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## HIGHLIGHTS

- **This is the largest cohort of patients with mixed connective tissue disease (MCTD) having associated interstitial lung disease (ILD);**
- **Upper gastrointestinal involvement and elevated C-reactive protein >5mg/L were associated with the presence of ILD;**
- **History of digital ulceration was identified as an independent major risk factor for severe ILD.**

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## The phenotype of mixed connective tissue disease patients having associated interstitial lung disease

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#### **Competing interests**

The authors have no financial or non-financial potential conflicts of interest to declare related to this project.

## **KEY MESSAGES**

- **This is the largest cohort of patients with mixed connective tissue disease (MCTD) having associated interstitial lung disease (ILD);**
- **Upper gastrointestinal involvement and elevated C-reactive protein >5mg/L were associated with the presence of ILD;**
- **History of digital ulceration was identified as an independent major risk factor for severe ILD.**

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**ABSTRACT**

**Objective:** We aimed to compare two matched populations of patients with MCTD with and without associated ILD and to identify predictive factors for ILD progression and severity.

**Methods:** This international multicenter retrospective study (14 tertiary hospitals), included MCTD patients who fulfilled at least one historical MCTD classification criteria. ILD was defined by the presence of typical chest high-resolution computed tomography (HRCT) abnormalities. Factors associated with ILD were assessed at baseline. Long-term progressive ILD was assessed in MCTD-ILD patients with multiple forced vital capacity (FVC) measurements.

**Results:** 300 patients with MCTD were included. Mean age at diagnosis was  $39.7 \pm 15.4$  years and 191 (63.7%) were women. Mean follow-up was  $7.8 \pm 5.5$  years. At baseline, we identified several factors associated with ILD presence: older age ( $p=0.01$ ), skin thickening ( $p=0.03$ ), upper gastro-intestinal (GI) symptoms ( $p<0.001$ ), FVC  $<80\%$  ( $p<0.0001$ ), diffusing capacity for carbon monoxide  $<80\%$  ( $p<0.0001$ ), anti-topoisomerase antibodies ( $p=0.01$ ), SSA/Ro antibodies ( $p=0.02$ ), cryoglobulinemia ( $p=0.04$ ) and elevated C-reactive protein ( $p<0.001$ ). Patients with MCTD-ILD were more likely to be treated with synthetic immunosuppressant agents ( $p<0.001$ ) in particular mycophenolate mofetil ( $p=0.03$ ). Digital ulcers (DU) were identified as a risk factor for FVC decline  $>10\%$ . During follow-up mortality was higher in the MCTD-ILD group ( $p<0.001$ ).

**Conclusion:** In this large international cohort of patients with MCTD, we identified different factors associated with ILD. Our findings also provide evidence that MCTD-ILD patients have increased mortality and that DU are associated with progressive lung disease.

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## INTRODUCTION

Mixed connective tissue disease (MCTD) is a rare systemic autoimmune condition characterized by overlapping features of systemic lupus erythematosus (SLE), systemic sclerosis (SSc), rheumatoid arthritis (RA) and dermatomyositis (DM) and by the presence of antibodies against the U1 small nuclear ribonucleoprotein autoantigen (anti-U1RNP) <sup>1,2</sup>. Raynaud's phenomenon (RP), puffy hands, arthritis and an increased risk of pulmonary involvement are considered typical clinical manifestations of MCTD <sup>3,4</sup>. Three classification criteria are currently used: Sharp, Kasukawa and Alarcon-Segovia <sup>5</sup>. Interstitial lung disease (ILD) has been described as a manifestation of connective tissue diseases, especially SSc and DM, and affects not only morbidity but also mortality <sup>6,7</sup>. Recently, data from the European Scleroderma Trials and Research Group (EUSTAR) showed that ILD patterns in SSc patients were highly heterogeneous and that a subset of patients developed progressive lung disease <sup>8</sup>. Moreover, numerous studies have consistently demonstrated that ILD is a leading cause of mortality in SSc <sup>9-12</sup>.

In the context of MCTD, data on the clinical phenotype of pulmonary disease are very scarce. Data from the Norwegian 126 MCTD patients' cohort showed that 52% had abnormal high-resolution chest CT (HRCT) findings and mortality was 20.8% in patients with severe lung disease (>50% of the lung parenchyma involved) after a mean 4.2 years' follow-up <sup>13</sup>. However, the precise clinical phenotype of patients with MCTD that develop ILD and prognostic factors for more severe lung disease are lacking. Therefore, the aim of this study was to compare two distinct populations of MCTD patients, with and without associated ILD, and to identify predictive factors for lung progression and severity.

## PATIENTS AND METHODS

### Patients

We conducted a longitudinal retrospective multicenter study in referral centers (n=14) in Norway, France, Italy, Iran, Japan, Portugal and Spain, during the period from December 2020 to November 2021. All physicians were asked to complete a standardized anonymized Excel form (Microsoft, Redmond, WA, USA). The inclusion criteria were (1) age  $\geq 18$  years at time of diagnosis; (2) a clinical diagnosis of MCTD verified by a rheumatologist; (3) a positive anti-U1RNP test; and (4) fulfillment of at least one of the three criteria sets for

MCTD (modified Sharps criteria<sup>1</sup>, criteria of Alarcón-Segovia or criteria of Kasukawa<sup>14</sup>). Overlap syndromes i.e. patients with a diagnosis of MCTD and evidence of another connective tissue disease (SLE, RA, SSc, Sjögren's syndrome or DM) were not excluded. Subjects were initially identified through electronic medical records search based on the positivity of anti-U1RNP antibodies. Subsequently, the final diagnosis of MCTD was determined based on the clinicians' description in the medical records. We asked each investigator to match any MCTD-ILD patient with one MCTD patient free of ILD based on the disease duration +/- 2 years. ILD cases were defined by the presence of any typical HRCT abnormalities i.e. ground-glass attenuations, airspace consolidation, reticular patterns, interseptal thickening, traction bronchiectasis and/or 'honeycombing'<sup>15,16</sup>. HRCT abnormalities were not standardized and were defined at each center radiologist discretion. Normal HRCT was not explicitly required for control patients, the absence of any documented findings suggestive of ILD (i.e. radiology reports, previous consultations with pulmonologists, or relevant diagnostic tests) helped in establishing the absence of significant ILD.

The patients' age, sex, smoking status, MCTD duration, clinical, laboratory, imaging data (HRCT), pulmonary function tests (PFTs) as well as treatments, were recorded at baseline and at the last available visit. For the MCTD-ILD group, available PFTs were recorded at 12±3 months after the initial diagnosis of ILD. Routine laboratory indicators of disease activity, including C-reactive protein (CRP) levels, were collected. The different lines of immunosuppressive agents (csDMARDs) and biologic targeted treatments (bDMARDs) were identified, including hydroxychloroquine (HCQ), azathioprine (AZA), mycophenolate mofetil (MMF), methotrexate (MTX), cyclophosphamide (CYC), tocilizumab (TCZ) and rituximab (RTX). NYHA class dyspnea, HRCT patterns and PFTs were assessed for MCTD-ILD patients at baseline.

Absolute forced vital capacity (FVC) decline of ≥10% between baseline and last available visit was used to define major ILD progression and was therefore selected in this study as the main outcome measure for progressive ILD. An absolute FVC decline comprised between 5% and 10%, which is greater than the estimated minimum clinically important difference at a group level<sup>17</sup> and had previously been associated with increased mortality in SSc<sup>8</sup>, was used to define moderate ILD progression. FVC stabilization/improvement was defined by a FVC decline <5%.



Absolute changes in FVC% were first evaluated over a 1-year period (12 months  $\pm$  3 months). Despite the limited availability of data at 12 months, we believe this time point provides valuable insights into the short-term outcomes of interest, and it allows for a preliminary assessment of the progression or stability of the studied parameters. Overall FVC% changes were then assessed prospectively from time of inclusion until the last follow-up visit in patients with available data using the definitions of progressive ILD described above.

All-cause mortality was assessed in all patients until last available follow-up.

### **Ethical considerations**

This study was conducted in compliance with the Good Clinical Practices protocol and Declaration of Helsinki principles. The written informed consent was not required due to study's design as being a retrospective chart review. Moreover, due to the de-identified and non-interventional nature of the study, it was determined to be exempt by each institutional review board except for the patients from Portugal. The institutional review board of the Centro Hospitalar Universitário Lisboa Norte, Lisbon, and Hospital Garcia de Horta, Almada, approved the present study. Ethics committee approval letters can be obtained upon request.

### **Statistical analysis**

Statistical analysis was performed using GraphPad Prism (v9.1.2) and Medcalc (v18.9.1). All data are presented as mean values  $\pm$  standard deviation (SD) or number and percentage (%) for continuous and categorical variables, accordingly. We used the unpaired *t*-test for two-group comparisons (continuous variables) and the chi-square test for differences in frequency (binary variables). Group comparisons of baseline variables were performed using the Mann–Whitney test for continuous variables or the  $\chi^2$  test for categorical variables. Logistic regression analysis were performed separately and adjusted for clinical and biological variables at baseline. Multivariable Cox regression models allowing for time-dependent variables were used to investigate the influence of sets of variables. The risk factors selected for multivariable analyses were based on a previous publication in the context of SSc (8). The starting point was the baseline visit. The risk of event was expressed as the hazard ratio (HR) and its 95% confidence interval (CI).

## RESULTS

### Study population and patient characteristics

The study population was composed of 300 MCTD patients including 150 patients with associated ILD. The number of included patients according to the recruitment country was as follows: Norway (n=118), France (n=76), Italy (n=36), Iran (n=20), Japan (n=20), Portugal (n=16), Spain (n=14). At the time of diagnosis patients had a mean age of  $39.7\pm 15.4$  years, 191 (63.7%) were women, 233 (77.7%) were Caucasian (**Table 1**). As stated in the previous section, patients were divided in two distinct groups according to the presence of diagnosed ILD: (1) MCTD-ILD group (n=150) and (2) MCTD without ILD group (n=150).

### Comparison between MCTD-ILD and MCTD without ILD patients at baseline

When comparing the two populations we observed that MCTD-ILD patients were older at the time of inclusion when compared to MCTD without ILD patients ( $42.2\pm 15.7$  vs  $37.5\pm 14.9$  years,  $p=0.01$ ) (**Table 1**). No significant differences were noted for sex ratio, percentage of non-Caucasian patients, smoking status or disease duration. MCTD-ILD patients had lower mean FVC% ( $80.4\pm 20.1\%$  vs  $95.3\pm 16.5\%$ ,  $p<0.001$ ) and lower mean diffusing capacity for carbon monoxide% (DLCO%) ( $62.4\pm 18.1\%$  vs  $74.8\pm 23.0\%$ ,  $p<0.001$ ) than patients without ILD. Moreover, skin thickening (38.7% vs 27.3%,  $p=0.03$ ) and upper gastro-intestinal (GI) symptoms (54.7% vs 30.7%,  $p<0.001$ ) were more frequent in the MCTD-ILD group. No significant differences were observed regarding the frequency of pulmonary hypertension (PH), digital ulcers (DU) or lower GI symptoms. The presence of a 'scleroderma pattern' on nailfold capillaroscopy was similar between both groups (22.0% vs 20.0%,  $p=0.45$ ). We identified 8 patients in the MCTD-ILD group who exhibited both skin sclerosis and SSc-specific antibodies. In contrast, none of the patients in the control group met these criteria ( $p=0.004$ ).

Regarding laboratory data, MCTD-ILD patients had more frequently increased CRP levels  $>5\text{mg/L}$  (54.7% vs 28.7%,  $p<0.001$ ), and higher percentages of anti-topoisomerase autoantibodies (4.0% vs 0.0%,  $p=0.01$ ), anti-SSA/Ro autoantibodies (29.3% vs 19.3%,  $p=0.02$ ) and cryoglobulinemia (5.3% vs 1.3%,  $p=0.04$ ).

Concerning therapeutic data, overall 62.0% of patients were taking corticosteroids at mean dose of  $20.1 \pm 16.5$  mg/day and the most frequently used csDMARD was MTX in 16.7% of patients. csDMARDs were more frequently used in MCTD-ILD patients (68.7% vs 49.3%,  $p < 0.001$ ), in particular MMF (7.3% vs 1.3%,  $p = 0.03$ ). There were no significant differences between the two groups regarding the use of corticosteroids, the use of csDMARDs other than MMF (AZA, MTX, CYC) or bDMARDs.

Logistic regression analysis adjusted for the following clinical variables: age, gender, smoking status, upper GI symptoms, digital ulcers (DU), skin thickening and the use of csDMARDs, revealed that older age (OR 1.03, 95% CI 1.01-1.05,  $p = 0.002$ ), presence of upper GI symptoms (OR 1.92, 95% CI 1.03 to 3.58,  $p = 0.04$ ) and csDMARDs at inclusion (OR 6.42, 95% CI 3.12 to 13.21,  $p < 0.001$ ) were independently associated with ILD. Furthermore, logistic regression adjusted for the following biological variables: rheumatoid factor (RF), anti-SSA/Ro autoantibodies, cryoglobulinemia and increased CRP  $> 5$ mg/L, identified CRP  $> 5$ mg/L (OR 8.78, 95% CI 0.26 to 2.91,  $p < 0.001$ ) as being independently associated with ILD in MCTD patients (**Table 2**).

### **Baseline lung features of MCTD-ILD group**

When focusing on the lung features of the 150 MCTD-ILD patients, 73% had dyspnea NYHA class 1 or 2 and 10% had dyspnea NYHA class 3 or 4 (**Table 3**). Nonspecific interstitial pneumonia (NSIP) was the most common HRCT pattern in 70% of cases followed by usual interstitial pneumonia (UIP) in 15% of cases. PFTs showed a mean FVC% of  $80.4 \pm 20.1$ % with 60 patients (40%) having FVC  $< 80$ %, DLCO% was  $62.4 \pm 18.1$ %. 10 patients (6.9%) presented exertional desaturation  $< 94$ % on 6-minute walking test (6MWT).

### **Longitudinal analysis**

In the longitudinal follow-up analysis, we present data at two key time points: 12 months and the last recorded follow-up visit. It is important to note that data at the 12-month mark were available for only 30% of the cohort. However, a more comprehensive set of follow-up data was obtained at the last recorded visit.

### Longitudinal analysis of MCTD-ILD group at 12 months $\pm$ 3 months

After a follow-up time of 12 months  $\pm$  3 months and with complete data for 45 (30.0%) MCTD-ILD patients, we observed a mean decrease in the FVC% of  $-2.4\pm 9.7\%$ . 40 (88.9%) MCTD-ILD patients were treated by a csDMARD most frequently HCQ, MTX and MMF. DLCO% showed an increase of  $1.3\pm 8.5\%$ . Overall, 8/45 (17.8%) patients had lost  $\geq 10\%$  of FVC and 7/45 (15.6%) patients had a loss of FVC comprised between 5% and 10%. No statistically significant associations were found between baseline characteristics and severe FVC decline  $\geq 10\%$ , moderate FVC decline 5-10% and FVC stabilization/improvement as defined by a FVC decline  $< 5\%$ . No deaths were recorded during this period.

### Longitudinal analysis of MCTD-ILD group at last follow-up

After a mean follow-up of  $7.8\pm 5.5$  years, with at least one follow-up visit for 124 (82.7%) and complete PFTs data for 109 (72.7%) MCTD-ILD patients, we observed a mean decrease in the FVC% of  $-4.6\pm 15.2\%$  and of  $-2.4\pm 18.0\%$  in the DLCO% (**Table 4**). 72 (66.1%) MCTD-ILD patients were treated by a csDMARD most frequently HCQ, MTX and MMF. The baseline characteristics of MCTD-ILD patients with and without at least one follow-up study on pulmonary function (n=109 and n=41, respectively) were comparable except for smoking status which was significantly more frequent in the former (28% vs 10%,  $p=0.02$ ) (**Supplementary Table 1**). Altogether 8/99 (8.0%) patients died during the follow-up period. Mortality was significantly higher in the MCTD-ILD group when compared to the MCTD without ILD group (8 vs 0 deaths,  $p<0.001$ ).

We examined the change from baseline in the FVC% and we observed a FVC decline of  $\geq 10\%$  in 33/99 (33.0%) patients, a FVC decline comprised between 5% and 10% in 14/99 (14.1%) patients and a stabilization/improvement in 52 (52.3%) patients (**Supplementary Figure 1**).

In an exploratory analysis, multivariate Cox analysis (included variables: age, gender, smoking status, skin thickening, SSc-specific autoantibodies and  $FVC < 80\%$ ) identified DU as a risk factor for ILD progression as defined by a FVC% decline of  $\geq 10\%$  (HR 16.7, 95% CI 2.80 to 100,  $p=0.002$ ) (**Supplementary Table 2**). On univariate analysis, there was a trend towards significance for the presence of RF as a protective factor against lung progression

(OR 0.32, 95% CI 0.10 to 1.00,  $p=0.05$ ). No association was seen with immunosuppressant treatment.

## DISCUSSION

Accumulating evidence suggests that ILD is a major cause of morbidity and mortality in many systemic connective tissue diseases (CTDs), including SSc, RA and myositis<sup>18-20</sup>.

ILD associated to CTDs has the potential to improvement and stabilization. However, a subset of patients will continue to have progressive lung fibrosis despite optimal treatment strategies<sup>21</sup>. In the few previous studies on adult MCTD, the reported frequencies of HRCT abnormalities compatible with lung fibrosis were up to 78%<sup>22-27</sup>.

To the best of our knowledge the present study is the largest cohort of patients with MCTD-ILD. On cross-sectional analysis at baseline of our MCTD sample we found that several variables such as older age at the time of diagnosis, presence of upper GI symptoms, and  $CRP > 5\text{mg/L}$  were independently associated with the presence of ILD. Moreover, MCTD-ILD patients were also more likely to be treated with csDMARDs at baseline. On the other hand, on the longitudinal analysis and after a mean of 7.8 years, mortality was higher in MCTD-ILD patients and a history of DU was strongly associated with lung progression.

Older age at diagnosis is a known risk factor for ILD progression and severity in CTD associated-ILD in particular SSc<sup>28</sup>. In the MCTD setting, our results showed that patients with ILD were significantly older than those without ILD at time of inclusion. This is in line with previous data from Gunnarsson et al<sup>13</sup>, where patients without ILD were younger than those with ILD (40.6 vs 46.2 (minor and moderate lung fibrosis) vs 50.3 years (severe lung fibrosis)). Similar findings were observed by Reiser et al<sup>25</sup> (41.6 (no ILD) vs 48.6 (stable ILD) vs 47.7 years (ILD progression)). In the latter, older age at baseline was also identified as a predictor of ILD progression (HR 1.1 (1.0, 1.1),  $p=0.028$ ), however this was not observed in our cohort. This discrepancy might be explained by the different definition of ILD progression used. In the former study ILD progression was defined by the extent of HRCT specific lesions between baseline and follow-up, whereas we opted for a definition purely based on FVC decline.

Upper GI symptoms were also identified as being independently associated with ILD in our

MCTD patients. Gastroesophageal reflux and esophageal motor disorders (hypomotility and/or dilatation of esophagus) are common in patients with MCTD<sup>29</sup>. Upper GI symptoms had been described to be associated with the development of ILD. In a previous series of 39 MCTD patients, Kawano-Dourado et al<sup>30</sup> observed that the prevalence of esophageal dilatation was associated with more severe ILD findings such as honeycombing and traction bronchiectasis. This was also observed in the study from Reiser et al<sup>25</sup>, where dilatation of esophagus on HRCT was significantly more frequent in patients with ILD. Similarly, in the latter, sclerodactyly was numerically more frequent in patients with ILD than those without ILD.

It is important to stress that according to the 2013 ACR/EULAR classification criteria<sup>31</sup> a small proportion of the included MCTD-ILD patients in our cohort could have been classified as having SSc, regardless of the presence of anti-U1 RNP autoantibodies. Anti-topoisomerase I positivity is a known factor associated with the development and severity of ILD in SSc patients<sup>32-34</sup>. Despite the 'SSc-like' clinical phenotype observed, and although our MCTD-ILD patients had higher percentages of anti-topoisomerase I and anticentromere autoantibodies than MCTD patients without ILD, their prevalence was very low (4.0% and 3.3%, respectively). Interestingly, in a previous study from Hoffmann-Vold et al the authors observed that 10% of patients with MCTD met the 2013 ACR/EULAR classification criteria for SSc<sup>35</sup>. Hence, based on these data, the overlapping clinical and serological features of these two diseases raise questions about the specificity of both SSc and MCTD classification criteria.

After univariate analysis we identified other biological variables that were associated with the presence of ILD: anti-SSA/Ro autoantibodies and cryoglobulinemia. Anti-SSA/Ro 52kDA had already been shown to be a promising marker for lung fibrosis in MCTD with a specificity of 81.3% and a negative predictive value of 76.3%<sup>36</sup>. Nonetheless, anti-SSA/Ro and cryoglobulinemia association with MCTD-ILD patients was not confirmed in our study after multivariate analysis.

Finally, in our cohort, increased CRP was strongly associated with the presence of ILD. Similar results were observed in Reiser et al study<sup>25</sup> where MCTD-ILD patients had higher erythrocyte sedimentation rates. Increased CRP levels had also been studied in the setting of SSc-ILD, being associated with rapidly progressive ILD and worse survival<sup>37-40</sup>. Therefore, further studies are required to identify biological markers that may help clinicians identifying

patients at risk of ILD development and progression as well as differentiate between MCTD-ILD and SSc-ILD patients.

Data on the course and outcome of MCTD-ILD are very scarce. In the study from Kawano-Dourado et al<sup>30</sup>, 10 year longitudinal data from 37 MCTD-ILD patients showed limited disease progression with a median percentage of ILD score increase from 7.5% to 11.2% (+3.7%), stable FVC% ( $2.58 \pm 0.54\%$  at baseline vs  $2.45 \pm 0.56\%$  at end of follow-up) and a modest, but significant decrease in DLCO% ( $17.7 \pm 5.1\%$  at baseline vs  $15.1 \pm 4.3\%$  at end of follow-up). Moreover, in the study from Reiserter et al<sup>25</sup> with data from 49 patients with MCTD-ILD and a mean follow-up of 6 years, the authors observed ILD progression as defined by HRCT in 23 patients (47%) with an increase of the extent of ILD from 8.8% (3-22) to 17.5% (6-43) (+8.7%). Patients with progressive lung disease had significantly lower FVC% and DLCO% levels at baseline than patients with stable lung disease. Although in our cohort a moderate and slow rate of decline in FVC% and DLCO% were observed in MCTD-ILD patients, roughly one in every three patients had a severe FVC decline of  $\geq 10\%$ . This rate of decline in FVC% seems to be greater than that observed in SSc patients. In a previous study from the EUSTAR database the authors reported a FVC decline of  $\geq 10\%$  in 23% of SSc-ILD patients at 5-year follow-up<sup>8</sup>. Interestingly, we identified a history of DU as a strong and independent predictor of ILD progression. In the study from Reiserter et al<sup>25</sup> the authors identified older age, male gender, higher anti-U1RNP levels and anti-Ro-52 autoantibodies to be independent predictors of ILD progression. However, no data is given on other risk factors, such as DU. Overall, our results seem to suggest that MCTD-ILD patients with more 'SSc-like' features are more likely to develop lung disease.

Despite the strength of our findings, the herein study had some limitations. The loss to follow-up of 17% of patients and PFTs data available for only 73% of patients may have introduced a selection bias. Furthermore, assessing the progression of lung function at the last follow-up visit rather than at a specific point in time may have introduced bias into the interpretation of the results. Also, in order to facilitate inclusion criteria we defined that patients had only to fulfill at least one of the historical classification criteria and this regardless of anti-U1RNP titers. Finally, because of its observational and multicentric nature, HRCT technique and interpretation were not standardized between centers which might have introduced some heterogeneity in the definition of ILD cases.

In summary, following the results observed in our study it appears reasonable to draw the following conclusions: ILD occurs more frequently in MCTD patients who are older at the time of diagnosis, who present a 'SSc-like' phenotype with skin thickening and GI symptoms and having elevated markers of systemic inflammation. Even if ILD extent was modest at baseline, approximately one third of patients had significant progression over an 8-year interval. DU were identified as an independent and strong risk factor for lung progression. Finally, ILD was associated with increased risk of mortality, and should therefore be considered by rheumatologists as a serious and potential life-threatening complication in MCTD.

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**Table 1.** Characteristics of MCTD patients stratified based on presence or absence of ILD on HRCT.

Characteristics	Total (n=300)	MCTD (n=150)	no-ILD	MCTD-ILD (n=150)	P-value
<b>Demographics</b>					
Age, mean (SD)	39.7	37.5 (14.9)		42.2 (15.7)	<b>0.01*</b>
Gender, n (%)	191	93 (62.0)		98 (65.3)	NS
African, n (%)	27	10 (6.7)		17 (11.3)	NS
Non caucasian, n (%)	67	30 (20.0)		37 (24.7)	NS
Smoking, n (%)	83	48 (32.0)		35 (23.3)	NS
<b>Clinical features</b>					
Disease duration, years, mean (SD)	16.8 (10.1)	16.6 (9.7)		16.9 (10.5)	NS
Time since MCTD diagnosis and ILD diagnosis, years, mean (SD)		5.8 (8.2)			
FVC%, mean (SD)	87.5	95.3 (16.5)		80.4 (20.1)	<b>&lt;0.001</b>
DLCO%, mean (SD)	68.5	74.8 (23.0)		62.4 (18.1)	<b>&lt;0.001</b>
PH, n (%)	10	4 (2.7)		6 (4.0)	NS

Skin thickening, n (%)	99	41 (27.3)	58 (38.7)	<b>0.03</b>
Digital ulcers, n (%)	42	16 (10.7)	26 (17.3)	NS
Polysynovitis, n (%)	109	55 (36.7)	54 (36.0)	NS
Myositis, n (%)	75	37 (24.7)	38 (25.3)	NS
Upper GI, n (%) **	128	46 (30.7)	82 (54.7)	<b>&lt;0.001*</b>
Lower GI, n (%) ***	72	37 (24.7)	35 (23.3)	NS
Capillaroscopy SSc pattern, n (%)	63	30 (20.0)	33 (22.0)	NS
<b>Biological features</b>				
Anti-topoisomerase I (Scl70), n (%)	6	0 (0.0)	6 (4.0)	<b>0.01</b>
Anticentromere, n (%)	7	2 (1.3)	5 (3.3)	NS
Rheumatoid factor, n (%)	85	38 (25.3)	47 (31.3)	NS
Anti-CCP, n (%)	20	8 (5.3)	12 (8)	NS
Anti-SSA/Ro, n (%)	73	29 (19.3)	44 (29.3)	<b>0.02</b>
Anti-SSB/La, n (%)	17	6 (4.0)	11 (7.3)	NS
Cryoglobulinemia, n (%)	10	2 (1.3)	8 (5.3)	<b>0.04</b>
CRP >10mg/L, n (%)	35 (11.7)	16 (10.7)	19 (12.7)	NS
CRP >5mg/L, n (%)	125 (41.7)	43 (28.7)	82 (54.7)	<b>&lt;0.001*</b>

Treatment				
Prednisone, n (%)	186 (62.0)	92 (61.3)	94 (62.7)	NS
csDMARD, n (%)	177 (59.0)	74 (49.3)	103 (68.7)	<0.001*
Azathioprine, n (%)	23 (7.7)	11 (7.3)	12 (8.0)	NS
Hydroxychloroquine, n (%)	100 (33.3)	48 (32.0)	52 (34.7)	NS
Methotrexate, n (%)	50 (16.7)	26 (17.3)	24 (16.0)	NS
MMF, n (%)	13 (4.3)	2 (1.3)	11 (7.3)	<b>0.03</b>
Cyclophosphamide, n (%)	9 (3.0)	2 (1.3)	7 (4.7)	NS
bDMARD, n (%)	5 (1.7)	2 (1.3)	3 (2.0)	NS

\*Significant after multivariate analysis

\*\*Upper gastrointestinal involvement included the following symptoms: dysphagia, gastroesophageal reflux, bloating, nausea/vomiting, early satiety and/or epigastric pain.

\*\*\*Lower gastrointestinal involvement included the following symptoms: abdominal pain, distension, diarrhea, constipation and/or fecal incontinence.

**Abbreviations:** MCTD, mixed connective tissue disease; ILD, interstitial lung disease; HRCT, high-resolution computed tomography; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; SSc, systemic sclerosis; GI, gastrointestinal; CRP, C-reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; MMF, mycophenolate mofetil; bDMARD, biological disease-modifying antirheumatic drug.

**Table 2.** Variables associated with MCTD-associated ILD analyzed by logistic regression.

Clinical variables	Univariable		Multivariable	
		P-value	OR (95% CI)	P-value
Older age		0.01	1.03 (1.01, 1.05)	0.002
Skin thickening		0.03	1.92 (1.03, 3.58)	0.04
Upper GI symptoms		<0.001	6.42 (3.12, 13.21)	<0.001
<b>Biological variables</b>				
CRP > 5mg/L		<0.001	8.78 (2.94, 26.22)	<0.001
Anti-topoisomerase I (Scl70) positivity		0.01		NS
Anti-SSA/Ro positivity		0.02		NS
Cryoglobulinemia		0.04		NS
csDMARD treatment		<0.001		NS
MMF treatment		0.03		NS

**Abbreviations:** MCTD, mixed connective tissue disease; ILD, interstitial lung disease; OR, odds ratio; GI, gastrointestinal; CRP, C-reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; MMF, mycophenolate mofetil.

**Table 3.** Baseline lung features of MCTD-ILD patients.

	<b>MCTD-ILD (n=150)</b>	<b>Variables available (n (%))</b>
<b>Clinical manifestations</b>		143 (95.3)
Dyspnea, n (%)	104 (71)	
NYHA 1-2, n (%)	109 (73)	
NYHA 3-4, n (%)	15 (10)	
<b>HRCT pattern</b>		150 (100)
NSIP, n (%)	101 (70)	
UIP, n (%)	22 (15)	
<b>Lung function tests</b>		129 (86)
FVC%, mean (SD)	80.4 ± 20.1	
FVC<80%, n (%)	60 (41)	
DLCO%, mean (SD)	62.4 ± 18.1	
Desaturation <94% on walking test, n (%)	10 (6.9)	

**Abbreviations:** MCTD, mixed connective tissue disease; ILD, interstitial lung disease; HRCT, high-resolution computed tomography; NSIP, nonspecific interstitial pneumonia; UIP, usual interstitial pneumonia; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide.

**Table 4.** Changes in lung features of MCTD-ILD patients during follow-up.

<b>Variables</b>	<b>Baseline (n=150)</b>	<b>Follow-up (n=99)</b>
Dyspnea NYHA ≥ 2, n (%)	82 (54.7)	65 (65.7)
FVC%, mean (SD)	80.4 (20.1)	77.2 (21.5)
FVC<80%, n (%)	60 (40)	52 (52.5)
DLCO%, mean (SD)	62.4 (18.1)	59.4 (18.7)
DLCO<80%, n (%)	98 (65.3)	85 (85.9)

**Abbreviations:** MCTD, mixed connective tissue disease; ILD, interstitial lung disease; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide.



**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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