Long-term follow-up of nailfold videocapillaroscopic microvascular parameters in mixed connective tissue disease *versus* systemic sclerosis patients: a retrospective cohort study

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ABSTRACT

Objective. *To retrospectively study nailfold videocapillaroscopy (NVC) changes in mixed connective tissue disease (MCTD) patients and to compare the capillary morphological abnormalities between patients affected by MCTD and systemic sclerosis (SSc) over time.*

Methods. *Ten MCTD patients on whom NVC had been performed, with a follow-up of three years, were selected. In addition, ten patients affected by SSc with similar age and disease duration of MCTD patients were enrolled to compare NVC abnormalities at baseline (T0).*

Results. *Seven out of ten patients with MCTD showed a "scleroderma-like pattern" at first NVC. No statistically significant variation of the detected NVC parameters was observed during the 3-year follow-up, and no statistically significant correlation was observed between capillary parameters and MCTD clinical aspects at first visit and during the follow-up. The scores of enlarged capillaries, giant capillaries and microhaemorrhages were significantly lower ($p<0.05$) in MCTD versus SSc patients at T0, moreover, the absolute number of total capillaries and normal capillaries was found significantly higher ($p<0.05$) in MCTD versus SSc patients.*

Conclusion. *This study suggests that nailfold microvascular damage does not seem to be significantly progressive in MCTD patients during a three-year follow-up. MCTD patients show significantly lower number of enlarged/giant capillaries, but higher number of total and normal capillaries than SSc patients at first nailfold capillaroscopy. The identification of a specific NVC pattern in MCTD patients is not yet possible.*

Introduction

Mixed connective tissue disease (MCTD) is a rare condition whose diagnosis necessarily requires the detection of high titre of anti-U1 RNP antibodies and the presence of several features of different connective tissue diseases (CTD), such as systemic sclerosis (SSc), dermatomyositis, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (1, 2).

MCTD was described by Sharp *et al.* for the first time in 1972 (3). Over the years, the existence of this isolated clinical condition has been questioned; nowadays, however, other studies supported the concept of MCTD as a different disease that may evolve into another CTD during follow-up (2, 3).

To date, the diagnostic criteria used for MCTD are the following three: Alarcón-Segovia, Kasukawa and Sharp (3-6). It is not yet clear what are the most appropriate diagnostic criteria to use, each of them has pros and cons (2). The Alarcón-Segovia criteria are simpler than the others, while the Kasukawa criteria are more appropriate for evaluating each sign and symptom of MCTD and have proved to be more sensitive and applicable in clinical practice (2). The original Sharp criteria, being more complicated, have been less used over time due to their high number of clinical features and the extractable nuclear antigen titre (2).

NVC is the best and non-invasive method to detect and to evaluate morphological microvascular abnormalities in patients affected by secondary Raynaud's phenomenon (RP) and therefore this technique permits early diagnosis of connective tissue diseases such as systemic sclerosis (7-8).

A microvascular pattern characterised by sequential and dynamic changes was found in patients affected by SSc and named "scleroderma-pattern", that consists in a cluster of alterations of the capillary distribution, shape, number and dimension and may be detected

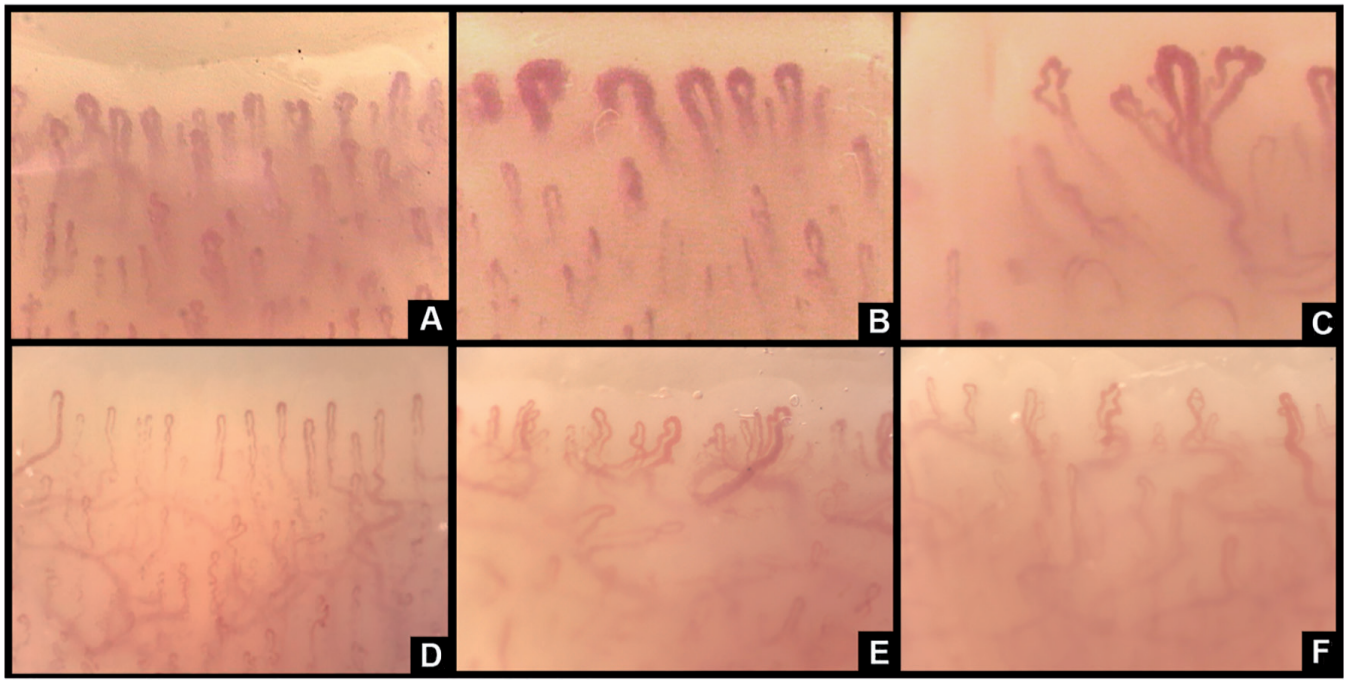

Fig. 1. Examples of "early" (**A**), "active" (**B**) and "late" (**C**) scleroderma patterns of microangiopathy in three patients with systemic sclerosis; normal nailfold capillary pattern (**D**) and nailfold capillary images of scleroderma-like patterns in three patients with mixed connective tissue disease (**E** and **F**). Nailfold videocapillaroscopy, magnification 200x.

also in other connective tissue disorders, including MCTD, the so called scleroderma-spectrum disorders (9-15).

Three defined major NVC patterns have been identified by Cutolo *et al*. in assessing the appearance and progression of the SSc microangiopathy (*early*, *active* and *late* patterns) (Fig. 1A-B-C) (16).

Even though MCTD may show markers of the SSc-pattern (giant capillaries, loss of capillaries, abormal shapes, *i.e*. ramifications, microhaemorrages), the relative proportions of these markers are different than in the SSc patterns and hence the terminology "scleroderma-like" pattern has been used to refer to those nailfold capillary abnormalities (14).

More specifically, in MCTD the presence of abnormal shapes, *i.e*. ramified/bushy capillaries often coexist along with giant capillaries, and capillary disorganisation may be sometimes extensive (17).

Until today, few studies described the main NVC changes in MCTD at basal time and during follow-up (2, 16). The aim of this retrospective study was to assess the nailfold microangiopathy in MCTD patients and to compare over 3 years the progression of capillary characteristics between patients affected by MCTD and SSc.

Materials and methods

Patients

Ten patients affected by MCTD with Raynaud phenomenon (Kasukawa's criteria) (2), have been retrospectively evaluated from the time they had undergone the first clinical assessment by NVC, and were followed for three years. All patients had antibodies against the U1 small nuclear ribonucleoprotein autoantigen (U1snRNP) according to Kasukawa's criteria. Patients with either anti-extractable nuclear antigen positivity different from U1snRNP or anti-dsDNA antibodies were excluded during the selection.

Also, 10 patients affected by SSc (according to EULAR/ACR 2013 criteria) (18) with the same mean disease duration of MCTD patients were enrolled and compared at the first NVC analysis.

This retrospective observational study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice, and all patient data were collected during the routine medical visits of follow-up in our Clinic. All the patients gave written informed consent to manage their clinical data and enter the study

Microvascular damage and nailfold capillaroscopy

NVC was performed using an optical probe, equipped with a 200x contact lens connected to image analysis software (Videocap, DS Medica, Milan, Italy). The same operator (CP) performed the NVC examinations in all patients, after a permanence in a comfortable room temperature of 22-24 °C for 20 minutes. Two millimetres in the middle of the nailfold were studied in each finger, and the average of capillary abnormalities from the eight fingers evaluated, as previously described (16, 19, 20). The number of images taken per patient was sixteen, two pictures per finger (thumbs excluded). The following qualitative parameters were assessed: normal capillaries (that include hairpin-shaped, tortuous or crossing capillaries with branch diameters <20 μm), dilated (enlarged) capillaries (irregular or homogeneous increase of capillary diameter between 20 and 50 μm), giant capillaries (homogeneously dilated normal shaped loops with a diameter ≥50 μm), microhaemorrhages (dark masses attributable to haemosiderin deposit), abnormally shaped

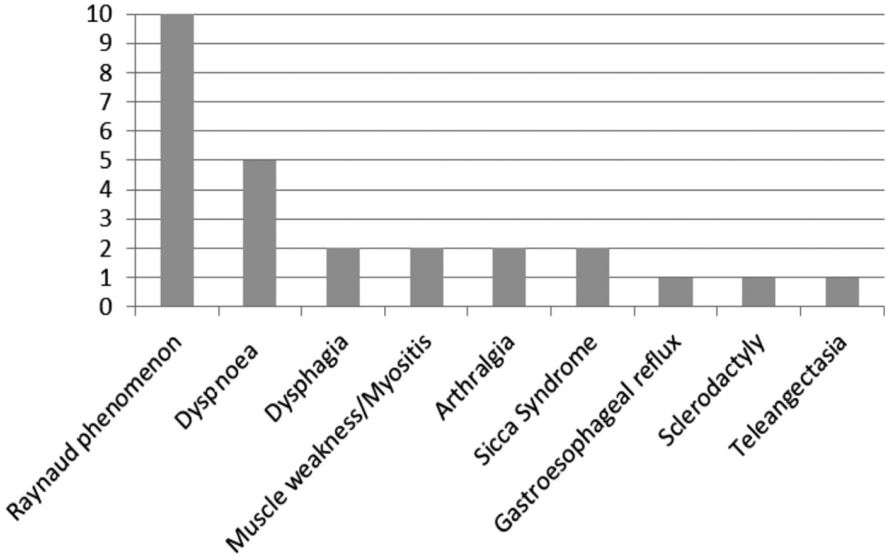

Fig. 2. Clinical symptoms in patients affected by mixed connective tissue disease at baseline visit (number of patients is reported in the ordinate).

i.e. ramified, branching, bushy capillaries, interconnected, originating from a single capillary), disorganisation of the microvascular array, capillary loss (reduction of the number of capillaries below nine) (9, 10, 19, 21).

All these parameters were also used to define the three validated NVC patterns of microangiopathy in SSc according to Cutolo *et al.* (*early*, *active* and *late*) (16, 22).

Using an already validated score, we have attributed to the different capillaroscopic parameters detected by NVC (dilated and giant capillaries, microhaemorrhages, abnormally shaped capillaries and disorganisation of the vascular array) a mean score value obtained from a semiquantitative rating scale (0=no changes, 1=less than 33% of capillary alterations/reduction, 2=33–66% of capillary alterations/reduction, 3=more than 66% of capillary alterations/reduction, per linear millimeter) (20, 22). Furthermore, the number of both total capillaries and normal capillaries were also counted.

Main NVC parameters, both scores and absolute capillary numbers, were assessed by NVC at baseline (T0, first NVC), after one (T1) and three years (T3) in MCTD, and at T0 in SSc patients.

Statistical analysis

Statistical analysis was achieved by non-parametric tests. Especially, the Mann-Whitney U-test was used to compare unpaired groups of variables and the Wilcoxon signed-rank test was used to compare paired groups of variables. Friedman test was adopted to identify differences across multiple related comparisons. Results are reported as median along with interquartile range (IQR). We considered p-values ≤ 0.05 as statistically significant.

Results

The ten MCTD patients enrolled were 8 females and 2 males: median age 49 years [IQR 34], median disease duration 5 [IQR 7] years. The other ten patients affected by SSc were all females (6 limited cutaneous and 4 diffuse cutaneous SSc), with similar median disease duration (5 [IQR 7] years) and age (51 [IQR 33] years) of the MCTD patients.

The clinical symptoms of patients affected by MCTD are reported in Figure 2.

MCTD patients were treated with different immunosuppressive drugs including cyclosporine A, methotrexate, hydroxychloroquine, mycophenolate, and all received low dose glucocorticoids. Care of SSc patients was in line with clinical practice guidelines (immunosuppressive drugs including cyclosporine A and mycophenolate, aminaphtone and cyclic i.v. iloprost, and all received low dose aspirin).

The diagnosis of MCTD did not change in any patient during the follow-up.

Seven out of ten MCTD patients (70%) showed a NVC "scleroderma-like" pattern at baseline (Fig. 1E-F). The other 3 patients showed normal capillaries (hairpin-shaped) (Fig. 1D), non-specific capillary variations (tortuous, crossing) or abnormalities, and/or capillaries with non-specific enlargements at T0. On the contrary, the ten SSc patients enrolled for this study showed, at baseline the *early* (40%), *active* (40%) and *late* (20%) NVC patterns (Fig. 1A-B-C).

Particularly, the capillaroscopic examination in MCTD patients showed at baseline enlarged capillaries in all patients and, in descending order of frequency, abnormal shapes, *i.e.* ramifications, reduced capillary number, microhaemorrhages and giant capillaries (when a "scleroderma-like" pattern was present). Moreover, in MCTD patients, at T0 the median absolute number of normal capillaries was 2.5 and the mean total capillary number was 8.0 (Table I).

After 3 years of follow-up enlarged capillaries were present in all patients, whereas abnormal shapes, *i.e.* ramifications and capillary loss were present in 70% of MCTD patients, microhaemorrhages in 57% of patients, disorganisation of the microvascular array and giant capillaries in 42% of patients. These percentages were similar to those observed at baseline. Interestingly, at T3 the median absolute number of normal capillaries was 2.0 and the total capillary number was 8.0 (Table I), therefore almost unchanged from baseline (p=n.s.).

During the 3-year follow-up of MCTD patients, no significant evolution of microangiopathy, detected by NVC was recognised with regard as to the scores as to the absolute capillary numbers (Table I). The analysed data showed no statistically significant correlations between the clinical aspects of MCTD (RP, teleangectasias, dyspnoea, dysphagia, sclerodactily, sicca syndrome and arthralgia) and microvascular damage at first visit, as well as during follow-up.

On the other hand, the scores of enlarged capillaries, giant capillaries and microhaemorrhages were found significantly higher in SSc patients when compared to MCTD patients at T0 (see Table II for statistical significances and

Table I. Median (and IQR) capillary parameter values in MCTD patients assessed by NVC at T0 (baseline) and after one (T1) and 3 years (T3).

	Absolute numbers			NVC scores					
	Normal capillaries	Total capillaries	Abnormal shaped (ramified) capillaries	Enlarged capillaries	Giant capillaries	Haemorrhages	Abnormal shaped (ramified) capillaries	Vascular array disorganisation	Capillary number
T0	2.5 (5.7)	8.0 (3.0)	2.0 (3.0)	2.0 (0.0)	1.0 (1.0)	1.0 (1.0)	1.0 (2.0)	0.5 (1.0)	1.0 (1.0)
T1	3.0 (4.0)	8.0 (3.2)	2.0 (2.1)	2.0 (0.7)	0.0 (1.0)	1.0 (1.0)	1.0 (0.7)	1.0 (1.5)	1.0 (1.5)
T3	2.0 (5.0)	8.0 (3.5)	1.0 (1.7)	2.0 (1.5)	0.0 (1.0)	1.0 (1.0)	1.0 (1.5)	0.0 (1.7)	1.0 (1.5)
p	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

MCTD: mixed connective tissue disease; NVC: nailfold videocapillaroscopy.
Statistical analysis: Wilcoxson and Friedman test.

Table II. Median (and IQR) capillary parameter values in MCTD *versus* SSc patients* at first (baseline) nailfold videocapillaroscopy (NVC).

	Absolute numbers			NVC scores					
	Normal capillaries	Total capillaries	Abnormal shaped (ramified) capillaries	Enlarged capillaries	Giant capillaries	Haemorrhages	Abnormal shaped (ramified) capillaries	Vascular array disorganisation	Capillary number
MCTD	2.5 (5.7)	8.0 (3.0)	1.5 (2.5)	2.0 (0.0)	1.0 (1.0)	1.0 (1.0)	1.0 (2.0)	0.5 (1.0)	1.0 (1.0)
SSc	0.0 (0.0)	5.0 (3.0)	0.5 (2.0)	2.5 (1.0)	1.5 (1.0)	1.0 (0.0)	1.0 (2.0)	0.5 (2.0)	1.5 (1.5)
p	0.04	0.009	n.s.	0.04	<0.02	0.05	n.s.	n.s.	0.05

*SSc patients with different NVC patterns at baseline.
Statistical analysis: Mann-Whitney test.
MCTD: mixed connective tissue disease; SSc: systemic sclerosis.

further details). In addition, the absolute number of both total and normal capillaries were found significantly lower at T0 in SSc patients *versus* MCTD patients (Table II).

On the contrary, no statistically significant difference was observed for the other capillary parameters (capillary abnormal shapes, *i.e.* ramifications, disorganisation of the vascular array) between the two groups of patients at baseline, even if the absolute number of capillary abnormal shapes, *i.e.* ramifications was found higher in MCTD than in SSc patients (median 1.5 *vs.* 0.5, respectively, p=0.11) (Table II).

Discussion

This study confirms that "scleroderma-like" capillary specific abnormalities are frequently observed in MCTD patients. However, the nailfold microvascular damage in MCTD seems less progressive than in SSc, as it was found not significantly changed after a three-year follow-up.

In addition, the study tried to identify by a detailed NVC analysis a specific NVC pattern of microvascular damage in MCTD patients and to compare it with the already validated NVC patterns detectable in patients affected by SSc. Nevertheless, due to the limited cohort of enrolled MCTD patients in this study, at present we could just elaborate some considerations and compare our data with those of previous studies.

As matter of fact, from years the question of whether MCTD is a distinct disease remains controversial and many authors have tried to solve this question. MCTD may be an evolutionary phase in connective tissue disease development, of whom, a proportion may develop SSc, some may develop lupus, some even dermatomyositis (2). However, the diagnosis of MCTD did not change in any patient of our cohort during follow-up, as patients with either anti-extractable nuclear antigen positivity different from U1snRNP or anti-dsDNA antibodies positivity had been excluded during the selection.

Nowadays MCTD has the characteristics of a distinct disease and there is the need to also identify predictors of organ involvement and evolution to start a suitable treatment in due time (2, 23). It has been hypothesised that capillaroscopy may be useful in predicting evolution of MCTD (23). However, in our study no statistically significant correlation was observed between capillary parameters and MCTD clinical aspects at first visit, as well as during the follow-up.

According to the data obtained from other studies, the presence of abnormal shapes, *i.e.* ramified or branched capillaries in MCTD patients evolving to SSc seems be the dominant hallmark (24, 25).

We detected the "scleroderma-like" pattern in 70% patients affected by MCTD, while the other patients showed a normal capillary pattern or non-specific capillary abnormalities.

These data are quite similar to those observed in other studies (12, 17). In fact, in two previous studies, the percentage of patients affected by MCTD in whom a "scleroderma-like" pattern was found, was 54% and 65%, respectively (12, 17). The most common altered capillary parameters detected in our patients, as mentioned, were enlarged capil-

laries, followed by abnormal shapes (angiogenetic vessels), capillary loss, microhaemorrhages and giant capillaries. Previous studies have also reported that the MCTD capillaroscopic features may include extremely convoluted, branched capillaries, sometimes termed as pseudoglomerulus or bushy capillary formation (7, 26, 27). Our study demonstrated that absolute number of abnormal shaped (ramified) capillaries was higher in MCTD than in SSc, but this did nott reach the statistical significance, perhaps due to little study population enrolled.

However, no investigation, including our study, has been able to describe a specific NVC pattern in MCTD patients if not a scleroderma-like pattern.

In 2012, an investigation elaborated by Wu PC et al. evaluated the clinical applicability of quantitative NVC in differential diagnosis of connective tissue diseases with RP in 67 patients (13). They found out that the sensitivity of the "scleroderma-like" pattern for MCTD was very low (20%), but with a high specificity (100%). Conversely they found that the sensitivity and specificity of the generic "scleroderma-pattern" in SSc was 89% and 80% respectively, whereas the specificity of the *early*, *active* and *late* scleroderma NVC patterns for SSc reached 87%, 97%, and 95%, respectively (13).

As noted, SSc shows a typical progression of the microangiopathy through the different NVC patterns (*early*, *active* and *late* patterns) (16, 28, 29).

On the contrary, in our study, no statistically significant variation of both the scores as well as the absolute numbers of the capillary parameters was observed in patients affected by MCTD during the 3 years follow-up, and similar findings have been recently reported also in patients with dermatomyositis (30).

According to a work by De Holanda Mafaldo *et al*., only 16% of MCTD patients might show a dynamic nature of capillary changes (17). Particularly, this study affirms that a significant reduction in the frequency of severe avascular areas was observed during the evolution, probably due to altered neoangiogenesis process (this may be the effect of the therapies). Despite our study didn't find significant differences during the follow up, we found out that the absolute number of capillaries slightly decreased in MCTD patients (7.6±1.6 at baseline and 7.4±2.2 after three years). The absolute number of capillaries detected by NVC, as shown in other studies, is the most sensitive among the NVC parameters at least in SSc (31-33).

Moreover, comparing SSc with MCTD patients (matched for age and disease duration at first capillaroscopy) we found out some further significant differences as reported in the result section. Of note, this is the first report comparing each singular NVC parameter between SSc and MCTD patients. According to these data, nailfold microvascular damage all together seems larger in SSc patients compared to patients with MCTD.

We are aware that our study presents some limitations. Firstly, this is a retrospective cohort study including a limited number of patients. Other limitations are the previous long disease duration already at the first capillaroscopic analysis and the different treatment background that might interfere with the microvascular damage progression and the results; however this condition was present in both MCTD and SSc patients (34). Another limitation of the study is that disease activity/severity were not properly assessed due to retrospective nature of the study; both disease activity and severity of SSc and MCTD at the baseline might have been a possible indicator for evolution of changes in both entities. Finally, it should be also considered that overlapping criteria for diagnosis of MCTD have been published by Sharp, Alarcón-Segovia, Kasukawa, and Kahn, and a comparison study determined that MCTD was best identified with the Alarcón-Segovia criteria, which have 62.5% sensitivity and 86.2% specificity, and the Kahn criteria. The Kasukawa criteria used in this study might have led to classification of patients as MCTD which would have otherwise been excluded by the Alarcon-Segovia or Kahn's classification criteria.

Therefore, larger longitudinal NVC studies need to better evaluate the progression over time of microangiopathy in MCTD patients and to search for an eventual more specific pattern reinforcing the role of NVC as diagnostic tool in complex autoimmune rheumatic diseases with complex pathophysiology (35-37).

In conclusion, in this retrospective cohort study on MCTD patients with an average disease duration of 6.4 years and a three year follow-up, the nailfold microangiopathy does not seem to be significantly progressive from the baseline observation. Patients with MCTD seem to show less enlarged/giant capillaries, but higher absolute number of both total and normal capillaries than SSc patients at first capillaroscopy. The identification of a specific NVC pattern in MCTD patients is not yet possible, however a "scleroderma-like" pattern may be observed in 70% of cases.

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References

1. CHAIGNE B, SCIRÈ CA, TALARICO R *et al*.: Mixed connective tissue disease: state of the art on clinical practice guidelines. *RMD Open* 2018; 4: e000783.
2. CAPPELLI S, BELLANDO RANDONE S *et al*.: "To be or not to be," ten years after: evidence for mixed connective tissue disease as a distinct entity. *Semin Arthritis Rheum* 2012; 41: 589-98.
3. SHARP GC, IRVIN WS, TAN EM, GOULD RG, HOLMAN HR: Mixed connective tissue disease an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA) *Am J Med* 1972; 52: 148-59.
4. ORTEGA-HERNANDEZ OD, SHOENFELD Y: Mixed connective tissue disease: an overview of clinical manifestations, diagnosis and treatment. *Best Pract Res Clin Rheumatol* 2012; 26: 61-72.
5. KASUKAWA R, TOJO T, MIYAWAKI S: Preliminary diagnostic criteria for classification of mixed connective tissue disease. *In*: KASUKAWA R, SHARP G (Eds.): Mixed connective tissue disease and antinuclear antibodies. Amsterdam, Elsevier 1987: 41-47.
6. ALARCÓN-SEGOVIA D, VILLAREAL M: Classification and diagnostic criteria for mixed

connective tissue disease. *In*: KASUKAWA R, SHARP G (Eds.): Mixed connective tissue disease and antinuclear antibodies. Amsterdam, Elsevier 1987: 33-40.
7. GRANIER F, VAYSSAIRAT M, PRIOLLET P, HOUSSET E: Nailfold capillary microscopy in mixed connective tissue disease. Comparison with systemic sclerosis and systemic lupus erythematosus. *Arthritis Rheum* 1986; 29: 189-95.
8. KOENIG M, JOYAL F, FRITZLER MJ *et al*.: Autoantibodies and microvascular damage are independent predictive factors for the progression of Raynaud's phenomenon to systemic sclerosis: a twenty-year prospective study of 586 patients, with validation of proposed criteria for early systemic sclerosis. *Arthritis Rheum* 2008; 58: 3902-12.
9. CUTOLO M, MELSENS K, HERRICK AL *et al*.: EULAR Study Group on Microcirculation in Rheumatic Diseases. Reliability of simple capillaroscopic definitions in describing capillary morphology in rheumatic diseases. *Rheumatology* (Oxford) 2018; 57: 757-59.
10. 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* (Oxford) 2016; 55: 883-90.
11. SELVA-O'CALLAGHAN A, FONOLLOSA-PLA V, TRALLERO-ARAGUÁS E *et al*.: Nailfold capillary microscopy in adults with inflammatory myopathy. *Semin Arthritis Rheum* 2010; 39: 398-404.
12. MARICQ HR, LEROY EC, D'ANGELO WA *et al*.: Diagnostic potential of in vivo capillary microscopy in scleroderma and related disorders. *Arthritis Rheum* 1980; 23: 183-88.
13. WU PC, HUANG MN, KUO YM, HSIEH SC, YU CL: Clinical applicability of quantitative nailfold capillaroscopy in differential diagnosis of connective tissue diseases with Raynaud's phenomenon. *J Formos Med Assoc* 2013; 112: 482-88.
14. 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: 1092-96.
15. 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.
16. CUTOLO M, SULLI A, PIZZORNI C, ACCARDO S: Nailfold videocapillaroscopy assessment of microvascular damage in systemic sclerosis. *J Rheumatol* 2000; 27: 155-60.
17. DE HOLANDA MAFALDO DIÓGENES A, BONFÁ E, FULLER R, CORREIA CALEIRO MT: Capillaroscopy is a dynamic process in mixed connective tissue disease. *Lupus* 2007; 16: 254-58.
18. VAN DEN HOOGEN F, KHANNA D, FRANSEN J *et al*.: Classification criteria for systemic sclerosis: an American college of rheumatology/ European league against rheumatism collaborative initiative. *Ann Rheum Dis* 2013; 72: 1747-55.
19. CUTOLO M, SULLI A, SMITH V: How to perform and interpret capillaroscopy. *Best Pract Res Clin Rheumatol* 2013; 27: 237-48.
20. 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: 885-87.
21. DE ANGELIS R: The most important capillaroscopic parameters in normal and pathological conditions. *In*: CUTOLO M (Ed.): Atlas of capillaroscopy in rheumatic diseases. Milan, Elsevier (I), 2008: 61-69.
22. CUTOLO M, SULLI A, SMITH V: Assessing microvascular changes in systemic sclerosis diagnosis and management. *Nat Rev Rheumatol* 2010; 6: 578-87.
23. ARINGER M, STEINER G, SMOLEN JS: Does mixed connective tissue disease exist? Yes. *Rheum Dis Clin North Am* 2005; 31: 411-20.
24. CUTOLO M, GRASSI W, MATUCCI-CERINIC M: Raynaud's phenomenon and the role of capillaroscopy. *Arthritis Rheum* 2003; 48: 3023-30.
25. SMITH V, VANHAECKE A, HERRICK AL *et al*.: "Fast Track algorithm": How to differentiate a scleroderma pattern from a non-scleroderma pattern? *Autoimmun Rev* 2019. [In Press].
26. ORTEGA-HERNANDEZ OD, SHOENFELD Y: Mixed connective tissue disease: an overview of clinical manifestations, diagnosis and treatment. *Best Pract Res Clin Rheumatol* 2012; 26: 61-72.
27. BELLANDO RANDONE S, CUTOLO M, CZIRJÁK L, MATUCCI-CERINIC M: Mixed connective tissue disease, a roundabout to rheumatic diseases? *Curr Rheumatol Rev* 2009; 5: 133-40.
28. SULLI A, PIZZORNI C, SMITH V, ZAMPOGNA G, RAVERA F, CUTOLO M: Timing of transition between capillaroscopic patterns in systemic sclerosis. *Arthritis Rheum* 2012; 64: 821-25.
29. SULLI A, RUARO B, SMITH V *et al*.: Progression of nailfold microvascular damage and antinuclear antibody pattern in systemic sclerosis. *J Rheumatol* 2013; 40: 634-39.
30. PIZZORNI C, CUTOLO M, SULLI A *et al*.: Long-term follow-up of nailfold videocapillaroscopic changes in dermatomyositis versus systemic sclerosis patients. *Clin Rheumatol* 2018; 37: 2723-29.
31. 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.
32. CUTOLO M, HERRICK AL, DISTLER O *et al*.: Nailfold videocapillaroscopic features and other clinical risk factors for digital ulcers in systemic sclerosis: a multicenter, prospective cohort study. *Arthritis Rheumatol* 2016; 68: 2527-39.
33. ORLANDI M, BARSOTTI S, LEPRI G *et al*.: One year in review 2018: systemic sclerosis. *Clin Exp Rheumatol* 2018; 36 (Suppl. 113): S3-23.
34. FRECH TM, MACHIN DR, MURTAUGH MA *et al*.: Implications of endothelial shear stress on systemic sclerosis vasculopathy and treatment. *Clin Exp Rheumatol* 2018; 36 (Suppl. 113): S175-82.
35. MASI AT, BIJLSMA JWJ, CHIKANZA IC, PITZALIS C, CUTOLO M: Neuroendocrine, immunologic, and microvascular systems interactions in rheumatoid arthritis: physiopathogenetic and therapeutic perspectives. *Sem Arthritis Rheum* 1999; 29: 65-81.
36. BURMESTER GR, BIJLSMA JWJ, CUTOLO M, MCINNES IB: Managing rheumatic and musculoskeletal diseases - past, present and future. *Nat Rev Rheumatol* 2017; 13: 443-48.
37. CUTOLO M, SOLDANO S, SMITH V: Pathophysiology of systemic sclerosis: current understanding and new insights. *Expert Rev Clin Immunol* 2019; 15: 753-64.