classified as sRP. Two patients in the VEDOSS group and one in each



	AUC	95% CI	P-value	Cutoff	Sensitivity	Specificity
Capillary loss score	0.905	0.869-0.942	**<0.0001**	1.105	85.7%	85.3%
Dilatation score	0.863	0.818-0.907	**<0.0001**	1.089	81.8%	75.2%
Giant Score	0.835	0.787-0.884	**<0.0001**	0.021	76.0%	87.6%
Microhemorrhages score	0.720	0.662-0.779	**<0.0001**	0.167	66.2%	65.9%
Ramification score	0.604	0.539-0.670	**0.003**	0.083	57.1%	54.3%

Fig. 2. ROC curves showing the accuracy of baseline capillaroscopic scores (capillary loss score, giant score, micro-haemorrhages score, ramifications score and dilatation score) in discriminating subjects with the scleroderma spectrum of diseases [Systemic sclerosis (SSc) and very early diagnosis of SSc] from patients with primary Raynaud's phenomenon, non-SSc secondary Raynaud's phenomenon and controls.

of the pRP and sRP groups developed overt SSc, while two more patients, one in each of the pRP and sRP groups, met criteria for VEDOSS, but not for SSc.

Differences in baseline capillaroscopy parameters between SSc patients and each of the pRP, non-SSc sRP and control groups, were confirmed in our study, as shown in Table I. ROC curve analysis was employed to evaluate the comparative performance of different baseline NVC scores at discriminating patients with SSc and VEDOSS at follow-up [SSc (n=142), VEDOSS (n=12)] from all other study participants [pRP (n=16), sRP (n=62) and controls (n=62)], excluding pRP and non SSc-sRP patients lost to follow-up. As shown in Figure 2, capillary loss score displayed the highest area under the curve [AUC (95% CI)=0.905 (0.869–0.942)], followed by dilatation score [0.863 (0.818–0.907)] and giant score [0.835 (0.787–0.884)], whereas micro-haemorrhages [0.720 (0.662–0.779)] and ramifications scores [0.604 (0.539–0.670)] seemed to be the least accurate in identifying patients with an SSc spectrum disorder.

Intra- and inter-rater variability

Intraclass correlation coefficient (ICC) for intra- and inter-rater variability analyses was very good for capillary loss score [intra- and inter-rater variability ICC (95% CI) 0.98 (0.93–0.99) and 0.97 (0.74–0.99), respectively], dilatation score [0.97 (0.93-0.99) and 0.94 (0.85–0.98), respectively], giant score [0.994 (0.985–0.998) and 0.98 (0.97–0.99), respectively] and micro-haemorrhages score [0.93 (0.84–0.97) and 0.94 (0.84–0.97), respectively]. The ramifications score, however, had only moderate inter observer reliability [0.52 (0.2–0.8)], despite a very good intra observer reliability [0.989 (0.971–0.995)]. Therefore, ramifications score was excluded from all further analyses in our study. The intra- and inter-rater proportion of agreement for the qualitative assessment of a patient as belonging to the normal, or the early, active, or late SSc pattern was 90% and 85%, respectively.

Clinical correlations of capillaroscopic parameters in SSc patients, at baseline

Associations of clinical characteristics of SSc patients with capillaroscopic fea-

Table III. Baseline correlations of capillaroscopic parameters with clinical and demographic features of SSc patients (n=138). Correlations by univariate analysis (ordinal logistic regression for capillaroscopy pattern and linear regression for each of the capillaroscopy scores under study) are shown; of them, those that remain significant in multivariate analysis are shown in bold. Number of patients with available data is shown in parenthesis.

	Capillaroscopy pattern	Capillary loss score	Giant score	Microhaemorrhages score	Dilatation score
Age	NS	NS	p=0.073 B=-0.005	NS	***p*=0.006**** **B=-0.009**
Male gender	NS	NS	NS	NS	NS
Disease duration	NS	p=0.046 B=0.001	NS	NS	NS
dcSSc disease subtype	p<0.0001 B=1.65 OR=5.12	p=0.036 B=0.156	NS	p=0.036 B=-0.127	NS
ACA+ (n=129)	NS	NS	p=0.087 B=0.196	NS	NS
Anti-Scl70+ (n=126)	NS	p=0.061 B=0.139	NS	NS	NS
Current smocking	NS	NS	***p*=0.008**§ **B=0.289**	NS	p=0.009** B=0.328
Digital ulcers (ever)	***p*=0.002*** **B=1.068** **OR=2.86**	p=0.004 B=0.206	NS	NS	p=0.014** B=0.234
mRSS	***p*<0.0001*** **B=0.163** **OR=2.86**	p=0.015 B=0.012	NS	NS	NS
RVSP >45 mmHg (n=131)	NS	NS	NS	NS	NS
$DLCOc_{SB}$ (n=115)	p=0.051 B=-0.017 OR=0.98	NS	NS	p=0.069 B=0.003	NS
TLC-He (n=108)	NS	NS	NS	***p*=0.001**# **B=0.006**	NS
FVC (n=133)	p=0.037 B=-0.018 OR=0.98	NS	p=0.051 B=0.004	p=0.004 B=0.004	NS
Interstitial lung disease (n=133)	NS	NS	p=0.033 B=-0.181	p=0.012 B=-0.149	NS
ESR	NS	NS	NS	NS	p=0.095 B=-0.004
HAQ-DI score	p<0.0001 B=1.062 OR=3.042	NS	p=0.029 B=-0.156	p=0.022 B=-0.116	NS
Need for vasodilation	p<0.013 B=0.825 OR=2.28	p=0.016 B=0.171	p=0.089 B=0.141	NS	p=0.057 B=0.182
Need for immunosuppression	NS	NS	NS	NS	NS

*Capillaroscopy pattern correlates significantly with mRSS (p=0.001, B=0.141, OR=1.151) and digital ulcers (p=0.024, B=0.872, OR=2.391) when adjusted for age, gender, disease subtype, FVC, HAQ-score and need for vasodilation (n=133).

§Giant score correlates significantly with current smoking status (p=0.028, B=0.262) when adjusted for age, gender, HAQ-DI score and interstitial lung disease on chest HRCT (n=133).

#Microhaemorrages score correlates significantly with TLC-He (p=0.035, B=0.006) when adjusted for age, gender, disease subtype, interstitial lung disease, FVC and HAQ-DI score (n=102).

**Dilatation score correlates significantly with age (p=0.030, B=-0.007) and current smoking status (p=0.047, B=0.248) and marginally with digital ulcers (p=0.092, B=0.163) when adjusted for gender (n=138).

tures at baseline are shown in Table III. Univariate analysis revealed that worse pattern in NVC correlates with the diffuse disease subtype (dcSSc), current or past digital ulcers, need for vasodilatory treatment, higher mRSS and HAQ-DI score and lower FVC. Multivariate ordinal regression analysis, adjusted for age, gender and the clinical features found statistically significant in univariate analysis, confirmed the association of worse capillaroscopic pattern with higher mRSS (p=0.001, B=0.141, OR=1.15), and digital ulcers ever in the disease course (p=0.024, B=0.872, OR=2.39). In univariate linear regression analysis capillary loss score was shown to correlate with longer disease duration, dcSSc, current or past digital ulcers, higher mRSS, and vasodilatory treatment. Likewise, giant score was shown to correlate with current smoking, lower HAQ-DI score and a lower probability for ILD. Micro-haemorrhages score correlated positively with FVC and TLC-He. An inverse association was found with dcSSc, ILD and HAQ-DI score. Finally, dilatation score correlated positively with smoking and digital ulcers and negatively with age. Multiple linear regression, adjusted for age, gender and the variables each time found statistically significant in univariate analysis, confirmed a direct association of giant score with current smoking (p=0.028, B=0.262), of micro-haemorrhages score with TLC-He (p=0.035, B=0.006) and of dilatation score with younger age (p=0.030, B=-0.007), smoking (p=0.047, B=0.248) and also marginally with digital ulcers (p=0.092, B=0.163).

Consistency over time of clinical correlations of capillaroscopic findings in SSc patients at two different time-points

To assess the consistency over time of correlations revealed between capillaroscopic findings and patients' clinical features, the same analysis as above was performed, at baseline and at follow-up, for 85 SSc patients with an available follow-up NVC, as shown in Table IV. The best match was found for giant score, for which only the baseline association with ILD indicated by uni-

Table IV. Lack of consistency between the majority of clinical correlations of capillaroscopic findings at baseline and after a mean±SD of 3.12±1.30 years in 85 SSc patients. Values shown in the table represent the results of univariate analysis (ordinal logistic regression for capillaroscopy pattern and linear regression for each of the 4 capillaroscopy scores under study). Correlations that remain significant in multivariate analysis are shown in bold. Number of patients with data available is shown in parenthesis.

		Capillaroscopy pattern	Capillary loss score	Giant score	Microhaemorrhages score	Dilatation score
Age	Baseline	NS	NS	NS	NS	NS
	Follow-up	NS	NS	NS	NS	*p*=0.013 B=-0.011
Male gender	Baseline	NS	NS	NS	NS	NS
	Follow-up	NS	NS	NS	NS	NS
Disease duration	Baseline	NS	*p*=0.079 B=0.0008	NS	NS	NS
	Follow-up	NS	NS	NS	NS	NS
dcSSc disease subtype	Baseline	*p*=0.007 B=1.277 OR=3.585	NS	NS	*p*=0.007 B=-0.206	NS
	Follow-up	***p*=0.003*** **B=1.319** **OR=3.740**	*p*=0.058 B=0.145	NS	*p*=0.068 B=-0.099	NS
ACA+	Baseline (n=80)	NS	NS	NS	*p*=0.073 B=0.180	NS
	Follow-up (n=80)	NS	NS	NS	NS	NS
Anti-Scl70+	Baseline (n=78)	NS	NS	NS	*p*=0.014 B=-0.196	NS
	Follow-up (n=78)	NS	*p*=0.036 B=0.164	NS	*p*=0.068 B=-0.102	NS
Current smocking	Baseline	NS	NS	*p*=0.090 B=0.243	NS	*p*=0.033** B=0.305
	Follow-up	NS	NS	NS	NS	*p*=0.095 B=0.143
Digital ulcers (ever)	Baseline	NS	NS	NS	NS	*p*=0.017 **B=0.264
	Follow-up	NS	***p*<0.0001**# **B=0.326**	*p*=0.061 B=0.185	***p*=0.005**$ **B=0.111**	***p*=0.001**** **B=0.450**
mRSS	Baseline	***p*=0.001*** **B=0.153** **OR=1.165**	NS	NS	NS	*p*=0.099 B=0.016
	Follow-up	***p*=0.009*** **B=0.108** **OR=1.113**	***p*<0.0001**# **B=0.029**	NS	NS	NS
RVSP >45 mmHg	Baseline (n=81)	NS	NS	NS	NS	NS
	Follow-up (n=73)	NS	NS	NS	NS	NS
$DLCOc_{SB}$	Baseline (n=72)	*p*=0.092 B=-0.020 OR=0.980	NS	*p*=0.092 B=0.005	*p*=0.073 B=0.004	NS
	Follow-up (n=72)	NS	NS	*p*=0.070 B=0.005	NS	NS
TLC-He	Baseline (n=68)	NS	NS	NS	*p*=0.005 B=0.007	NS
	Follow-up (n=66)	NS	NS	NS	NS	NS
FVC	Baseline (n=80)	*p*=0.098 B=-0.020 OR=0.980	NS	*p*=0.054 B=0.006	*p*=0.001 B=0.007	NS
	Follow-up (n=78)	NS	NS	NS	NS	NS
Interstitial lung disease	Baseline (n=82)	NS	NS	***p*=0.00**& **B=-0.328**	*p*=0.001 B=-0.255	NS
	Follow-up (n=81)	NS	NS	NS	NS	NS

		Capillaroscopy pattern	Capillary loss score	Giant score	Microhaemorrhages score	Dilatation score
ESR	Baseline	NS	NS	NS	NS	NS
	Follow-up	p=0.060 B=0.024 OR=1.02	NS	NS	NS	NS
HAQ-DI score	Baseline	p=0.043 B=0.829 OR=2.29	NS	NS	p=0.063 B=-0.127	NS
	Follow-up	NS	NS	NS	NS	NS
Need for vasodilation	Baseline	NS	p=0.090 B=0.135	NS	NS	NS
	Follow-up	NS	p=0.003 B=0.226	NS	NS	NS
Need for Immunosuppression	Baseline	p=0.073 B=0.797 OR=2.22	NS	NS	NS	**p=0.013**[**] **B=0.282**
	Follow-up	p=0.064 B=0.778 OR=2.18	NS	NS	NS	NS

*At baseline capillaroscopy pattern correlates significantly with mRSS (p=0.004, B=0.145, OR=1.156) when adjusted for age, gender, disease subtype and HAQ-DI score (n=85). At follow-up capillaroscopy pattern correlates significantly with mRSS (p=0.046, B=0.085, OR=1.09) and disease subtype (p=0.022, B=1.066, OR=2.90) when adjusted for age and gender (n=85).

[#]At follow-up capillary loss score correlates significantly with digital ulcers (p=0.001, B=0.282) and mRSS (p=0.011, B=0.019) when adjusted for age, gender, need for vasodilation and anti-Scl70 positivity (n=78).

[&]At baseline giant score correlates significantly with interstitial lung disease (p=0.005, B=-0.328) when adjusted for age and gender (n=82).

[$]At follow-up micro-haemorrhages score correlates significantly with digital ulcers (p=0.048, B=0.121) when adjusted for age and gender (n=85).

**At baseline dilatation score correlates significantly with immunosuppressive treatment (p=0.015, B=0.273) and marginally with digital ulcers (p=0.069, B=0.197) and current smocking status (p=0.062, B=0.259) when adjusted for age and gender (n=85). At follow-up dilatation score correlates significantly with digital ulcers (p=0.006, B=0.374) when adjusted for age and gender (n=85).

Table V. Occurrence of a composite adverse disease outcome (FVC deterioration >10% and/or DLCO deterioration >15% and/or mRSS deterioration >3.5 and/or new digital ulcer formation in patients without prior such history and/or death) after a mean±SD follow-up of 3.31±1.40 years in 94 SSc patients can be predicted by baseline capillaroscopy pattern, as shown by multivariate binary logistic regression analysis.

		p-value	Odds Ratio	95% CI	
Male gender		0.272	2.413	0.502	11.600
Age		0.309	1.018	0.984	1.054
dcSSc disease subtype		0.632	1.322	0.422	4.143
Disease duration		**0.014**	**0.991**	**0.984**	**0.998**
Capillaroscopy pattern	**Active (vs. early)**	**0.043**	**3.305**	**1.037**	**10.537**
	Late (vs. early)	**0.023**	**6.900**	**1.305**	**36.472**
Smoking at baseline		0.406	1.736	0.472	6.387
Need for vasodilation at baseline		0.812	0.888	0.334	2.361
FVC at baseline		0.716	0.995	0.969	1.022
mRSS at baseline		0.314	1.049	0.560	1.151

variate analysis at baseline and shown to persist when adjusted for age and gender (p=0.005, B=-0.328) was not replicated at follow-up. A good match was also found for capillaroscopy pattern, for which univariate analysis showed a significant correlation with dcSSc and mRSS, both at baseline and follow-up. However, an association with HAQ-DI score was present only at baseline. Multivariate analysis adjusted for age, gender and the variables that were significant in the univariate model, confirmed the correlation of capillaroscopy pattern with mRSS, both at baseline (p=0.004, B=0.145, OR=1.16) and at follow-up (p=0.046, B=0.085, OR=1.09) and with dcSSc at follow-up (p=0.022, B=1.066, OR=2.90), but failed to confirm the association with dcSSc at baseline. In the case of capillary loss score, univariate associations with anti-Scl-70 positivity, need for vasodilation, current or past digital ulcers and mRSS were found only at follow-up, with the latter two being further confirmed by multivariate analysis (p=0.001, B=0.282 for digital ulcers, p=0.011, B=0.019 for mRSS, respectively). Micro-haemorrhages score correlated positively at baseline with TLC-He and FVC and inversely with dcSSc, anti-Scl-70 positivity and ILD, but these associations were not confirmed by multivariate analysis. On the contrary, at follow-up, a significant association of micro-haemorrhages score with digital ulcers persisted after adjustment for age and gender (p=0.048, B=0.121). Finally, in univariate analysis, dilatation score correlated significantly at baseline with current smoking, current or past digital ulcers and immunosuppressive treatment. Association with immunosuppressive treatment (p=0.015, B=0.273) remained significant, while that with digital ulcers (p=0.069, B=0.197) and current smoking status (p=0.062, B=0.259) became marginal after correcting for age and gender. At follow-up, dilatation score was found to correlate with age

and digital ulcers in univariate analysis, with only digital ulcers remaining significant (p=0.006, B=0.374) after adjusting for age and gender.

Evaluation of the predictive capacity of NVC in SSc patients

Next we attempted to evaluate the ability of baseline NVC to predict the occurrence of a composite adverse disease outcome in SSc patients. Among 105 SSc patients attending our clinic for a follow-up evaluation and 10 additional patients deceased at follow-up, sufficient information to define occurrence of the composite outcome was provided for 94 subjects, who were included in the analysis. Mean ± SD time to follow-up for this population was 3.31±1.40 years. Of the NVC parameters under study, only capillaroscopy pattern (active pattern: p=0.023, OR=3.374, late pattern: p=0.007, OR=6.400) correlated significantly with the composite adverse disease outcome in univariate analysis. A multivariate model was constructed including capillaroscopy pattern, age, gender, disease subtype, disease duration, smoking, vasoactive treatment, FVC and mRSS at baseline. As shown in Table V, SSc patients with active (p=0.043, OR=3.305) or late capillaroscopic pattern (p=0.023, OR=6.900) at baseline had higher risk for the composite adverse disease outcome, compared to patients with an early baseline pattern. In this model disease duration also displayed a negative correlation with the composite outcome (p=0.014, OR=0.991). No interaction existed between disease duration and capillaroscopy pattern in this model.

Finally, to detect possible correlations of baseline capillaroscopic features with death as an outcome in SSc patients, we performed univariate binary logistic regression analysis in 115 SSc patients for which follow-up survival data were available. After a mean±SD follow-up of 3.22±1.42 years no baseline NVC parameter was found to correlate significantly with death in univariate analysis and only a marginal inverse correlation was found for giant (p=0.076, OR=0.203) and micro-haemorrhages score (p=0.064, OR=0.074), which ceased to exist after adjusting for age, gender, disease duration, disease subtype, smoking, FVC, mRSS and vasoactive therapy at baseline.

Discussion

In our study we confirm the importance of NVC in discriminating patients presenting RP in the context of the SSc spectrum of disorders from those with primary RP, non-SSc secondary RP and from healthy controls (11). ROC curve analysis indicated that capillary loss score, followed by dilatation score and giant score, are the most reliable parameters in differentiating patients belonging to the SSc spectrum of disorders. By contrast, micro-haemorrhages and ramifications scores did not perform equally well. It is therefore conceivable that micro-haemorrhages and ramifications, which represent the most common capillaroscopic finding in systemic lupus erythematosus (12) and are also frequent in Sjögren's syndrome (13), rheumatoid arthritis (14, 15) and diabetes mellitus (16] are less specific for the SSc spectrum of disorders compared to capillary loss, dilatation and giant capillaries.

Sekiyama *et al*. used ROC curve analysis to assess the diagnostic performance of the number of capillaries/mm as measured by NVC, in discriminating 101 SSc patients from 151 non-SSc patients (healthy controls, primary RP patients, and undifferentiated CTD patients) and reported an AUC of 0.894 (p<0.001, 95% CI 0.850-0.938), which is comparable to the AUC=0.905 we found for capillary loss score (17). In another interesting paper, Ingegnoli *et al*. reported that only the presence of giant loops (HR 2.64) and microhaemorrhages (HR 2.33) and the number of capillaries in NVC were significant for predicting the 5-year transition from primary RP to RP secondary to the SSc spectrum of disorders, whereas enlarged or branching loops and capillary disorganization were not (18). In our study by contrast we showed that dilatation of capillary loops performs better than microhaemorrhages in identifying patients with RP secondary to scleroderma. Finally, in a French-Italian study of 66 VEDOSS patients loop diameter and apex width were significantly higher among patients progressing to SSc (N=21) compared to non-progressors (N=45) (19).

In concert with the results of a previous study by Smith *et al*. (20) our data show that the inter-rater ICC for capillary ramifications was 0.52, much lower than for all other qualitative and semi-quantitative assessments. Given this limitation, the fact that our protocol was configured in 2011, at a time when ramifications score was still considered a reliable parameter in capillaroscopy and the recent publication by the EULAR Study Group on Microcirculation in Rheumatic Diseases of an optimised set of simple definitions for describing capillary morphology (normal, abnormal, non-evaluable), with an excellent reliability, even among novice assessors (21, 22), we decided to exclude ramifications score from all further analyses evaluating the clinical correlations and the predictive role of NVC in our study.

Previous studies have extensively investigated the cross-sectional correlations of capillaroscopy findings with clinical disease features in SSc (23). Late scleroderma pattern and lower capillary density in NVC have been associated with dcSSc (24-27), anti-Scl70 positivity (24, 25, 27, 28), longer disease duration (28), digital ulcers (24, 26, 28-32), more extensive skin (26, 28, 29), heart (26) and lung involvement (24, 26, 28, 29, 33-35) pulmonary arterial hypertension (24, 34, 36-38), muscle weakness, joint contractures, synovitis, tendon friction rubs, conduction blocks (24), esophageal dysmotility (28, 33), acro-osteolysis and calcinosis on hand radiographs (39) and increased severity (29) or activity indices (24, 26, 40, 41). Our findings corroborate the association of worse NVC pattern with dcSSc (OR: 5.12), increased mRSS (OR: 2.86), decreased FVC (OR: 0.98), decreased DLCO (OR: 0.98) and current or past digital ulcers (OR: 2.86), as well as the association of capillary loss score with dcSSc, higher mRSS and current or past digital ulcers. Regarding mega-capillaries, their presence in SSc has been associated with younger age, lower extent of reticular pattern on chest HRCT (29), and less risk for new digital ulcers (42). Our results also in-

dicate a marginally significant negative correlation of mega-capillaries with the presence of ILD on HRCT of the lungs and a marginal positive correlation with FVC.

To the best of our knowledge, this is the first study to explore the consistency over time of the correlations found between capillaroscopic features and clinical characteristics of SSc patients. Interestingly, clinical correlations derived from univariate and multivariate analysis at baseline and at follow-up did not coincide for most of the capillaroscopic indices investigated. The best match was found for giant score and capillaroscopy pattern. In our cohort a considerable proportion of SSc patients have been receiving immunosuppressive and/or vasodilating therapy. In fact, treatment with vasodilating agents was administered to 55 (48%) of the 115 SSc patients with follow-up data available, at baseline and to 65 patients (57%) at follow-up. Endothelin receptor antagonists, alone or in combination with iloprost, have shown a beneficial effect on the structure and function of the microvasculature in SSc patients, promoting angiogenesis and leading to an increase in capillary number (43–45). It is possible that the lack of consistency over-time in the correlations between capillaroscopic and clinical findings observed in our SSc cohort, could reflect the effect of treatment on the microvasculature of our patients, even more so since many of the capillaroscopic indices we examined showed an association with current use of vasodilation.

Our data show that after 3.31 years of follow-up, SSc patients with a late baseline NVC pattern had nearly 7-fold higher probability for disease deterioration or death, whereas those with an active baseline NVC pattern had nearly 3-times higher risk for this adverse outcome, compared to patients with an early baseline pattern. Disease duration also displayed a weak negative correlation with the composite adverse outcome in our predictive model. Since ours was not an incident cohort, some SSc cases with severe disease were not included, as they died early, thus causing the association of longer disease duration with better outcome to emerge.

Two previous large prospective studies evaluating the prognostic capacity of NVC with regard to disease progression in SSc have identified worsening baseline capillaroscopy pattern as an important determinant of new severe organ involvement in SSc patients, after a follow-up of 18-24 months (46, 47). Besides, a third study performed by Avouac *et al*. in a cohort of 140 French SSc patients followed up annually, both clinically and capillaroscopically for 3 years, indicated that the major prognostic capillaroscopic parameter predictive of overall disease progression was progressive loss of capillaries over time (42). In this latter study progressive loss of capillaries over time was also associated with new digital ulcer occurrence, lung vascular progression, skin score deterioration and worsening of the Medsger severity score (42). In our study we prospectively evaluated both capillaroscopy pattern and loss of capillaries and found a significant correlation of disease outcome at 3-years only with the active and late disease pattern, thus corroborating previous results. Moreover, in the studies by Smith *et al*. (46, 47) the predictive model constructed using multiple logistic regression analysis, was adjusted only for disease duration, disease subset and vasoactive medication, whereas in our analysis we also corrected for age, gender, smoking and baseline clinical parameters, such as FVC and mRSS.

As far as mortality is concerned, we found no significant correlations of NVC parameters with death and only a trend towards lower mortality with increased giant and micro-haemorrhages score. A previous study in SSc patients reported an avascular score>1.5 as an independent predictor of death in multivariate analysis (48). Other studies have identified an active NVC pattern (49) or an increased capillary loss (50-52) and capillary dilatation (50) at baseline as non-independent risk factors for mortality.

Our study has several limitations. First of all the wide range of follow-up time of our patients (18-73 months) could have affected the association of baseline capillaroscopic indices with future outcomes, as not all patients had the same timespan to develop the outcomes under study. As already mentioned, ours was not an inception cohort, therefore, patients with the most severe clinical course who died early, have been, a priori, excluded. In addition, the number of deaths that occurred in our cohort is small to allow for definitive conclusions regarding the role of NVC as a predictor of mortality in SSc. Finally, of the 138 patients originally enrolled in the SSc group, 17% were lost to follow-up or declined re-evaluation, which represents a considerable rate of attrition.

Conclusions

In this prospective study of primary and secondary RP patients, referred to our tertiary university rheumatology clinic for capillaroscopy, we confirmed the pivotal role of NVC in discriminating patients with the SSc spectrum of disorders from those with RP of other etiology and from controls. After comparing the diagnostic accuracy of the main 5 semi-quantitative components of NVC, we concluded that capillary loss score performs best for identifying RP patients with the SSc spectrum of disorders, followed by dilatation and giant score, while micro-haemorrhages and ramifications scores were not as useful. In addition, we showed that individual clinical correlations of NVC parameters change over time in SSc patients, possibly due to treatment effect on disease physical history. Finally, our data confirmed the predictive values of NVC in SSc patients, showing that active and late capillaroscopy pattern at baseline are prognostic of an adverse disease outcome.

References

1. WIGLEY FM, FLAVAHAN NA: Raynaud's Phenomenon. *N Engl J Med* 2016; 375: 556-65.
2. RUARO B, SULLI A, SMITH V *et al*.: Advances in nailfold capillaroscopic analysis in systemic sclerosis. *J Scleroderma Relat Disord* 2018; 3: 122-31.
3. 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 con-

nective tissue disease: A follow-up study of 3,029 patients with primary Raynaud's phenomenon. *Rheumatol Int* 2012; 32: 3039-45.
4. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *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.
5. PAXTON D, PAULING JD: Does nailfold capillaroscopy help predict future outcomes in systemic sclerosis ? A systematic literature review. *Semin Arthritis Rheum* 2018: 48: 484-94.
6. MINIER T, GUIDUCCI S, BELLANDO-RANDONE S *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.
7. CUTOLO M, SULLI A, PIZZORNI C, ACCARDO S: Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol* 2000; 27: 155-60.
8. SULLI A, SECCHI ME, PIZZORNI C, CUTOLO M: Scoring the nailfold microvascular changes during the capillaroscopic analysis in systemic sclerosis patients. *Ann Rheum Dis* 2008; 67: 885-7.
9. SULIMAN YA, BRUNI C, JOHNSON SR *et al*.: Defining Skin Ulcers in Systemic Sclerosis: Systematic Literature Review and Proposed World Scleroderma Foundation (WSF) Definition. *J Scleroderma Relat Disord* 2017; 2: 115-20.
10. KHANNA D, FURST DE, CLEMENTS PJ *et al*.: Standardization of the modified Rodnan skin score for use in clinical trials of systemic sclerosis. *J Scleroderma Relat Disord* 2017; 2: 11-8.
11. BUKHARI M, HOLLIS S, MOORE T, JAYSON MI, HERRICK AL: Quantitation of microcirculatory abnormalities in patients with primary Raynaud's phenomenon and systemic sclerosis by video capillaroscopy. *Rheumatology* (Oxford) 2000; 39: 506-12.
12. CUTOLO M, MELSENS K, WIJNANT S *et al*.: Nailfold capillaroscopy in systemic lupus erythematosus: A systematic review and critical appraisal. *Autoimmun Rev* 2018; 17: 344-52.
13. TEKTONIDOU M, KASKANI E, SKOPOULI FN, MOUTSOPOULOS HM: Microvascular abnormalities in Sjögren's syndrome: nailfold capillaroscopy. *Rheumatology* (Oxford) 1999; 38: 826-30.
14. SAG S, SAG MS, TEKEOGLU I, KAMANLI A, NAS K, AYDIN Y: Nailfold videocapillaroscopy results in patients with rheumatoid arthritis. *Clin Rheumatol* 2017; 36: 1969-74.
15. CUOMO G, FRONGILLO E, PIRRO L, MASTRO A DEL, ROMANO C: THU0098 The nailfold capillaroscopy in rheumatoid arthritis: quantitative analysis and clinical and serological correlation. Poster Present., vol. 76, *BMJ and EULAR*; 2017; 237.2-237.
16. UYAR S, BALKARLI A, EROL MK *et al*.: Assessment of the relationship between diabetic retinopathy and nailfold capillaries in type 2 diabetics with a noninvasive method: nailfold videocapillaroscopy. *J Diabetes Res* 2016; 2016: 7592402.
17. SEKIYAMA JY, CAMARGO CZ, ANDRADE LEC, KAYSER C: Reliability of widefield nailfold capillaroscopy and videocapillaroscopy in the assessment of patients with Raynaud's phenomenon. *Arthritis Care Res* (Hoboken) 2013; 65: 1853-61.
18. INGEGNOLI F, BORACCHI P, GUALTIEROTTI R *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.
19. VASILE M, AVOUAC J, SCIARRA I *et al*.: From VEDOSS to established systemic sclerosis diagnosis according to ACR/EULAR 2013 classification criteria: a French-Italian capillaroscopic survey. *Clin Exp Rheumatol* 2018; 36 (Suppl. 113): S82-7.
20. SMITH V, PIZZORNI C, DE KEYSER F *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.
21. SMITH V, BEECKMAN S, HERRICK AL *et al*.: An EULAR study group pilot study on reliability of simple capillaroscopic definitions to describe capillary morphology in rheumatic diseases. *Rheumatology* 2016; 55: 883-90.
22. CUTOLO M, MELSENS K, HERRICK AL *et al*.: Reliability of simple capillaroscopic definitions in describing capillary morphology in rheumatic diseases. *Rheumatology* 2018; 57: 757-9.
23. SOULAIDOPOULOS S, TRIANTAFYLLIDOU E, GARYFALLOS A, KITAS GD, DIMITROULAS T: The role of nailfold capillaroscopy in the assessment of internal organ involvement in systemic sclerosis: A critical review. *Autoimmun Rev* 2017; 16: 787-95.
24. INGEGNOLI F, ARDOINO I, BORACCHI P *et al*.: Nailfold capillaroscopy in systemic sclerosis: Data from the EULAR scleroderma trials and research (EUSTAR) database. *Microvasc Res* 2013; 89: 122-8.
25. CUTOLO M, PIZZORNI C, TUCCIO M *et al*.: Nailfold videocapillaroscopic patterns and serum autoantibodies in systemic sclerosis. *Rheumatology* 2004; 43: 719-26.
26. CARAMASCHI P, CANESTRINI S, MARTINELLI N *et al*.: Scleroderma patients nailfold videocapillaroscopic patterns are associated with disease subset and disease severity. *Rheumatology* 2007; 46: 1566-9.
27. SATO LT, KAYSER C, ANDRADE LEC: Nailfold capillaroscopy abnormalities correlate with cutaneous and visceral involvement in systemic sclerosis patients. *Acta Reumatol Port* 2009; 34: 219-27.
28. BREDEMEIER M, XAVIER RM, CAPOBIANCO KG *et al*.: Nailfold capillary microscopy can suggest pulmonary disease activity in systemic sclerosis. *J Rheumatol* 2004; 31: 286-94.
29. DE SANTIS M, CERIBELLI A, CAVACIOCCHI F *et al*.: Nailfold videocapillaroscopy and serum VEGF levels in scleroderma are associated with internal organ involvement. *Auto Immun Highlights* 2016; 7: 5.
30. HERRICK AL, MOORE TL, MURRAY AK *et al*.: Nail-fold capillary abnormalities are associated with anti-centromere antibody and severity of digital ischaemia. *Rheumatology* 2010; 49: 1776-82.
31. ALIVERNINI S, DE SANTIS M, TOLUSSO B *et al*.: Skin ulcers in systemic sclerosis: Determinants of presence and predictive factors of healing. *J Am Acad Dermatol* 2009; 60: 426-35.
32. ENNIS H, MOORE T, MURRAY A, VAIL A, HERRICK AL: Further confirmation that digital ulcers are associated with the severity of abnormality on nailfold capillaroscopy in patients with systemic sclerosis. *Rheumatology* 2014; 53: 376-7.
33. MARINO CLAVERIE L, KNOBEL E, TAKASHIMA L *et al*.: Organ involvement in Argentinian systemic sclerosis patients with "late" pattern as compared to patients with "early/active" pattern by nailfold capillaroscopy. *Clin Rheumatol* 2013; 32: 839-43.
34. MARKUSSE IM, MEIJS J, DE BOER B *et al*.: Predicting cardiopulmonary involvement in patients with systemic sclerosis: complementary value of nailfold videocapillaroscopy patterns and disease-specific autoantibodies. *Rheumatology* 2017; 56: 1081-8.
35. CASTELLVÍ I, SIMEÓN-AZNAR CP, SARMIENTO M *et al*.: Association between nailfold capillaroscopy findings and pulmonary function tests in patients with systemic sclerosis. *J Rheumatol* 2015; 42: 222-7.
36. RICCIERI V, VASILE M, IANNACE N *et al*.: Systemic sclerosis patients with and without pulmonary arterial hypertension: a nailfold capillaroscopy study. *Rheumatology* 2013; 52: 1525-8.
37. HOFSTEE HMA, NOORDEGRAAF AV, VOSKUYL AE *et al*.: Nailfold capillary density is associated with the presence and severity of pulmonary arterial hypertension in systemic sclerosis. *Ann Rheum Dis* 2009; 68: 191-5.
38. ONG YY, NIKOLOUTSOPOULOS T, BOND CP, SMITH MD, AHERN MJ, ROBERTS-THOMSON PJ: Decreased nailfold capillary density in limited scleroderma with pulmonary hypertension. *Asian Pacific J Allergy Immunol* 1998; 16: 81-6.
39. MORARDET L, AVOUAC J, SAMMOUR M *et al*.: Late nailfold videocapillaroscopy pattern associated with hand calcinosis and acro-osteolysis in systemic sclerosis. *Arthritis Care Res* (Hoboken) 2016; 68: 366-73.
40. SAMBATARO D, SAMBATARO G, ZACCARA E *et al*.: Nailfold videocapillaroscopy microhaemorrhage and giant capillary counting as an accurate approach for a steady state definition of disease activity in systemic sclerosis. *Arthritis Res Ther* 2014; 16: 462.
41. ANDRACCO R, IRACE R, ZACCARA E *et al*.: The cumulative number of micro-haemorrhages and micro-thromboses in nailfold videocapillaroscopy is a good indicator of disease activity in systemic sclerosis: a validation study of the NEMO score. *Arthritis Res Ther* 2017; 19: 133.
42. AVOUAC J, LEPRI G, SMITH V *et al*.: Sequential nailfold videocapillaroscopy examinations have responsiveness to detect organ progression in systemic sclerosis. *Semin Arthritis Rheum* 2017; 47: 86-94.

43. CUTOLO M, ZAMPOGNA G, VREMIS L, SMITH V, PIZZORNI C, SULLI A: Longterm effects of endothelin receptor antagonism on microvascular damage evaluated by nailfold capillaroscopic analysis in systemic sclerosis. *J Rheumatol* 2013; 40: 40-5.
44. TROMBETTA AC, PIZZORNI C, RUARO B *et al*.: Effects of longterm treatment with bosentan and iloprost on nailfold absolute capillary number, fingertip blood perfusion, and clinical status in systemic sclerosis. *J Rheumatol* 2016; 43: 2033-41.
45. CESTELLI V, MANFREDI A, SEBASTIANI M *et al*.: Effect of treatment with iloprost with or without bosentan on nailfold videocapillaroscopic alterations in patients with systemic sclerosis. *Mod Rheumatol* 2017; 27: 110-4.
46. SMITH V, RICCIERI V, PIZZORNI C *et al*.: Nailfold capillaroscopy for prediction of novel future severe organ involvement in systemic sclerosis. *J Rheumatol* 2013; 40: 2023-8.
47. SMITH V, DECUMAN S, SULLI A *et al*.: Do worsening scleroderma capillaroscopic patterns predict future severe organ involvement? A pilot study. *Ann Rheum Dis* 2012; 71: 1636-9.
48. KAYSER C, SEKIYAMA JY, PRÓSPERO LC, CAMARGO CZ, ANDRADE LEC: Nailfold capillaroscopy abnormalities as predictors of mortality in patients with systemic sclerosis. *Clin Exp Rheumatol* 2013; 31 (Suppl. 76): S103-8.
49. SIMEÓN CP, ARMADANS L, FONOLLOSA V *et al*.: Mortality and prognostic factors in Spanish patients with systemic sclerosis. *Rheumatology* (Oxford) 2003; 42: 71-5.
50. SCUSSEL-LONZETTI L, JOYAL F, RAYNAULD J-P *et al*.: Predicting mortality in systemic sclerosis: analysis of a cohort of 309 French Canadian patients with emphasis on features at diagnosis as predictive factors for survival. *Medicine* (Baltimore) 2002; 81: 154-67.
51. HISSARIA P, LESTER S, HAKENDORF P *et al*.: Survival in scleroderma: results from the population-based South Australian Register. *Intern Med J* 2011; 41: 381-90.
52. PAVAN TR, BREDEMEIER M, HAX V *et al*.: Capillary loss on nailfold capillary microscopy is associated with mortality in systemic sclerosis. *Clin Rheumatol* 2018; 37: 475-81.