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Nailfold capillaroscopy abnormalities and pulmonary hypertension in mixed connective tissue disease and systemic sclerosis patients

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Abstract. – OBJECTIVE: Pulmonary arterial hypertension (PAH) represents an important vascular complication of mixed connective tissue disease (MCTD) and systemic sclerosis (SSc). Microvascular involvement in these diseases can be investigated by means of nailfold capillaroscopy (NFC). Microvascular involvement detected in the nailfold bed is the mirror of the microvascular damage occurring in the entire body, further indicating the involvement of the target organs. The aim of this study was to evaluate the microvascular involvement in MCTD patients with or without PAH, compared to that found in SSc patients with or without PAH.

PATIENTS AND METHODS: This cross-sectional study was performed in the Department of Internal Medicine and Department of Rheumatology, Timișoara, Romania, during the time period between January 2017 and December 2022, on a group of 26 patients with MCTD and 26 SSc patients. Antinuclear antibodies, anti-U1-RNP, anti-Scl 70, anti-centromere, anti-cardiolipin antibodies (aCL) (IgM, IgG), anti-β2-glycoprotein I (aβ2GPI) (IgM, IgG) antibodies, and lupus anticoagulant (LAC) were determined in both the groups. PAH was evaluated through cardiac ultrasonography, determining the sPAP (systolic pulmonary artery pressure). Nailfold capillaroscopy was performed using a USB Digital Microscope and 2.0-megapixel digital camera recording capillaries density, giant capillaries, enlarged capillaries, capillaries hemorrhages, avascular areas, ramified/bushy capillaries scores. Data were recorded and presented as mean ± standard deviation. Statistical analyses were performed using the Student's *t*-test, ANOVA test, and Pearson's correlation. Differences were considered statistically significant if *p*-value < 0.05.

RESULTS: Among the MCTD patients, PAH was identified in 12 patients (46.15%), while among the SSc patients PAH was identified in 14 patients (53.84%). Development of PAH in MCTD patients was associated with lower capillaries density (*p*-value < 0.00001), higher scores of giant capillaries, ramified/bushy capillaries, and capillary hemorrhages (*p*-value < 0.00001, for each of them). Anti-U1-RNP, aCL, aβ2GPI antibodies and LAC were also found to be involved in PAH-associated MCTD development. Unlike MCTD patients, SSc patients with PAH presented with lower capillaries density and ramified/bushy capillaries scores (*p*-value < 0.05).

CONCLUSIONS: The MCTD patients who presented significant NFC abnormalities (especially active and late scleroderma-like capillaroscopic pattern) are prone to PAH development. Capillary density reduction is the most important factor associated with the occurrence of PAH. Differences in NFC findings (especially capillary density and ramified/bushy capillaries) were detected among patients with MCTD and SSc having PAH.

Key Words:
Mixed connective tissue disease, Nailfold capillaroscopy, Pulmonary hypertension, Systemic sclerosis.

Introduction

Mixed connective tissue disease (MCTD), which was first described by Sharp et al[1] in 1972, is a condition that associates specific features of systemic lupus erythematosus (SLE), poly/dermatomyositis (PM/DM), systemic sclerosis (SSc) and rheumatoid arthritis (RA), in the presence of

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high titers of antibodies targeting the U1 small nuclear ribonucleoprotein particle (U1snRNP)[1,2]. It is a rare condition; its incidence is between 0.2 and 2.1/100,000 in adults and has a prevalence of approximately 3.8/100,000 adults[3-6], with females being more frequently affected.

Although, in the past, it was considered that MCTD had a milder evolution, the latest research in literature in this area suggests that due to its potential to cause organ damage, it can be associated with a negative prognosis. Pulmonary involvement, in the form of interstitial lung disease and pulmonary arterial hypertension (PAH), is the most severe organ damage witnessed[2]. PAH is present in a proportion between 3.4% and 23.8% of MCTD patients, depending on the criteria for defining MCTD and PAH[7,8]. However, PAH appears in SSc patients, too, and is commonly associated with PAH (30-50% of patients)[2].

PAH is defined as elevated mean pulmonary artery pressure (≥ 25 mmHg) at rest on right heart catheterization with pulmonary capillary wedge pressure lower than 15 mmHg and increased pulmonary vascular resistance[9,10]. In connective tissue diseases, several mechanisms are involved in the PAH appearance, such as primary arteriopathy of pulmonary vessels, interstitial lung disease, and, less frequently, pulmonary thromboembolic disease and postcapillary left ventricular dysfunction. In MCTD-associated PAH, histopathological examination usually reveals intimal proliferation, medial hypertrophy of the pulmonary vessels, and intraluminal thrombi[11]. The study published by Kawano-Dourado et al[12] documented that the progression of MCTD-related lung involvement does not seem to be rapid in comparison to the progression witnessed in SSc or PM/DM.

However, due to the fact that peripheral microvasculature is affected in both MCTD and SSc patients, these patients exhibit Raynaud's phenomenon, which can precede years before the occurrence of other manifestations of the disease[13]. The study of microvascularization in these diseases is performed through nailfold capillaroscopy (NFC). NFC is a non-invasive method frequently used in rheumatology for visualizing the microvascular network[14-16]. Microvascular involvement detected in the nailfold bed is the mirror of the microvascular damage occurring across the body, directly proportional to the involvement of the target organs[17,18]. NFC not only allows the differential diagnosis between the primary and secondary Raynaud's phenomenon, establishing the early diagnosis of connective tissue diseases (mainly SSc), but also provides information regarding the prognosis of the disease based on the severity of the target organ damage in these diseases[18]. Abnormalities of nailfold capillaries appear in 70-80% of SSc patients[2], and 9-27% of non-SSc patients, respectively[19].

The aim of this study was represented by the assessment of microvascular involvement in patients with MCTD, respective SSc, both with and without PAH.

Patients and Methods

Patients

This cross-sectional study was performed in the Department of Internal Medicine, in the Rheumatology Unit, of Railway Clinical University Hospital in Timișoara, Romania, between January 2017 and December 2022, on a group of 26 patients having MCTD and 26 patients with SSc. The 26 patients with MCTD fulfilled the Alarcon-Segovia criteria[20], and the 26 patients with SSc fulfilled the 2013 Classification Criteria for Systemic Sclerosis[21]. Exclusion criteria included age under 18 years, patients' refusal to participate in this study, pregnancy or breastfeeding women, connective tissue diseases other than MCTD or SSc, previous pulmonary diseases with or without PAH not associated with MCTD or SSc, severe cardiovascular or liver diseases, and current smokers. All the participants signed the written informed consent. This study was approved by the Ethics Committee of Railway Clinical University Hospital, Timișoara, Romania (registration number: 28/01.2018). The study was conducted in accordance with the Declaration of Helsinki.

Methods

Patient medical files were sifted, and details regarding medical history, complete clinical and paraclinical examination results, and current treatment plans were noted. Laboratory tests (antinuclear antibodies, anti-U1-RNP, anti-Scl 70, anti-centromere antibodies, lupus anticoagulant, anti-cardiolipin, and anti-β2-glycoprotein I antibodies), nailfold capillaroscopy, and cardiac ultrasonography were performed in all the studied patients.

Antinuclear antibodies, anti-U1-RNP, anti-Scl 70, and anti-centromere antibodies were determined using indirect immunofluorescence (HELMED) (for detection) and immunoenzymatic method (EIA, serum) (for their titration). Lupus

anticoagulant (LAC) (LA-sensitive PTT), anti-cardiolipin antibodies (aCL) (IgM, IgG) (EIA, serum), and anti-β2-glycoprotein I antibodies (aβ2GPI) (IgM, IgG) (EIA, serum) were performed in all the studied patients.

In the present study, PAH was evaluated through cardiac ultrasonography, determining the sPAP (systolic pulmonary artery pressure) with Siemens Acuson X300 Ultrasound System (Siemens Healthineers, Erlangen, Germany) 2.5 MHz transducer. The sPAP measurement was performed using Bernoulli's equation, as sPAP = 4 × $(TRV)^2$ + right atrial pressure (RAP), where: TRV = tricuspid regurgitation velocity, measured by means continuous wave Doppler, RAP = right atrial pressure, estimated from inferior vena cava diameter and its inspiratory collapse. PAH was defined as a PASP over 30 mmHg[9,22,23].

Nailfold capillaroscopy was performed using a Dino-Lite USB digital microscope with 2.0-megapixel digital camera (Dino-Lite, ANMO Electronics Corporation, Manuscriptstraat 12-14 - 1321 NN Almere, The Netherlands). Before this procedure, the patients took place in a room with a stable temperature of 20-22°C for at least 15 minutes in order to avoid capillaries vasoconstriction, which could induce false positivity for avascular areas. The 2nd, 3rd, 4th, and 5th fingers of both hands were examined. Giant capillaries, enlarged capillaries, capillaries hemorrhages, avascular areas, ramified/bushy capillaries, and capillary architecture were the recorded nailfold capillaroscopic parameters. The patients were classified as having normal, unspecific patterns and scleroderma-like patterns. The patients who presented scleroderma-like patterns were classified as early, active, and late. The recorded nailfold capillaroscopic parameters were semi-quantitatively assessed on a scale from 0 to 3. Score 0 represented the absence of any pathological change in the nailfold capillaries, score 1 meant that pathological changes were present in less than 33% of the capillaries analyzed, score 2 defined the presence of changes between 33 and 66% of the analyzed capillaries, and score 3 defined the presence of changes over 66% of the analyzed capillaries. The mean score for each parameter was calculated from the analysis of all fingers except the thumbs[24,25].

Statistical Analysis

Data were recorded and presented as mean ± standard deviation. Statistical analyses were performed using the Student's *t*-test, ANOVA test, and Pearson's correlation. Differences were considered statistically significant if p-value < 0.05.

Results

The baseline participant characteristics are presented in Table I. A female predominance was observed in both diseases. No statistically significant difference was found in the age of the studied patients (p-value > 0.05). However, the mean length of disease evolution was shorter in SSc patients than in MCTD patients (p-value < 0.0001). Among SSc patients, 18 had diffuse SSc (69.23%), and 8 had limited SSc (30.76%).

The patients benefited from immunosuppressive treatment (Azathioprine, Cyclophosphamide, Mycophenolate mofetil), respectively, for pulmo-

Table I. Baseline participant characteristics.

Parameter	Mean ± standard deviation		*p*-value
	MCTD	SSc	
Sex [n (%)]	26	26	> 0.05
Males	6 (23.07%)	8 (30.76%)	
Females	20 (76.92%)	18 (69.23%)	
Mean age (years)	48.57 ± 6.48	51.34 ± 5.16	> 0.05
Mean length of disease evolution (years)	10.88 ± 3.19	7.42 ± 2.88	< 0.0001
Mean length of Raynaud's phenomenon (years)	6.76 ± 6.02	8.88 ± 3.27	> 0.05
The drugs used by the patients in the moment of investigation			
Azathioprine	8 patients	7 patients	
Cyclophosphamide	8 patients	8 patients	
Mycophenolate mofetil	10 patients	11 patients	
Calcium blockers	20 patients	22 patients	
Endothelin receptor antagonists	0 patients	4 patients	

MCTD = mixed connective tissue disease; SSc = systemic sclerosis.

nary hypertension (calcium blockers, respective endothelin receptor antagonists), as can be seen in Table I.

Raynaud's phenomenon was present in 20 MCTD patients (76.92%) (9 patients without PAH and 11 patients with PAH) and in all of the SSc patients (100%). The mean length of Raynaud's phenomenon did not show statistically significant differences ($p > 0.05$).

PAH was identified in 12 MCTD patients (42.30%), respective in 14 SSc patients (53.84%). The mean age of the patients with MCTD without PAH (MCTD-PAH) was higher (50 ± 4.93 years) than that of MCTD patients with PAH (MCTD+PAH) (46.91 ± 7.82 years), but without a statistically significant difference ($p > 0.05$). In the case of SSc patients no statistically significant differences were highlighted between the patients with PAH *vs.* without PAH (SSc-PAH: 50.75 ± 6.26 years *vs.* SSc+PAH: 51.85 ± 4.18 years) ($p > 0.05$). However, the mean length of disease evolution of the MCTD-PAH was reduced (9.85 ± 1.65 years) as compared to that of MCTD+PAH (12.08 ± 4.12 years) patients. The SSc patients without, respective with PAH, presented the same trend (SSc-PAH: 4.44 ± 1.33 years *vs.* SSc+PAH: 7.50 ± 2.50 years). All the differences were statistically significant ($p < 0.0001$) (Table II).

Analyzing the patients with MCTD+PAH and those with SSc+PAH, the following aspects were found. No statistically significant differences were identified in terms of patient age (46.91 ± 7.82 years in MCTD patients *vs.* 51.85 ± 4.18 years in SSc patients, $p > 0.05$), but it was found that the duration of MCTD was significantly longer than that of SSc until PAH development (12.08 ± 4.12 years *vs.* 7.50 ± 2.50 years, $p < 0.0001$). PAH associated with MCTD developed more slowly than that associated with SSc.

In the appearance of PAH associated with MCTD, other factors contributed besides the duration of the disease evolution. The intervention of autoantibodies in the appearance of PAH is presented in Table II.

Anti-U1-RNP antibodies were associated with the PAH development in MCTD ($p < 0.0001$). They were not identified in any SSc patient.

On the other side, some patients with MCTD presented LAC, aCL (IgG, IgM), or aβ2GPI (IgG, IgM) antibodies. These antibodies were associated with the development of PAH, probably through microthrombosis developed in the pulmonary microcirculation. In some studied SSc+PAH patients, these antibodies were identified, but their titers were lower than in BMTC+PAH patients, which was statistically significant.

Nailfold capillaroscopy revealed different patterns among MCTD and SSc patients. SSc patients presented early scleroderma capillaroscopic pattern (3 patients), active scleroderma capillaroscopic pattern (12 patients) (Figure 1), and late scleroderma capillaroscopic pattern (11 patients) (Figure 2). MCTD patients presented the following capillaroscopic patterns: normal (7 patients), non-specific (8 patients), and scleroderma-like pattern (11 patients). The NFC-monitored parameters are presented in Table III.

It had been shown that in MCTD+PAH patients, capillaries density was lower ($p < 0.00001$), giant capillaries, ramified/bushy capillaries, and microhemorrhages scores were higher than in MCTD-PAH patients, statistically significant ($p < 0.0001$) (Figure 3). Despite no statistically significant differences in sPAP ($p > 0.05$), capillary density was significantly lower in patients with SSc+PAH than those with MCTD+PAH ($p < 0.05$). Ramified/bushy capillaries

Table II. Monitored parameters in studied patients.

Parameter	MCTD-PAH	MCTD+PAH	SSc-PAH	SSc+PAH	*p*-value
Mean age (years)	50 ± 4.93	46.91 ± 7.82	50.75 ± 6.26	51.85 ± 4.18	> 0.05
Mean length of disease evolution (years)	9.85 ± 1.65	12.08 ± 4.12	4.44 ± 1.33	7.50 ± 2.50	< 0.0001
sPAP (mmHg)	25.35 ± 2.56	64.58 ± 16.48	23.58 ± 3.28	63.07 ± 14.05	< 0.0001
aCL (IgG)	10.62 ± 12.22	55.96 ± 27.93	4.35 ± 15.09	22.67 ± 25.14	< 0.0001
aCL (IgM)	4.64 ± 8.17	28.45 ± 16.51	0.55 ± 1.93	21.76 ± 23.68	< 0.0001
aβ2GPI (IgG)	3.06 ± 1.07	39.55 ± 40.93	1.20 ± 4.18	10.06 ± 16.79	< 0.0001
aβ2GPI (IgM)	1.26 ± 0.72	32.58 ± 28.66	1.06 ± 3.69	4.42 ± 9.52	< 0.0001
anti-U1-RNP	40.07 ± 10.44	88.58 ± 35.51	0	0	< 0.0001

MCTD = mixed connective tissue disease; PAH = pulmonary arterial hypertension; SSc = systemic sclerosis; sPAP = systolic pulmonary artery pressure; aCL = anti-cardiolipin antibodies.

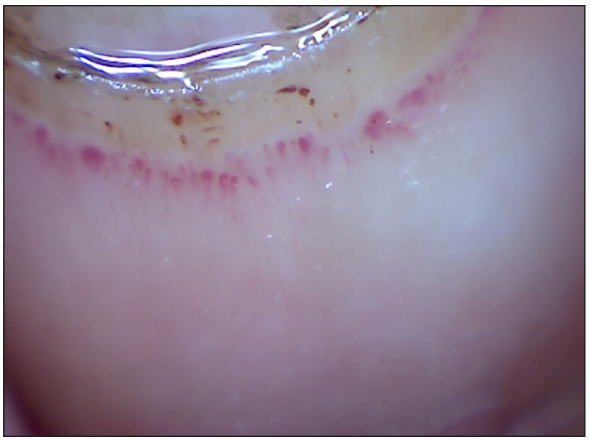

Figure 1. NFC (×50); SSc: giant capillaries, microhemorrhages (A.C. collection). SSc = systemic sclerosis.

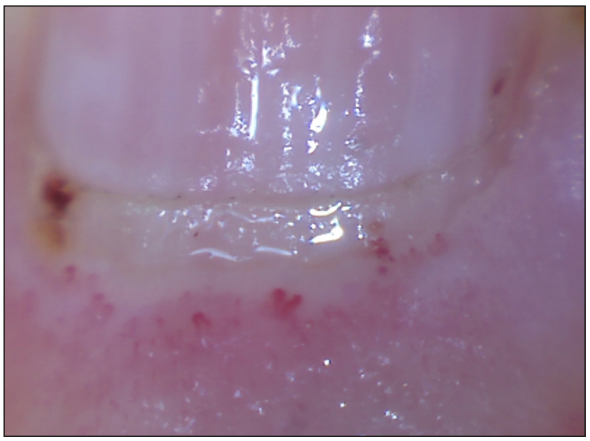

Figure 2. NFC (×50); SSc: decreased capillaries density, avascular areas, rare microhemorrhages (A.C. collection). SSc = systemic sclerosis

and microhemorrhage scores were higher in MCTD+PAH than in those with SSc+PAH ($p < 0.0001$) (Figure 4). Giant capillaries score did not show statistically significant differences between MCTD+PAH patients and SSc+PAH patients ($p > 0.05$).

Statistical analysis showed the existence of correlations between sPAP and NFC parameters (Table IV). In MCTD-PAH, statistically significant correlations were found between sPAP and ramified/bushy capillaries score ($p = 0.000012$) and giant capillaries score ($p = 0.001624$). MCTD microangiopathy, evaluated by means of NFC, has a dynamic evolution. In MCTD, PAH has developed over several years of evolution, and significant changes in NFC findings appear in parallel. In the presence of PAH, the MCTD patients presented lower capillaries density and higher giant capillaries, ramified/bushy capillaries, and hemorrhage scores. In MCTD+PAH patients, strong correlations were found between sPAP and capillaries density ($p = 0.000272$) and respective micro hemorrhages score ($p = 0.000653$). SSc+PAH patients presented significant correlations between sPAP and capillaries density ($p = 0.000017$) and mega capillaries score ($p = 0.000012$).

Discussion

While many studies about PAH and NFC abnormalities in SSc patients have been published in literature, not many studies have been published[10,16,23] about abnormalities in MCTD ones. The present study showed that the MCTD patients who presented significant NFC abnormalities were prone to PAH development. Capillary density reduction was the most important factor associated with the occurrence of PAH.

The studied MCTD patients fulfilled the Alarcon-Segovia criteria. The three diagnostic criteria used for MCTD are Alarcón-Segovia, Kasukawa, and Sharp. The Alarcón-Segovia criteria are the simplest of them. On the other hand, the Kasukawa criteria are considered to be beneficial in assessing the symptoms and signs of MCTD[26].

Table III. Nailfold capillaroscopic parameters in studied patients.

Parameter	MCTD-PAH	MCTD+PAH	SSc-PAH	SSc+PAH	*p*-value
sPAP (mmHg)	25.35 ± 2.56	64.58 ± 16.48	23.58 ± 3.28	63.07 ± 14.05	< 0.00001
Capillaries density/mm	9.94 ± 0.92	6.99 ± 1.53	9.12 ± 0.73	4.91 ± 1.54	< 0.00001
Giant capillaries score	0.40 ± 0.68	1.35 ± 0.26	2.90 ± 0.19	1.42 ± 0.49	< 0.00001
Ramified/bushy capillaries score	0.70 ± 0.73	1.77 ± 0.20	0 ± 0	1.04 ± 0.70	< 0.00001
Microhemmorhages score	0.30 ± 0.66	1.14 ± 1.17	1.56 ± 0.97	0.50 ± 0.77	< 0.05

MCTD = mixed connective tissue disease; PAH = pulmonary arterial hypertension; SSc = systemic sclerosis; sPAP = systolic pulmonary artery pressure.

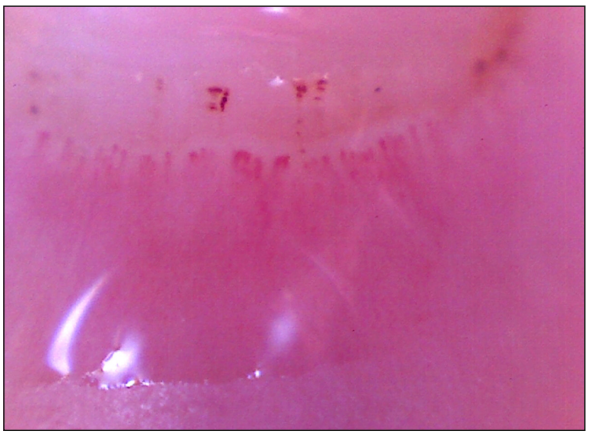

Figure 3. NFC (×50); MCTD: giant capillaries, microhemorrhages with "comb-like" pattern (A.C. collection). MCTD = mixed connective tissue disease.

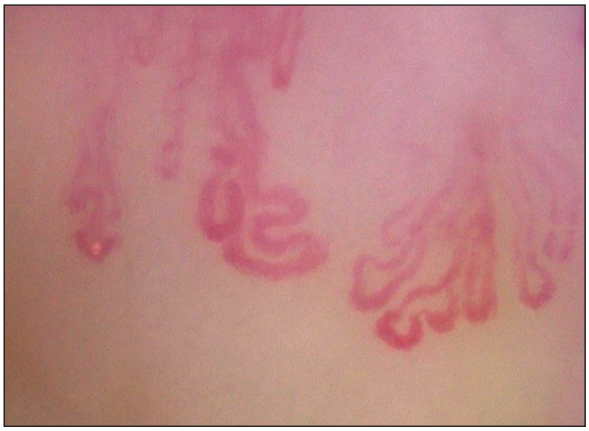

Figure 4. NFC (×200); MCTD: ramified capillaries (A.C. collection). MCTD = mixed connective tissue disease.

Morbidity and mortality associated with MCTD are secondary to pulmonary involvement, which is particularly complex and multifaceted. Pulmonary hypertension represents a serious problem of diagnosis and treatment of these patients[16]. Pulmonary arterial hypertension represents a hemodynamic state characterized by an increase in mean pulmonary artery pressure over 25 mmHg at rest

Table IV. Correlations between sPAP and NFC parameters.

MCTD-PAH patients **Correlation between sPAP and:**	**r**	***p*-value**
Capillaries density	-0.4704	0.076801
Giant capillaries score	0.7396	0.001624
Ramified/bushy capillaries score	0.8830	0.000012
Micro hemmorhages score	0.5808	0.023185
MCTD+PAH patients **Correlations between sPAP and:**	**r**	***p*-value**
Capillaries density	-0.8869	0.000272
Giant capillaries score	-0.4741	0.070341
Ramified/bushy capillaries score	0.5825	0.060046
Micro hemorrhages score	0.8615	0.000653
SSc-PAH patients **Correlations between sPAP and:**	**r**	***p*-value**
Capillaries density	-0.7426	0.021911
Giant capillaries score	-0.7099	0.032149
Ramified/bushy capillaries score	0	0.999999
Micro hemorrhages score	0.8069	0.008576
SSc+PAH **Correlations between sPAP and:**	**r**	***p*-value**
Capillaries density	-0.8477	0.000017
Giant capillaries score	-0.8549	0.000012
Ramified/bushy capillaries score	0.6753	0.002932
Micro hemorrhages score	-0.6814	0.002595

MCTD = mixed connective tissue disease; PAH = pulmonary arterial hypertension; SSc = systemic sclerosis; sPAP = systolic pulmonary artery pressure.

on right heart catheterization with pulmonary capillary wedge pressure lower than 15 mmHg and increased pulmonary vascular resistance. It can be idiopathic or associated with other conditions like drugs, toxins, or underlying diseases. Among these diseases, connective tissue diseases are the most frequent conditions associated with PAH development, representing one-fourth of the total PAH patients[10,23,27].

Among the connective tissue diseases, systemic sclerosis, mixed connective tissue disease, and systemic lupus erythematosus are most commonly associated with the development of PAH. Epidemiological data related to the occurrence of PAH highlights the differences between different population groups. In American and European patients, SSc is the most frequent disease associated with PAH development, whereas in Asian patients, SLE, MCTD, and Sjögren's syndrome are the most frequent diseases associated with PAH. But, regardless of the connective tissue disease in which it occurs, PAH has a more unfavorable evolution and prognosis than idiopathic PAH[27-31].

Many studies[2,3,11,32,33] have shown that the prevalence of PAH is within wide limits, from 3.4% and 23.8% in MCTD, respective between 5% to even 50% in SSc patients, depending on the diagnostic criteria, and the measurement methods of pulmonary pressure.

In the present study, PAH was identified in 12 MCTD patients (46.15%), with a female predominance (81.81% females *vs.* 18.18% males, $p < 0.0001$), respectively in 14 SSc patients (53.84%), also predominantly in female patients (70.58% females *vs.* 29.41% males, $p < 0.001$). No statistically significant difference was observed regarding the age of MCTD patients, respectively SSc ones ($p > 0.05$), but those with MCTD had a longer duration of evolution as compared to those with SSc until PAH development ($p < 0.0001$).

Studying the histological specimens of patients with MCTD, Sasaki et al[33], Felis-Giemza et al[34], and Zanatta et al[35] described that the lesions in pulmonary small arteries and arterioles are represented by fibrous intimal thickening, development of plexiform lesions, thrombosis, whereas fibrinoid vasculitis is rarely detectable[34-36].

Raynaud's phenomenon represents the peripheral expression of the vasculopathy that accompanies MCTD and SSc[13]. Raynaud's phenomenon was identified in all the SSc patients and in 76.92% of MCTD ones. Among the MCTD patients, Raynaud's phenomenon was reported in all the patients with PAH and only in 60% of non-PAH patients. Grader-Beck et al[13] described in their review the association between Raynaud's phenomenon and the PAH development in MCTD patients. Jeon et al[26] reported that MCTD patients with Raynaud's syndrome and abnormal NFC findings are prone to the development of PAH. Koulouri et al[36] highlighted the relationship between Raynaud's phenomenon and PAH, especially in the presence of anti-U1-RNP antibodies.

The obligatory serological criterion for MCTD diagnosis is represented by the presence of anti-U1 RNP antibodies[2]. In some MCTD patients, the titers of these antibodies are correlated with the disease activity and can be considered as prognostic factors. Anti-U1-RNP antibodies are associated with the PAH development in MCTD[37,38]. In the present study, it was identified that the mean values of these antibodies were lower in the MCTD patients without PAH than in those with PAH ($p < 0.0001$). Many studies[28,36,39] confirmed these findings because chronic inflammation and autoimmunity may generate and contribute to the progression of PAH associated with MCTD. The role of anti-U1-RNP antibodies in PAH genesis in MCTD patients is complex. These antibodies activate the endothelial cells, generating their damage and upregulating the expression of intracellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1, and MHC class II molecules. Endothelial cell damage, impaired vascular regeneration, and abnormal progressive vascular remodeling lead to the final result, which is represented by intimal hyperplasia, obliterated vasculopathy, and pulmonary arterial hypertension[38,39]. Felis-Giemza et al[34] studied the PAH associated with MCTD in a group of 79 patients with a median age of 44 years. Among them, 12 patients (15.2%) developed PAH. Anti-U1-RNP antibodies were present in all the patients who developed PAH but only in 76% of those who did not develop PAH. Grau Garcia et al[40] demonstrated in their study that the patients with high anti-U1-RNP levels showed more Raynaud's phenomenon and vascular involvement ($p < 0.001$, $p = 0.008$). In our SSc patients, anti-U1-RNP antibodies were absent. In general, these antibodies are detected in less than 10-14% of SSc patients, more often in a limited form of SSc than in the diffuse form of this disease. The presence of these antibodies is correlated with pulmonary fibrosis in SSc patients[41-43]. Ho and Reveille[42] reported that the presence of anti-U1-RNP antibodies is associated with a better prognosis in SSc patients.

The present study revealed that the nailfold capillaries morphology changes correlated with target organ damage in MCTD. Detection of local microvascular abnormalities was correlated with systemic vascular involvement. Interestingly, the structural abnormalities in the capillary bed in NFC often precede the onset of systemic manifestations of MCTD and SSc[17,18,26].

One of the assessment methods of peripheral microcirculation is NFC. The studied MCTD patients presented the following NFC patterns: normal (7 patients), unspecific (8 patients), and scleroderma-like pattern (11 patients). In MCTD patients, data from the literature show that the NFC findings can be very variable: from normal to severe changes, represented by capillary dilatation, giant capillaries, branched and bushy capillaries, and avascular areas. Among these changes, a scleroderma-like pattern is associated with further development of pulmonary involvement, especially PAH[44-47]. On the other hand, the studied SSc patients presented early scleroderma capillaroscopic pattern (3 patients), active scleroderma capillaroscopic pattern (12 patients), and late scleroderma capillaroscopic pattern (11 patients).

A scleroderma-like pattern was found in 42.30% of studied MCTD patients. The frequency of the scleroderma-like pattern found in patients with MCTD varies by study, ranging from 54% to 70%. It is important to note that even if MCTD patients show the markers of the scleroderma-like pattern (giant capillaries, ramified capillaries, microhemorrhages, avascular areas), the markers proportion is different from SSc[26,48-50]. Ramified/bushy capillaries and giant/enlarged capillaries are the most frequent findings in MCTD patients[26,49,51-53]. The NFC findings in MCTD patients evolve over time[52,54].

The present study revealed that capillaries density was lower in SSc patients (6.85 ± 2.46/mm) than in MCTD ones (8.57 ± 1.93/mm), statistically significant ($p < 0.00001$). Giant capillaries score was higher in SSc patients (2.10 ± 0.84) than in MCTD patients (0.84 ± 0.71), statistically significant ($p < 0.001$), too. In contrast, the microhemorrhage score did not show statistically significant differences between the two groups of patients (0.99 ± 1.01 in SSc *vs.* 0.69 ± 1.00 in MCTD, $p > 0.05$). However, the ramified/bushy capillaries score was lower in SSc patients than in MCTD ones (0.56 ± 0.73 in SSc, *vs.* 1.19 ± 0.77 in MCTD, $p < 0.001$). These aspects can be explained by the fact that 88.46% of SSc patients presented active and late scleroderma capillaroscopic patterns, and of those with MCTD, only 42.30% presented scleroderma-like capillaroscopic patterns. On the other hand, the ramified/bushy capillaries represent an important capillaroscopic feature in MCTD patients[47]. Many MCTD patients presented LAC, aCL, and aβ2GPI antibodies, and specific NFC findings in these patients are the hemorrhages presence[46,47,55].

The pulmonary involvement in connective tissue diseases is correlated with NFC findings[17]. Ciang et al[53] reported that the PAH was identified more frequently in patients with MCTD and severe NFC changes. Another study conducted by Celinska-Löwenhoff et al[17] reported that the high number of giant capillaries was associated with pulmonary involvement in MCTD patients. They recommended that NFC should be considered in all MCTD patients, and scleroderma-like capillaroscopic patterns should raise the suspicion of lung involvement.

In studied MCTD patients, PAH appearance was associated with lower capillaries density and higher scores of giant capillaries, ramified/bushy capillaries, and microhemorrhages, statistically significant ($p < 0.00001$). Among the MCTD and SSc patients who developed PAH, there appeared to be some differences. Capillaries density was lower in SSc patients (4.91 ± 1.54/mm) than in MCTD ones (6.99 ± 1.53/mm), showing statistical significance ($p < 0.05$), although no statistically significant differences were found between sPAP in the two conditions ($p > 0.05$). As seen in Table II, MCTD+PAH patients had LAC, as well as elevated aCL (IgM, IgG) and aβ2GPI (IgM, IgG), compared to SSc+PAH patients. These findings highlight the role of anti-phospholipid (aPL) antibodies in the PAH appearance in MCTD patients. However, MCTD+PAH patients tended to have higher levels of aPL, suggesting its potential role in PAH development. Several studies[2,27,28,39,56,57] reported the relationship between aPL and PAH development in collagen tissue disorders. These antibodies generate endothelial dysfunction and induce the secretion of adhesion molecules. Ramified/bushy capillaries score was higher in MCTD patients (1.19 ± 0.77) than in SSc ones (0.56 ± 0.73), showing statistical significance ($p < 0.05$), while no statistically significant differences were found between sPAP in the two conditions ($p > 0.05$).

It is known[26,46,47,49,55] that the ramified/bushy capillaries characterize NFC findings in MCTD patients. Giant capillaries score was higher in SSc patients than in MCTD ones, with no statistical significance (1.42 ± 0.49 *vs.* 1.35 ± 0.26, $p > 0.05$).

On the other hand, the hemorrhage score was higher in MCTD patients than in SSc patients but without being statistically significant ($p > 0.05$). The higher hemorrhage score in MCTD patients is explained by the fact that those patients positive for aPL antibodies have multiple microhemorrhages[46,55]. Paolino et al[25] reported in their study that the MCTD patients showed a significantly lower number of enlarged/giant capillaries, but a higher number of total capillaries than SSc patients at their first nailfold capillaroscopy.

Statistical analysis showed the presence of correlations between the values of pressure in the pulmonary artery and capillaroscopic parameters. These correlations depended on the presence or absence of PAH in MCTD patients, as well as in the case of those with SSc (Table IV). Thus, in the case of MCTD without PAH, significant correlations were established between sPAP and ramified/bushy capillaries score ($p = 0.000012$) and giant capillaries score ($p = 0.001624$). In the presence of PAH, significant correlations were shown between CPAP and capillaries density ($p = 0.000272$) and hemorrhage score ($p = 0.000653$). These aspects can be explained based on the dynamics of capillaroscopy changes in MCTD; in the early stages, ramified/bushy and giant capillaries predominate, while in the late stages, the capillary density is reduced[26,45,55]. Ciang et al[53] reported that the PAH is associated with Raynaud's phenomenon and significant NFC abnormalities in MCTD patients. Paolino et al[25] identified that in MCTD patients, ramified/bushy capillaries often coexist along with giant capillaries. The ramified/bushy capillaries presence was suggestive of MCTD. For diagnostic purposes, bushy capillaries displayed 72% sensitivity, 80% specificity, and 87.2% negative predictive value[47,50]. In the late stages of evolution, the disorganization of the capillary network with the reduction of their density represents their main feature[28]. Scleroderma-like capillaroscopic pattern (giant capillaries, ramified/bushy capillaries, avascular areas, and hemorrhages), identified in more than half of MCTD patients, is associated with PAH development[44].

In SSc patients, sPAP was correlated with capillaries density ($p = 0.000012$), giant capillaries score ($p = 0.000042$), ramified/bushy capillaries score ($p = 0.005835$), and hemorrhages score ($p = 0.003945$), reflecting the dynamics of vascular changes both at the level of microcirculation and macrocirculation. Several studies in literature performed on SSc patients showed strong correlations between NFC abnormalities (especially reduced capillaries density) and the severity of PAH[58]. These findings supported that microcirculation abnormalities are involved in PAH development[58]. Riccieri et al[58], in their study performed on 24 SSc patients, reported greater devascularization and a higher frequency of the active and late NFC pattern compared with the early pattern among patients who developed PAH. The authors showed significant correlations between mPAP values and the NFC score ($p < 0.005$) and with the avascular areas score ($p < 0.001$). PAH development is associated with active and late scleroderma capillaroscopic patterns. These results are confirmed by other studies[59-62] in SSc patients.

Limitations of the Study

First of all, the number of studied patients was low; moreover, they were enrolled from the southwestern region of Romania, and all of them were Caucasians. Due to the small number of patients, the proportion of patients with PAH was higher than in other studies[31,32], which were performed on a large number of patients. On the other hand, pulmonary artery pressure was evaluated through cardiac ultrasonography, not by right heart catheterization, which can be considered as a limitation of the study. Future studies on larger cohorts can help provide more concrete information regarding the role of NFC in PAH development.

Conclusions

The present study revealed that the nailfold capillaries morphology changes correlate with target organ damage in MCTD. The MCTD patients who presented significant NFC abnormalities (especially active and late scleroderma capillaroscopic pattern) were prone to PAH development. Capillary density reduction was the most important factor associated with the occurrence of PAH. Among patients with MCTD and SSc with PAH, there were differences in NFC findings (especially capillaries density and ramified/bushy capillaries).

Authors' Contribution

Conceptualization: A.C. Methodology: A.C., A.M., N.R.K. Investigation: A.C., A.M., N.R.K. Resources: A.C., A.M., N.R.K. Data curation: A.C., A.M., Writing – original draft preparation: A.C. Writing – review and editing: A.C., A.M., N.R.K. Visualization: A.C., N.R.K. Supervision: A.C. All the authors have read and agreed to the published version of the manuscript.

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Ethics Approval
This study was approved by the Ethics Committee of Railway Clinical University Hospital, Timişoara, Romania (registration number: 28/01.2018). The study was conducted in accordance with the Declaration of Helsinki.

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Informed Consent
Written informed consents were obtained from all individuals involved in the study.

Conflict of Interest
The authors declare no conflict of interest.

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