ORIGINAL RESEARCH

# Microvascular damage in autoimmune connective tissue diseases: a capillaroscopic analysis from 20 years of experience in a EULAR training and research referral centre for imaging

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## ABSTRACT

**Objective** Nailfold videocapillaroscopy (NVC) allows the detection of microvascular damage in autoimmune connective tissue diseases (CTDs). The prevalence of the morphological capillary findings was retrospectively evaluated in a wide cohort of patients with Raynaud's phenomenon secondary to a CTD at the time of the first single NVC, independently from their current treatment, autoantibody profile and comorbidities.

**Methods** One-thousand-one-hundred-eighty-one patients affected by CTDs were included from 2001 to 2021. The considered CTDs were systemic sclerosis (SSc), undifferentiated connective tissue disease (UCTD), mixed connective tissue disease (MCTD), dermatomyositis (DM), systemic lupus erythematosus, Sjögren's syndrome and primary antiphospholipid syndrome (aPS). The capillaroscopic parameters were distinguished between scleroderma patterns and non-scleroderma patterns.

**Results** Giant capillaries were significantly more frequent in SSc, DM and MCTD than in other CTDs (respectively, in 73%, 73% and 61% of patients, p<0.001 when comparing each rate vs the other CTDs). The mean capillary count was significantly lower in SSc, DM and MCTD (respectively, 7.04±0.18 vs 6.5±0.75 vs 7.7±2 capillaries/linear mm) compared with the other CTDs (p<0.001 for each rate vs the other CTDs). The non-specific abnormalities of capillary morphology were significantly more frequent in SSc, MCTD and aPS (respectively, in 48%, 41% and 36% of cases, all p<0.001 vs each other CTDs).

**Conclusion** This large size sample of patients with CTDs, collected over 20 years of analysis, confirms the highest prevalence of specific capillaroscopic alterations in patients with SSc, DM and MCTD, when compared with other CTDs.

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Raynaud's phenomenon (RP) secondary to autoimmune connective tissue diseases (CTDs) is characterised by the presence of microangiopathic findings in nailfold videocapillaroscopy (NVC).
⇒ There is a lack of data related to the comparative prevalence of the microvascular damage across all the CTDs with recent standardised definitions in capillaroscopy.

## WHAT THIS STUDY ADDS

⇒ Our survey reports a comparative detailed NVC analysis in the widest published cohort of secondary RP affected by CTDs.
⇒ Patients with systemic sclerosis, dermatomyositis and mixed connective tissue disease display a microangiopathy with a higher prevalence of specific alterations compared with other CTDs.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our retrospective study might support the diagnostic approach of secondary RP by providing large-scale data inherent to the prevalence of specific and non-specific findings in capillaroscopy across autoimmune CTDs.

## INTRODUCTION

Raynaud's phenomenon (RP) is a frequent clinical feature of altered microcirculation and it is a common manifestation in autoimmune connective tissue diseases (CTDs) with a prevalence widely ranging from 11% to 90% according to the considered CTD.[1] Patients with systemic sclerosis (SSc) show RP as a first clinical manifestation in up to 90% of cases, whereas lower rates have been reported for other CTDs such systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS)

where it can be observed, respectively, in up to 46% and 13% of cases.[2 3]

The assessment of RP secondary to a CTD can be safely performed by nailfold videocapillaroscopy (NVC), which is considered the gold standard method for the detection of the morphological microvascular abnormalities secondary to autoimmune rheumatic diseases and helps distinguishing the secondary from the primary RP which is purely functional with absent morphological microvascular changes.[4] Indeed, the suggested criteria for primary Raynaud's phenomenon (PRP) include, besides the absence of pathological NVC findings, symmetric attacks, the absence of tissue necrosis, ulceration or gangrene, the absence of a secondary cause, negative tests for antinuclear antibodies and a normal erythrocyte sedimentation rate.[5]

Conversely, the specific microvascular abnormalities such as giant capillaries (homogeneous dilation with a diameter>50 µm) or the combination of severe capillary loss with abnormal shapes identify patients with NVC 'scleroderma pattern'.[6–9]

The abnormal morphological parameters specific to different stages of disease progression in SSc microangiopathy have been categorised as 'early', 'active' and 'late' patterns, but NVC patterns have been searched also in other CTDs.[10]

NVC 'scleroderma-like' pattern has been described for CTDs beyond SSc and consists in a cluster of alterations of the capillary distribution, shape, number and dimension (mixing together the aspects observed in detail in the three SSc NVC patterns). This pattern has been reported for idiopathic inflammatory myopathies, such dermatomyositis (DM) or the antisynthetase syndrome, and mixed connective tissue disease (MCTD).[11–13]

The aim of this study was to detect the prevalence of each morphological microcirculatory alteration, retrospectively evaluated, in a wide cohort of patients with RP secondary to a definite diagnosis of CTD at the time of the first single NVC, independently from their current disease status, treatment, autoantibody profile and comorbidities and to compare capillary findings in each CTD with SSc.

## METHODS
### Study population
The considered CTDs, diagnosed through the most recent international classification criteria available at the time of the enrollment, were: SSc, undifferentiated connective tissue disease (UCTD), MCTD, DM, SLE, SS and primary antiphospholipid syndrome (aPS). Patients with overlap syndromes were excluded from our analysis. This retrospective study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. All the patients signed the mandatory written informed consent to manage their clinical data collection/analysis as a standard hospital procedure according to the rules of the University Hospital at the time of their first NVC in our clinic. The code of the written consent form, allowing the retrospective utilisation of anonymised images in our division, is CONSAZHQA_0001. All the patients were evaluated for RP at the time of their referral to the centre. The patients were in treatment with different conventional synthetic or biological disease modifying antirheumatic drugs, glucocorticoids and vasoactive agents (see table 1).

### Nailfold videocapillaroscopy
NVC was performed using an optical probe, equipped with a 200× contact lens connected to image analysis software (Videocap, DS Medica, Milan, Italy). The operator performed the NVC examinations in all patients, after an acclimatisation period of 20 min at 22–24°C, according to the standardised procedures.[6 7]

Two millimetres in the middle of the nailfold bed were assessed for each finger. According to the most recent validated literature evidence, the NVC parameters were defined as following: normal capillaries (hairpin shaped), non-specific capillary variations of morphology (tortuous or crossing capillaries with branch diameters<20 µm), dilated capillaries (irregular or homogeneous increase of capillary diameter between 20 µm and 50 µm), giant capillaries (homogeneously dilated normal shaped loops with a diameter≥50 µm), microhaemorrhages (dark masses attributable to hemosiderin deposit), abnormal shapes (ie, ramified capillaries, non-covex head of capillaries, neoangiogenesis and originating from a single capillary) and lower capillary density (reduction of capillary number below normal range of 7 capillaries per linear mm, counted at the distal row).[6] We recorded the frequency of patients presenting at least one NVC finding (ie, giant capillaries, microhemorrhages or abnormal shapes, see table 2).

The previously mentioned NVC parameters were collected in the cohort of patients at baseline (first visit in our clinic). The capillaroscopic parameters were collected according to the Fast Track Algorithm and distinguished between scleroderma pattern (specific NVC alterations) and non-scleroderma patterns (non-specific NVC alterations and normal findings).[14] According to these standardised definitions, specific capillaroscopic alterations include the presence of giant capillaries and/or the loss of capillaries combined with abnormally shaped capillaries.[6] We defined 'scleroderma-like' pattern as the NVC findings in other CTDs beyond SSc which are characterised by a mix of the specific abnormalities of all the SSc patterns without fitting the single definition of 'early', 'active' or 'late' pattern (figure 1).[7]

### Statistical analysis
Categorical variables, such as frequencies, were compared with the $\chi^2$ test. Normal distribution of metric data was checked before each statistical test both analytically, with the Kolmogorov-Smirnov test, and graphically with Q–Q plots. In case of normal distribution, parametric tests were used, such as the independent t-test

**Table 1** Demographic and clinical features of the cohort

| Demographic and clinical features | SSc (n=601) | DM (n=30) | MCTD (n=70) | UCTD (n=315) | aPS (n=22) | SLE (n=108) | SS (n=35) |
|---|---|---|---|---|---|---|---|
| Sex (F, %) | 558, 92.8% | 24, 80% | 59, 84.2% | 298, 94% | 17, 77.2% | 99, 91.6% | 32, 91.4% |
| Age (years, mean±SD) | 57.6±15 | 53±18 | 49±16 | 52±15 | 50±14 | 47.4±13.13 | 56.6±15.7 |
| Disease duration (years, mean±SD) | 5.4±4.6 | 6.6±5.7 | 4.4±3 | 5.4±3 | 7.6±5.7 | 12.3±4.1 | 2.8±1.6 |
| **ANA profile pattern** | | | | | | | |
| Homogeneous (%) | 19% | 15% | 0% | 15% | 8% | 76% | 0% |
| Speckled (%) | 28% | 73% | 100% | 56% | 4% | 0% | 76% |
| Nucleolar (%) | 18% | 7% | 0% | 21% | 0% | 18% | 0% |
| Centromeric (%) | 25% | 3% | 0% | 8% | 0% | 0% | 0% |
| Mixed (%) | 6% | 0% | 0% | 0% | 3% | 0% | 0% |
| Cytoplasmic (%) | 0% | 0% | 0% | 0% | 0% | 0% | 15% |
| Negative (%) | 4% | 2% | 0% | 0% | 85% | 6% | 9% |
| **Autoantibody profile (ENA or other specific autoantibodies)** | | | | | | | |
| | ACA 43.6% | Negative MSA in 13% of patients | Anti-RNP 100% | ACA 12% | ACL IgM or IgG 72% | Anti-dsDNA 52% | Anti-SSA 78% |
| | ATA 28.8% | Anti-Jo1 47%, | | Anti-SSA 6% | Anti-β2GPI IgM or IgG 72% | ACL IgM or IgG 30 % | Anti-SSB 43% |
| | ARA 3.9% | Anti-Mi2 13% | | ATA 6% | LAC 27% | Anti-β2GPI IgM or IgG 27 % | |
| | Anti-RNP 5.9% | Anti-NXP2 10% | | Anti-Ro52, 4% | | Anti-Sm 19% | |
| | Anti-Th/To 1.6% | Anti-TIF-1γ 7% | | Anti-SSB 2% | | LAC 15% | |
| | Anti-fibrillarin 1.3% | Anti-SAE1-2 7% | | Anti-Jo1 2% | | Anti-RNP 8% | |
| | Anti-Ku 0.5% | Anti-MDA5 3% | | | | Anti-SSA 6% | |
| | Anti-Jo1 0.6% | Negative MSA 13% | | | | Anti-SSB 4% | |
| | Anti-SSA 2.5% | | | | | | |
| | Anti-SSB 1.1% | | | | | | |
| | Anti-Ro52 9.5% | | | | | | |
| **Ongoing treatment** | | | | | | | |
| Vasodilators (ie, CCB, PDE5-I), n (%) | 538 (89.5%) | 6 (20%) | 23 (32.6%) | 75 (23.8%) | 0 (0 %) | 2 (1.8%) | 3 (8.5%) |
| Glucocorticoids, n (%) | 42 (6.9%) | 23 (77%) | 40 (56.5%) | 192 (61%) | 6 (27%) | 100 (92.5%) | 27 (77.1%) |
| csDMARDs, n (%) (ie, HCQ, MTX, CsA, MMF, CYC) | 389 (64.7%) | 25 (83%) | 46 (65.2%) | 130 (41.3%) | 5 (22.7%) | 95 (88%) | 22 (62.8%) |

Continued

**Table 1**  Continued

| Demographic and clinical features | SSc (n=601) | DM (n=30) | MCTD (n=70) | UCTD (n=315) | aPS (n=22) | SLE (n=108) | SS (n=35) |
|---|---|---|---|---|---|---|---|
| bDMARDs/ tsDMARDs, n (%) (ie, RTX, BEL, NIB, JAKi) | 38 (6.3 %) | 2 (7%) | 1 (1.4%) | 0 (0 %) | 0 (0%) | 12 (11%) | 0 (0 %) |
| Comorbidities | | | | | | | |
| Type two diabetes mellitus, n (%) | 36 (6%) | 3 (10%) | 1 (1.4%) | 2 (0.6 %) | 1 (4.5%) | 15 (13.8%) | 0 (0 %) |
| Dyslipidaemia, n (%) | 84 (14%) | 3 (10%) | 6 (8.5%) | 34 (10.8%) | 1 (4.5%) | 9 (8.3%) | 2 (5.7%) |
| Arterial hypertension, n (%) | 204 (34%) | 8 (27%) | 17 (24%) | 74 (23.4%) | 9 (40.9%) | 34 (31.5%) | 5 (14.3%) |
| Chronic renal failure, n (%) | 48 (8%) | 1 (3%) | 2 (3%) | 2 (0.6%) | 1 (4.5%) | 20 (18.5%) | 2 (5.7%) |

ACA, anticentromere autoantibodies; ACL, anticardiolipidin antibodies; ANA, antinuclear antibodies; aPS, antiphospholipid syndrome; ARA, anti-RNA polymerase III antibodies; ATA, antitopoisomerase I; BEL, belimumab; CCB, calcium channel blockers; CsA, cyclosporine A; cs/bDMARDs, conventional synthetic or biological disease modifying antirheumatic drugs; CYC, cyclophosphamide; DM, dermatomyositis; ENA, extractable nuclear antigens antibodies; F, females; HCQ, hydroxychloroquine; methotrexate; IgM/IgG, immunoglobulin M/G; JAKi, janus kinase inhibitors; LAC, lupus anticoagulant; MCTD, mixed connective tissue disease; MMF, mycophenolate mofetil; MSA, myositis specific autoantibodies; m±SD, mean and SD; MTX, methotrexate; NIB, nintedanib; PDE5-I, inhibitors of phosphodiesterase-5; RNP, ribonucleoprotein; RTX, rituximab; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSA, Sjögren's-syndrome-related antigen A; SSB, Sjögren's-syndrome-related antigen B; SSc, systemic sclerosis; UCTD, undifferentiated connective tissue disease; β2GPI, beta2-glycoprotein1.

if the considered samples were two or by the analysis of variance in case of multiple samples. When data were distributed non-symmetrically, non-parametric tests such as Mann-Whitney or Kruskall-Wallis tests were chosen. P values <0.05 were considered as statistically significant.

Statistical analysis was performed with DATAtab.

## RESULTS
### Main clinical features of the study population
One-thousand-one-hundred-eighty-one patients affected by CTDs were included from 2001 to 2021 in the Rheumatology Division of San Martino Polyclinic University Hospital (Genoa, Italy). One-thousand sixty-five patients were females (90.1% of the cohort), whereas the mean age and the mean disease duration of the whole cohort were, respectively, 54.1±16.2 years and 5.5±4 years. The other descriptive clinical–demographical features, including, the current treatment, the autoantibody profile and the presence of comorbidities potentially influencing the microvascular findings are reported in table 1 for each CTD.

### NVC findings
Giant capillaries, capillary dilations and microhemorrhages were significantly more frequent in SSc and DM compared with other CTDs (respectively, in 73%, 99% and 70% of patients with SSc and in 73%, 96% and 70% of patients with DM; non-significant p values for the

comparison between SSc vs DM but p<0.001 for comparisons between SSc and the other CTDs, see table 2 and figure 2). The non-specific abnormalities of capillary morphology, such as the ramifications (expression of neoangiogenesis), were significantly more frequent in SSc, MCTD and aPS among all the CTDs (respectively, in 48%, 41% and 36% of cases; non-significant differences between the three of them but p<0.05 for the comparison between each of the 3 vs the other CTDs).

Interestingly, giant capillaries and abnormal shapes were detected, respectively, in 61% and 41% of patients with MCTD. In patients with aPS, the most significant prevalence of microhaemorrages (50%) was observed compared with other CTDs, to the exclusion of SSc and DM where microhemorrhages were detected in 70% of cases.

Among CTDs, the mean capillary density (number of capillaries/linear mm) was significantly lower in SSc and DM (respectively, 7.04±0.18 vs 6.5±0.75) compared with other CTDs (figure 2). A non-significant difference was observed for the mean capillary density between SSc and DM (p=0.11), whereas a higher capillaries count was detected in MCTD versus SSc (p=0.04). When SSc and DM were excluded, MCTD was the CTD with the lowest capillary numerosity compared with other CTDs (p<0.01 for each comparison). 'Early', 'active' and 'late' scleroderma patterns were detected in 34%, 38% and 16% of patients with SSc, whereas scleroderma-like pattern was

**Table 2** Main capillaroscopic findings in autoimmune CTDs. All the alterations are compared, as a reference, with SSc

| NVC features | SSc n=601 | DM n=30 | MCTD n=70 | UCTD n=315 | aPS n=22 | SLE n=108 | SS n=35 |
|---|---|---|---|---|---|---|---|
| Capillary density | 7.04 (1–12) | 6.5 (4-10) p=0.11 | 7.7 (3–11) p=0.04 | 9.17 (3–13) p=0.001 | 9.5 (7–12) p<0.001 | 9.6 (4–12) p<0.00001 | 9.5 (6–12) p<0.00001 |
| Patients with reduction of the capillary number (<7/linear mm) | 324 (54 %) | 16 (53%) p=0.95 | 24 (34%) p=0.002 | 39 (12%) p<0.0001 | 0 (0%) p<0.0001 | 7 (6%) p<0.0001 | 1 (3%) p<0.0001 |
| Giant capillaries | 442 (73%) | 22 (73%) p=0.97 | 43 (61 %) p=0.03 | 70 (22%) p<0.00001 | 2 (9 %) p<0.00001 | 16 (15 %) p<0.00001 | 5 (14 %) p<0.00001 |
| Dilations | 597 (99%) | 29 (96%) p=0.1 | 63 (90%) p<0.0001 | 288 (91%) p=0.001 | 19 (86 %) p=0.00004 | 89 (82%) p<0.00001 | 24 (69%) p<0.00001 |
| Microhaemorrhages | 424 (70%) | 21 (70%) p=0.61 | 32 (46%) p<0.0001 | 129 (41%) p<0.0001 | 11 (50%) p=0.004 | 5 (5%) p<0.001 | 2 (6%) p<0.00001 |
| Non-specific abnormal capillary morphologies | 290 (48%) | 13 (12%) p<0.00001 | 29 (41 %) p=0.28 | 84 (27%) p<0.00001 | 8 (36 %) p=0.27 | 13 (12%) p<0.00001 | 7 (20%) p<0.00001 |
| NVC pattern | 'Early': 201 (34%) 'Active': 227 (38%) 'Late': 97 (16%) NL: 17 (2%) NSA:59 (10%) | NL: 0 (0%) p=1 NSA: 8 (26%) p<0.00001 Scleroderma pattern: 11 (37%) Scleroderma-like pattern: 11 (37%) | NL: 2 (3%) p=0.45 NSA: 26 (36 %) p<0.00001 Scleroderma pattern: 21 (30%) Scleroderma-like: 22 (31%) | NL: 16 (5%) p=0.09 NSA: 229 (73%) p<0.00001 Scleroderma pattern: 14 (4%) Scleroderma-like: 56 (18%) | NL: 4 (18%) p=0.05 NSA: 16 (73%) p<0.00001 Scleroderma-like pattern: 2 (9%) | NL: 15 (14%) p<0.00001 NSA: 78 (71%) p<0.00001 Scleroderma pattern: 2 (2%) Scleroderma-like pattern: 14 (13%) | NL: 12 (35%) p<0.00001 NSA: 18 (51%) p<0.00001 Scleroderma-like pattern: 5 (14%) |

*Statistically significant p values are indicated in bold type

aPS, antiphospholipid syndrome; CTDs, connective tissue diseases; DM, dermatomyositis; MCTD, mixed connective tissue disease; NL, normal; NSA, non-specific alterations; NVC, nailfold videocapillaroscopy; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSc, systemic sclerosis; UCTD, undifferentiated connective tissue disease.
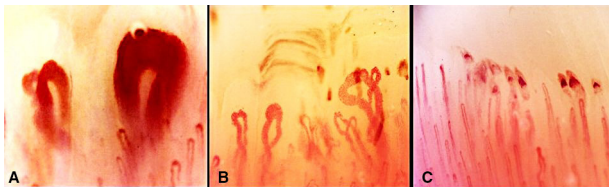
**Figure 1** Microvascular morphological alterations detectable on NVC in autoimmune CTDs. (A) Giant capillaries in an SSc patient with an 'active' scleroderma pattern. (B) Scleroderma-like pattern in a patient with MCTD. (C) 'Comb-like' microhaemorrhages in a patient with primary aPS. aPS, antiphospholipid syndrome; CTDs, connective tissue diseases; MCTD, mixed connective tissue disease; NVC, nailfold videocapillaroscopy; SSc, systemic sclerosis.

detected more frequently in DM (37 %) and MCTD (31%) compared with other CTDs beyond SSc (p<0.02, non-significant p value between DM and MCTD, p=0.6).

Scleroderma-pattern was detected in 37% of patients with DM, 30% of patients with MCTD, 4% of patients with UCTD and 2% of patients with SLE. In aPS and SS, no patients with scleroderma-pattern were found.

## DISCUSSION

The results of the NVC analysis in a wide cohort of patients with CTD suggest that the microvascular damage in SSc, DM and MCTD displays more specific NVC findings compared with other CTDs. Indeed, these patients showed a higher prevalence of giant capillaries and a more severe reduction of capillary density when compared with the other autoimmune CTDs. These NVC specific alterations are regarded as the crucial elements, which can permit the differentiation of a 'scleroderma pattern' from a 'non-scleroderma' pattern according to recent standardised guidelines in capillaroscopy.[6]

From a diagnostic perspective, NVC findings of 'scleroderma pattern' and specific autoantibodies have been shown to be strong predictors of SSc development in a cohort of patients with secondary RP.[15–17] Additionally, NVC findings have also been introduced in the 2013 classification criteria for SSc, a fact which emphasises its importance especially in the early diagnosis of the disease.[18]

Follow-up studies have reported that the progressive reduction of capillary density is correlated with the overall organ damage in SSc and might be a promising adjunct outcome measure in randomised clinical trials for monitoring the efficacy of vasoactive and/or immunosuppressive agents.[19–21] Additionally, NVC findings have been shown to be predictors of peripheral vascular injury and lung involvement in patients with SSc.[22–24]

While the progression of microangiopathy in SSc has an established literature, exploratory and follow-up studies related to NVC findings in the other CTDs are fewer and more contrasting and even fewer are the comparative reports of the different microangiopathies among CTDs.

In DM, Manfredi et al have shown that patients having a shorter disease duration (<6 months) exhibit a reduced mean capillary density and a higher rate of giant capillaries compared with patients with longer disease duration (>6 months).[12] The latter showed, however, an increased frequency of abnormal shapes compared with individuals with early disease. NVC findings in DM also appear to correlate, according to different studies, to skin, muscle, joint and lung involvement.[25–27] Nevertheless, these correlations with disease activity are heterogeneous: the small sample sizes of the different reports, the variable inclusion of patients with diverse autoantibodies phenotype and the heterogeneous methods of clinical assessment make difficult the generalisation of NVC
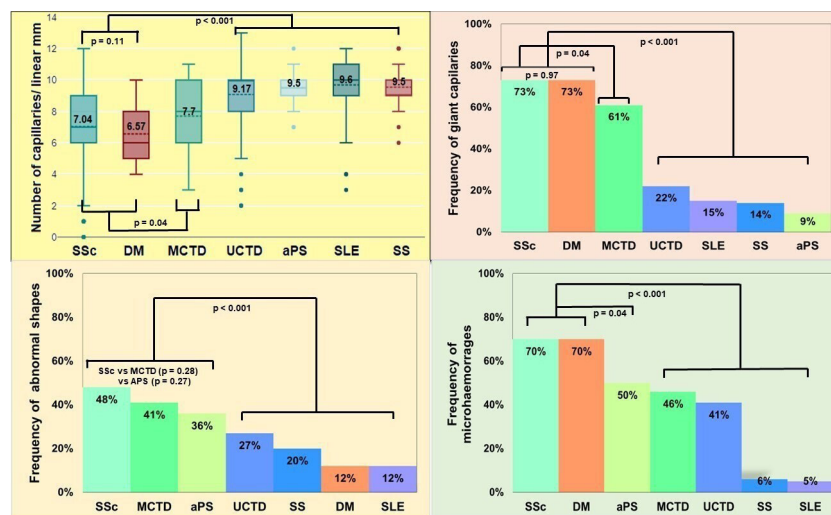


**Figure 2** Mean capillary density, rates of giant capillaries, abnormally shaped capillaries and microhaemorrhages in CTDs. aPS, antiphospholipid syndrome; CTDs, connective tissue diseases; DM, dermatomyositis; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; SS, Sjögren's syndrome; SSc, systemic sclerosis; UCTD, undifferentiated connective tissue disease.

findings with the overall clinical features in patients with DM.[28]

From comparative studies between DM and SSc, a lower density in SSc versus DM has been detected: more specifically, by comparing patients with DM with each subgroup of patients with SSc divided according to the stages of 'scleroderma pattern', it has been shown that patients with DM display a more severe capillary loss than the 'early' NVC SSc pattern but present a lower extent of capillary reduction when compared with 'active' and 'late' NVC SSc patterns.[29] Another difference was reported in the longitudinal follow-up of the microangiopathies in these diseases: while the neoangiogenesis rate increased in both during the follow-up; on the contrary, capillary count recovered in DM while worsened in SSc, suggesting today more chances of reversibility with treatment in patients with DM.[29] Characteristics of stability in DM microangiopathy have been previously observed by our group compared with patients with SSc who more frequently displayed features of progression.[13]

Despite our study did not show a statistically significant difference in the capillary count between patients with SSc and patients with DM, this could be explained, partly, by the relatively low percentage of 'late' NVC pattern in patients with SSc (16%) where capillary count typically drops.

Our findings also showed a non-significant difference in the rate of giant capillaries between SSc and DM (both detected in 73% of cases) and a higher frequency of abnormal shapes in SSc (48% vs 12%). This might be explained by the relatively high percentage of enrolled patients with 'early' and 'active' NVC SSc pattern, where giant capillaries are mostly expressed.

After SSc and DM, we detected a reduced capillary count in MCTD compared with other CTDs. In literature, there are reports showing a correlation between reduced capillary density in NVC and interstitial lung disease and muscle involvement indicating that microangiopathy might be a potential proxy in MCTD for identifying patients with a higher disease activity and organ damage.[30 31]

In our study, most of the patients with MCTD showed non-specific alterations (60%) but 'scleroderma pattern' and 'scleroderma-like' pattern were detected in, respectively, 30% and 31% of patients indicating the presence of specific NVC microvascular markers of damage. This rate of 'scleroderma-like' pattern in MCTD patients is in line with literature findings.[32 33] In a recent study published by our group, we have shown that MCTD patients also display a more severe microvascular phenotype compared with patients with UCTD and this concept is confirmed also in this large cohort, especially when the difference in the 'scleroderma-like' pattern is considered (31% in MCTD vs 18% of UCTD).[34]

However, follow-up studies will be necessary to understand if the 'scleroderma-like' pattern is a predictor of poor outcomes in patients with MCTD.

In our cohort of patients with UCTD, the rate of scleroderma pattern and 'scleroderma-like' pattern is in line with other published cross-sectional studies.[35 36] The prognostic value of these patterns has shown, at the moment, features of predictivity toward development of SSc, especially when combined in a multivariate complex model, which also includes the titre of antinuclear antibodies, the presence of specific SSc-related autoantibodies and the presence of giant capillaries and/or avascular areas at NVC.[37] Further longitudinal studies should be addressed to quantify the value of the isolated scleroderma pattern in UCTD in predicting SSc development.

In aPS, no specific NVC pattern is known, nevertheless the so called 'comb-like' appearance, consisting in multiple parallel/linear microhemorrhages beneath normal-shaped capillaries arranged perpendicularly to the nailfold bed, has been described.[38] This has been previously associated with the presence of anticardiolipin antibodies (microthromby), which were positive in 72% of our patients.[39] Moreover, the most diffused capillary modification in aPS is capillary dilation of either the afferent, apical or efferent branch.[40] Our study confirms these literature data, showing a prevalence of non-specific abnormalities in 73% of patients and with the most frequent microvascular findings being capillary dilations (86%) and microhaemorrhages (50%).

In SLE, according to the literature, non-specific alterations were mainly described, above all tortuous capillaries, microhemorrhages and abnormally shaped capillaries.[41] Moreover, capillary abnormalities have been detected in up to 36% of patients with SLE, with described correlations with autoantibodies against Sjögren's-syndrome-related antigen A/B (anti-SSA/SSB) and anti-U1 ribonucleoprotein (U1RNP), but a 'scleroderma-like' pattern has been rarely observed (2.4%–15%), percentages in line with our results.[42]

The importance of RNP positivity, influencing the severity of NVC findings on patients with SLE, has been recently stressed by an observational study where significant correlations emerged between this autoantibody profile and the reduction of capillary number.[43] Our findings are in line also with the literature reports showing a prevalence of non-specific alterations (84%), with the dilatation being the most diffuse NVC feature.

In SS, no specific NVC pattern has been identified.[44] A 'scleroderma pattern' may be present in a variable percentage of patients with SS (10%–30%), especially in those who show RP and have an overlap syndrome with SSc or primary biliary cholangitis.[32]

Generally, capillary density stays within the normal range, even if some reports suggest a lower capillary density compared with healthy controls, especially in the presence of RP and/or anti-centromere antibodies.[32 44 45] The results of our study agree with literature data, showing a prevalence of non-specific abnormalities in 60% of patients with SS and a 'scleroderma-like' pattern in 14% of SS individuals. It has still to be understood if

'scleroderma-like' pattern in SS is associated with overlap syndromes or with systemic vasculitis SS related.[46]

Among the main limitations, we acknowledge the variability in the sample sizes between each CTD: however, this derives partly from the retrospective study design. Furthermore, treatment, autoantibody profile, ethnicity, sex and comorbidities might have influenced microangiopathic findings, but separate subgroup analyses were not performed for reasons of statistical power.

To the best of our knowledge, however, our retrospective evaluation, represents a detailed comparative NVC analysis in the widest published cohort of secondary RP affected by CTDs.

Despite such type of comparison has been previously done,[32] the added value of this survey derives from the higher sample size and from the implementation of the most recent standardised and validated definitions in capillaroscopy.[6]

## CONCLUSIONS

Our results, collected over 20 years of analysis, confirm that NVC in SSc, DM and MCTD shows a more specific microangiopathy compared with other CTDs. This is documented by the higher prevalence of specific NVC abnormalities: higher rate of giant capillaries and reduced capillary density combined with abnormally shaped capillaries when compared with other CTDs.

Nevertheless, the microvascular findings detected in other CTDs, despite being mostly non-specific, might be worthy of longitudinal NVC evaluation since endothelial damage might play a role in their pathophysiology. NVC might identify phenotypes of patients displaying a more severe microvascular domain with potential clinical correlations with other extravascular features or evolving toward overlap syndromes within the scleroderma-spectrum disorders.

The correlations between organ and microvascular damage in CTDs different from SSc are being studied in ongoing projects. These will have, as aims, the enrollment of higher sample sizes, which might permit more accurately the evaluation of these associations.

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