

## Abstract

In 1972, Sharp and colleagues described a new autoimmune rheumatic disease which they called mixed connective tissue disease (MCTD), characterized by overlapping features of systemic sclerosis (SSc), systemic lupus erythematosus (SLE), polymyositis/dermatomyositis (PM/DM), high levels of anti-U1snRNP and low steroid requirement use with good prognosis. MCTD was proposed as a distinct disease. However, soon after the original description, questions about the existence of such a syndrome as well as disputes over the features initially described began to surface. The conundrum of whether MCTD is a distinct disease entity remains controversial. We undertook a literature review focusing on the articles reporting new data about MCTD published in the last decade, to determine whether any new observations help to answer the conundrum of MCTD. After reviewing recent data, we question whether the term MCTD is appropriately retained, preferring to use the term “undifferentiated autoimmune rheumatic disease”.

# Mixed Connective Tissue Disease– Enigma Variations?

## **Introduction**

Over forty years ago mixed connective tissue disease (MCTD) was envisaged as a condition characterized by high levels of antibodies to ribonucleoprotein (RNP), Raynaud's phenomenon, swollen fingers, esophageal dysfunction and the absence of lung and renal disease. These patients required low steroids and the prognosis was good[1]. Within ten years, major doubts about the original claims emerged. Nimelstein et al[2] in reviewing the original 25 cases observed that some patients lacked high levels of anti-RNP, and three could not be identified. Furthermore lung and renal disease occurred, the steroid requirement could be high and the prognosis seemed closer to that of lupus patients. These and other data reviewed elsewhere[3]:[4] led to major doubts about MCTD as originally defined and as a separate disease entity.

There have been four attempts to develop classification criteria and the conundrum of whether MCTD should be renamed continues. Here, we focus on the literature published about 'MCTD' in the past decade. We sought to discover if any genuinely original insights have emerged to help unravel the MCTD mystery.

## **Methodology**

Our literature review focused on articles in English published from 2005-2015. We identified 479 articles listing MCTD or anti-RNP as key words. We excluded case reports and articles focusing on undifferentiated connective tissue disease (UCTD) but

did not really consider MCTD at all. Around 100 articles remain, which formed the basis of this review.

### **Diagnostic Criteria & Disease Evolution**

Four sets of classification criteria for MCTD have been published: Sharp's[5], Kasukawa's[6], Alarcon-Segovia's[7], and Kahn's[8]. Previous literature concluded that those of Alarcon-Segovia had the highest sensitivity and specificity, while Sharp's criteria had a lower specificity[9][10]. One recent study[11] evaluated 161 MCTD patients at diagnosis and after a mean follow up of 7.9 years observing their evolution. Kasukawa's criteria were more sensitive (sensitivity 75%) compared to Alarcón-Segovia's (73%) or Sharp's (42%) criteria. The percentage of patients at final follow up who satisfied 1 or more of the 3 classification criteria was lower compared to the time at diagnosis (Kasukawa: 53% versus 75%; Alarcon: 44% versus 73%; Sharp: 32% versus 42%). Even using Kasukawa's criteria >40% were no longer diagnosable as having MCTD. Of the remainders, 17.3% evolved into systemic sclerosis (SSc), 9.1% into systemic lupus erythematosus (SLE), 2.5% into rheumatoid arthritis (RA) and 11.5% were reclassified as UCTD. The percentage of patients evolving into other autoimmune rheumatic diseases (ARDs) was lower in patients with disease duration of 0-5 years than in those >5 years. Using multiple variable regression, a significant association was found between anti-dsDNA at the first visit and evolution into SLE in those initially diagnosed with MCTD by Kasukawa's criteria (OR 1.3;  $P=0.012$ ) and Alarcón-Segovia's (OR 1.4;  $P=0.001$ ). In patients with a first diagnosis of MCTD (Kasukawa's criteria), sclerodactyly (OR 1.2;  $P=0.034$ ) and esophageal hypomotility or dilation (OR 1.4;  $P=0.001$ ) were associated with evolution into SSc.

Another study[12] reported that the point prevalence showed no statistical difference between the three criteria sets (Sharp's, Alarcón-Segovia's or Kasukawa's), indicating that they may be comparable[12]. The point prevalence of MCTD in living adults in Norway was 3.8 per 100 000, while the incidence of adult-onset MCTD in Norway from 1996-2005 was 2.1 per million per year.

A study[13] involving 280 MCTD patients reported different disease evolution observations. During follow-up, MCTD patients developed new symptoms; but did not show progression to other ARDs. This finding contrasted markedly with earlier studies, which demonstrated that most patients with anti-U1RNP developed into classified ARDs within 5 years of presentation. In these studies, Sharp's criteria were often used, which may be less specific than other criteria[14]·[15]. In other reports, the classification criteria used for diagnosis were not confirmed[16], which might have influenced the evolution rate.

**Clinical Features** (Table 1 summarizes new findings in recent 10 years)

### *Pulmonary hypertension*

Pulmonary hypertension (PH) is a major clinical feature in MCTD, with prevalence from 10%to 50%. There are three recent prevalence studies. In one, PH occurred at a frequency of 3.4%[17]. In the others, the prevalence of pulmonary arterial hypertension ranged from 14% to 17.9% in MCTD[13]·[18]. PH associated with ARDs is classified into five subgroups:[19]

- Group 1: pulmonary arterial hypertension (PAH);
- Group 2: PH due to left heart disease;
- Group 3: PH due to lung disease or hypoxia;
- Group 4: chronic thromboembolic pulmonary hypertension (CTEPH);
- Group 5: PH with unclear or multifactorial mechanisms.

Thus, PAH is just one subgroup in PH. The 3.4% prevalence of PH in the nationwide Norwegian multicentre cohort of 147 adult MCTD patients included isolated PAH and PH due to interstitial lung disease (ILD)[17]. The lower than anticipated PH prevalence was possibly explained by the selection of the study population. While previous studies usually assessed PH in MCTD referred to tertiary centres, this study screened unselected MCTD patients throughout Norway, perhaps leading to a lower prevalence. Different studies have used different cut offs on echocardiograms to screen for PH before subjecting the patients to right heart catheterization (RHC). One used pulmonary artery systolic pressure (PASP) of >40 mmHg as a cut off[17], while two others used right ventricular systolic pressure (RVSP) of 25 mmHg for screening[13][18]. PASP is considered equal to RVSP in the absence of pulmonary outflow tract obstruction. This discrepancy might significantly affect the ascertainment rate for PH.

Anti-U1RNP, anti-antiendothelial cell (AECA), anticardiolipin antibodies (ACL) and IgG anti-beta-2-glycoprotein I (anti- $\beta$ 2-GPI) might be associated with development of PAH; suggested by higher levels of these antibodies in MCTD patients with PAH compared to those without. AECA were also associated with high serum thrombomodulin and von Willebrand Factor antigen (vWFAg) levels, indicating endothelial cell activation and damage[13][18][20]. Furthermore, anti-U1RNP might have a direct pathogenic role. Considering antiphospholipid antibodies, previous studies

showed that ACL were not associated with thromboembolism in MCTD, and most ACL in MCTD sera are beta-2-glycoprotein I independent, which may explain the absence of associated clotting events[21]:[22]. However, as MCTD-PAH patients tended to have higher levels of antiphospholipid antibodies[13]:[20], suggesting their potential role in PAH. PAH in MCTD was noted to be more commonly associated with Raynaud's syndrome with abnormal nailfold capillaroscopy[23].

In MCTD, diastolic function of left ventricle was worse compared to a control group. For right ventricular function, there was global failure of the right ventricle function in cases of MCTD complicated with PAH, while right ventricle function in those without PAH was not different from the controls[24].

The pathophysiology of PAH in MCTD is similar to other ARDs, especially SSc. But interstitial lung disease (ILD) was not significantly related to PAH in MCTD[25]:[26]. Among ARDs with associated PAH, one-year survival and discharge from hospital were lower in MCTD with PAH when compared to SLE, SSc and RA with PAH[27]. One recent review recommended that all patients with SSc, CREST syndrome, or MCTD should undergo annual screening with echocardiography for PAH, though RHC is the standard for definitive diagnosis. Cardiac MRI is complementary for diagnosing PAH[28].

### Interstitial lung disease

Two studies involving more than 100 MCTD patients showed that 47.1% and 52% had evidence of ILD[13]:[29]. Another study evaluated ILD and esophageal dysfunction

and reported HRCT abnormalities in up to 78 % among 50 MCTD patients[30]. 19% had severe lung fibrosis, these patients had lower functional status. The mortality was significantly worse in severe lung fibrosis. These patients had shorter mean disease duration at study inclusion than patients with minor or moderate fibrosis, indicating a more rapidly progressive disease[29].

Concerning ILD in MCTD, the most frequent histology is non-specific interstitial pneumonia (NSIP), followed by usual interstitial pneumonia (UIP) and lymphocytic interstitial pneumonia (LIP)[28]. Anti-U1RNP antibodies may contribute to disease manifestations. They induce pulmonary injury in murine models[31][32]. A correlation was found between diffusing capacity for carbon monoxide (DLCO) and ILD, but not between total lung capacity and ILD[30]. This is probably because reduced DLCO is the most sensitive test for predicting ILD. Esophageal abnormalities may also be related to ILD. In MCTD, 3 different sub-phenotypes can be seen:

1<sup>st</sup> subgroup: AECA and antiphospholipid antibodies associated with PAH, Raynaud's, livedo reticularis and vascular thrombosis.

2<sup>nd</sup> subgroup: presence of ILD, esophageal dysmotility and myositis.

3<sup>rd</sup> subgroup: higher prevalence of anti-CCP antibodies and erosive arthritis

In cluster 2, the incidence of ILD was 98.7%, significantly higher than in groups 1 and 3 ( $p < 0.001$ ). Nailfold capillaroscopy might help to differentiate the subgroups, as abnormal scleroderma like capillary pattern was found most commonly in the first subgroup (68.7% vs 26.5% and 33.3% in the other two subgroups). This SSc capillary pattern also correlated with ACL, AECA ( $p < 0.0001$ ) and PAH ( $p < 0.05$ ). The worst overall survival probability belonged to cluster 1, where 11 of 17 patients died, eight from PAH[33].

### Esophageal involvement

64% of MCTD patients had severe esophageal dysfunction, and 50% of patients had distal abnormal acid reflux. However, there was no statistically significant relation between esophageal dysfunction and acid reflux. ILD was significantly higher among patients with esophageal dilatation (92% vs. 45%;  $p < 0.01$ ) and severe esophageal dysfunction (90% vs. 35%;  $p < 0.001$ ). No statistically significant differences in the frequencies of ILD between patients with normal or abnormal acid reflux were found[30], i.e. there was correlation between severe esophageal dysfunction and lung damage in the absence of acid reflux. Thus ILD may be associated with food reflux instead of acid reflux in MCTD. This observation was reminiscent of another study demonstrating abnormal lung function tests and significant delay in the clearance of the nucleotide in the scintigraphy[34].

### Musculoskeletal involvement

Arthritis in MCTD has a possible association with rheumatoid factor (RF) and anti-CCP. Whereas RF was positive in 30 to 100% of MCTD patients, anti-CCP was found in only 9%. Anti-U1RNP may be a predictor of more aggressive erosive arthritis[22]. Recent observations[35] included significantly higher levels of serum IgM-, IgG- and IgA-RF in MCTD patients when compared with controls( $p < 0.05$ ). The frequency of IgM-RF in MCTD patients was 48% vs. 77% in RA. A recent review[36] reported 75-95% MCTD patients had arthritis. Around 50% of patients with frank arthritis are erosive. HLA-DR4 is associated with polyarthritis in MCTD.

There have been no major new observations of myositis in MCTD. Up to 2/3 of MCTD patients have overt myositis ranging from mild to severe[22]. When myositis patients with anti-RNP antibodies were compared to those without, the former group responded better to corticosteroids. Histologically, myositis in MCTD is indistinguishable from dermatomyositis (DM). Immunoglobulin deposits were found in muscles and it is an immune-complex-driven disease[25][37][38].

### Cardiovascular involvement

The prevalence of cardiac involvement varies from 13% to 65%[39]. Pericarditis was the most common cardiac diagnosis with prevalence of 30% and 43% in 2 prospective studies. Non-invasive cardiac tests detected subclinical cardiac abnormalities in 6%–38% of patients. Diastolic dysfunction and accelerated atherosclerosis were well-documented in a case–control study[24]. Two older and one recent prospective studies revealed an overall mortality of 10.4% over an ensuing 13–15 years. 2.1% of patients died of direct cardiac causes[13][40][41].

Endothelium-dependent vasodilation, assessed by flow-mediated dilation (FMD), was significantly impaired in MCTD (Alarcón-Segovia's criteria) versus controls[42]. The percentage of FMD was even lower in MCTD with cardiovascular diseases (CVD), than in those without it. The percentage of nitrate-mediated dilation (NMD) did not differ between MCTD vs. controls, and MCTD patients with CVD vs. those without. Mean carotid intima-media thickness (IMT) was higher in MCTD patients than in controls.

### Renal involvement

The original MCTD description stressed the paucity of renal involvement[1] but later review showed that it occurred with a frequency between 5% and 36%[43]. In one review[44], 12 of 30 MCTD patients (40%) had renal involvement. The majority had membranous glomerulonephritis (GN), followed by mesangial GN. Some patients had proliferative GN and a few had scleroderma pattern[45]. Patients with renal disease have more systemic manifestations than those without. 72% of nephropathy patients experienced resolution or improvement with steroids. Electron microscopy revealed immune complex deposition in the glomeruli[36][44]. In MCTD patients, the anti-U1snRNP seemed to have no correlation with nephropathy[44]. But in a study of a murine model[46], immunization of mice with RNP antigen induced anti-RNP and MCTD clinical manifestations, typically ILD but not renal disease. In contrast, for mice deficient in Toll-like receptor 3 (TLR-3), RNP antigen exposure induced SLE-like GN. Exposure to RNP antigen in an appropriate context may induce autoimmunity and MCTD features, while changes in innate immunity or TLR signaling with the same trigger may lead to the development of SLE-like nephritis.

### Raynaud's phenomenon (RP) & capillaroscopy

Capillaroscopy findings of nailfold capillaries are usually classified as normal, nonspecific, or scleroderma (SD) like. The prevalence of RP observed in scleroderma and MCTD is generally 90% or more in most studies. There are 2 different types of SD pattern:

1)Slow: Irregularly enlarged or giant loops with no or minimal capillary loss

2)Active: Definite capillary loss and neoformation of capillaries.

MCTD patients often demonstrate a slow SD pattern. RP tends to be less severe in MCTD than in scleroderma, with fewer digital ulcers or loss of digits[47].

In a recent prospective study enrolling over 3000 patients with primary RP, initially none of the patients with primary RP had symptoms or signs of ARDs and all had normal nailfold capillaries. After a mean FU of 4.8 years, 1,660 (54.8%) patients still had primary RP, but 246 (8.1%) had suspected secondary RP, and 1,123 (37.1%) developed ARDs (363 UCTD, 263 SSc, 143 SLE, 24 MCTD). Suspected secondary RP meant patients had no clinical signs of ARD but had serological findings or abnormal nailfold capillaroscopy. SD pattern in capillaroscopy was significantly associated with development of SSc, dermatomyositis, overlap syndrome with signs of SSc and MCTD. The SD pattern had a better negative than positive predictive value for possible development of an ARD. Among the 24 patients who developed MCTD, half had normal capillaries, the other half had nonspecific or SD type capillary changes. The SD pattern was present in 9/24 (37%) patients with MCTD[48]. Another study involving over 1000 patients with RP reported similar findings and noted that SD type capillary changes with RP was indicative of the development of an ARD, despite the absence of other disease symptoms[49].

In a Hungarian study[13], the number of MCTD patients with the SD pattern on capillaroscopy increased over time. The typical “SD pattern” at the time of diagnosis of MCTD (Alarcon’s criteria) was found in 31.4%. During a mean 13.1 years’ follow up, there was a modest progression to 40.3%. More deceased MCTD patients had SD

pattern compared with those MCTD patients who survived (38.3% of 258 living patients vs. 63.3% of 22 deceased,  $p < 0.02$ ). Another nailfold capillaroscopy study[50] performed on 63 MCTD patients (Kasukawa's criteria) noted that SD-pattern was observed in 65% patients at entry and in 71.5% patients at previous capillaroscopy before inclusion. Capillaroscopy changed with treatment apparently, as a reduced capillary density was more frequently observed in patients taking immunosuppressant than those without medication (66.7 vs 33.3%,  $P = 0.001$ ). Nailfold capillary changes in MCTD seem to be a dynamic process.

MCTD demonstrates nailfold capillary abnormalities more reminiscent of SSc than SLE. But branched "bushy" capillary formations are believed by some to be characteristic of MCTD[25]. Despite the high frequency of RP in SLE in one study[51], only 2% SLE patients had an SD pattern compared with 54% of MCTD patients. A relationship between anti-U1RNP and Raynaud's phenomenon was reported. Anti-U1RNP may contribute directly to the vasculopathy.

### **Serology & Immunology** (Table 2 summarizes new findings in recent 10 years)

U1-snRNP is composed of U1-RNA, seven common core Sm proteins, and three U1-specific proteins (U1-70K, U1-A, and U1-C)(25). The components of the spliceosome complex that anti-U1snRNP recognized include the U1-RNA and the U1-specific polypeptides 70kD, A, and C in a study using Kasukawa's criteria. Anti-U1-70K were found in 75–90% of MCTD patients (Sharp's criteria), and were the most commonly detected component. They were only found in 20–50% of SLE patients who were positive for anti-RNP. Antibodies against the RNA component of U1-snRNP were found in 38% of anti-RNP positive patient sera[21]:[37]:[52].

Most MCTD patients have high titer anti-U1snRNP[25], but anti-U1snRNP is not exclusive to MCTD. In a study of 161 MCTD patients, between 20-40% of SLE patients, 2-14% with SSc, and 6-9% with myositis were positive for anti-U1snRNP. Patients with RA usually lacked anti-U1snRNP antibodies[11]. IgM anti-U1snRNP titers were significantly higher in SLE patients than either MCTD patients (Alarcon's criteria) or healthy controls ( $p \leq 0.05$ ). IgG anti-U1snRNP was significantly higher in SLE and MCTD populations than in the healthy group; but IgG reactivity was similar in both ARDs. Combining IgM anti-U1C and anti-U1A it was possible to classify SLE and MCTD patients with an accuracy of 71.3%, which was rather unsatisfactory[53]. Lower titer anti-U1RNP were found in SLE and were usually of the IgM isotype associated with anti-Sm[22]. Another review[25] also reported MCTD patients were less likely than SLE to retain IgM U1-snRNP antibodies, and claimed that long-standing high titer IgG U1-snRNP are typical for MCTD conveying high specificity. This observation differs from the finding in the study[53] above.

MCTD also differs from SLE and RA in response to another spliceosome-associated protein, the heterogeneous nuclear RNP (hnRNPA2). Recent study claimed that antibodies to Sm-D, which are present in the U1-snRNP and positive in 10% of white SLE patients, are largely specific for SLE. Patients who fulfill MCTD criteria rarely have antibodies to Sm[25]. Another cross sectional comparative analysis of immunological markers in sera from 51 SLE patients and 10 MCTD patients and 59 controls reported different observations. Levels of anti-SmBB' or anti-SmD were similar in SLE and MCTD sera, though antibodies to SmD were more frequent in SLE. Also, as sera from MCTD had higher levels of anti-U1-70kD than sera from SLE

patients, high anti-U1-70kD were useful for diagnosing MCTD[54]. Notably, 94% of MCTD patients vs. 20% of SLE patients had sera with antibodies against U1-A protein[37].

Anti-U1snRNP seemed to be robust markers of MCTD onset. Their emergence preceded the onset of clinical manifestations[55]. Individual U1-RNP antibodies have been evaluated as markers of disease activity, measured by activity scales derived from lupus. Anti-U1-RNA titers correlated with activity, unlike anti-U1-70K titers[21]. During remission, antibodies to U1-70K, U1-A and U1-C reduced, suggesting a correlation between the autoantibodies and disease activity[37]. A control study which assessed B cell subsets in 46 MCTD patients (Alarcon's criteria) vs. 20 healthy individuals showed that anti-U1RNP levels decreased after treatment ( $p < 0.0467$ )[56].

Antigen modification is important in the pathogenesis of MCTD. One study of alterations in post-translational modifications (PTMs) on U1snRNP 68k subunit in 4 MCTD, 4 SLE, 4 RA patients and 3 healthy donors showed that MCTD and SLE patients were characterized by increase of low phosphorylated U1-68k. PTMs on autoantigens were involved in the production of antibodies as altered self-proteins created novel epitopes to which the immune system has not been tolerized[57]. The 70kD polypeptide of U1-RNP is susceptible to multiple forms of antigen modification including PTM and caspase cleavage during apoptosis or oxidative cleavage in response to stress. Apoptotically modified 70kD is antigenically distinct from intact 70kD and may have clinical implications in breaking immune tolerance. Autoantibodies reactive with apoptotic 70kD are superior markers to those against intact 70kD for MCTD[21][55]. In 53 MCTD patients, 29(54%) preferentially recognized the apoptotic

form of 70K compared to intact 70K[31]. The appearance of autoantibodies to U1-snRNP components follows a characteristic order. Antibodies to U1-70K and Sm-B/BV generally appear early. Anti-U1A and U1C and Sm-D are detected later. U1-70K is a major early immunogen, this together with the fact that U1-70K is modified during apoptosis, suggest apoptotic modifications on U1-70K protein might be important for triggering immune response to U1snRNP[58].

A cohort study evaluated 15 peptides in 68 SLE and 29 MCTD patients and 26 healthy individuals. U1-70K was the best at predicting which samples were in the SLE group from healthy samples and the second best at separating MCTD from healthy samples ( $p=0.0001$ ). Another two peptides from U1A protein, were the 2 best predictors of SLE vs. MCTD ( $p=0.167$  and  $p=0.206$ ). Though they are not capable of definitively segregating SLE from MCTD, U1A may be the most likely protein that can distinguish the 2 diseases[59].

There are 2 proposed mechanisms of pathogenic role by anti-U1RNP:

- 1) Binding to endothelial cells, leads to endothelial cell activation and damage leading to vascular disease pathogenesis, inducing RP, puffy hands, sclerodactyly, PH, possibly ILD and esophageal dysmotility.
- 2) Forming immune complexes that might activate complement, induce myositis, (non-erosive) arthritis and perhaps ILD. The pathogenesis of erosive arthritis is unclear[22].

A recent study reported that the proportion of transitional B cells, naive B cells and double negative (DN) B lymphocytes was higher in MCTD patients (Alarcon's criteria)

than in controls. The memory B cell population had a close correlation with disease activity measured by the systemic lupus activity measure (SLAM). The number of plasma cells was also increased and there was an association between their number and anti-U1RNP levels. Cyclophosphamide, methotrexate, and corticosteroid treatment decreased the number of DN and CD27<sup>high</sup> B cells[56].

In a cross-sectional study involving 21 MCTD patients (Alarcon-Segovia's criteria), CD4<sup>+</sup>CD25<sup>high</sup> T regulatory cells decreased with increasing levels of disease activity, though the correlation was not significant[60]. In another study of T regulatory (Treg) cells involving 48 MCTD patients(Alarcon-Segovia's criteria), the percentage and absolute number of CD4<sup>+</sup>CD25<sup>+</sup>high Treg cells were lower in the MCTD patients than in controls ( $p < 0.04$ ), and were further decreased in active MCTD and lower than in the inactive stage ( $p < 0.01$ ). There was an increase in percentage and absolute number of CD4<sup>+</sup>IL-10<sup>+</sup> Treg cells in MCTD patients compared to the healthy controls ( $p < 0.02$ ). The percentage of CD4<sup>+</sup>IL-10<sup>+</sup> Treg cells was higher during active disease than during remission ( $p < 0.005$ ). The role of these cells in immunoregulation and inflammation is reviewed elsewhere[61].

Immune mechanisms that contribute to U1-snRNP immunogenicity include epitope spreading through B and T-cell interactions and apoptosis induced modifications. Spread of immunogenicity can occur within a single antigen to multiple epitopes on the same protein (intramolecular spreading) or to other epitopes within a greater macromolecular complex (intermolecular epitope spreading)[37].

## **Genetics**

Novel genetic associations within the major histocompatibility complex (MHC) on chromosome 6 and select regions on chromosome 3 have been claimed for MCTD. The frequency of HLA-DR4 was increased in MCTD compared to healthy controls in world-wide population-based studies. In MCTD patients, no association was found with MHC haplotypes associated with SLE (HLA-DR3) or scleroderma (HLA-DR5)[55]. A significant association of U1RNP disease with HLA-DR4 and DR154–61 was noted, which was different from SLE or SSc[22]. Thus HLA evidence seems to favour MCTD being distinct from other ARDs, and as a disease that is T-cell dependent, given the HLA class II association. In contrast, other evidence implied HLA-DR4 seemed primarily to be related to U1-RNP antibody formation rather than disease expression. Patients with or without MCTD did not differ with respect to DR4 frequency. In these studies, some allotyped MCTD patients used SLE patients as controls, while others used healthy individuals. In certain studies, the MCTD patients exhibited a heterogeneous clinical picture with a few fulfilling the classification criteria for SLE or SSc. The classification criteria for recruiting MCTD patients also varied with Sharp's and Alarcon-Segovia's criteria being used in different studies. Thus the claims made about the association of MCTD with HLA-DR4 remain confused[25].

## **Treatment**

In the past decade there has been a lack of randomized trials. Management relies on extrapolation of guidelines for equivalent manifestations in SLE; SSc and RA.

Immunosuppressive therapy and steroids remain the therapeutic mainstay for MCTD. A retrospective study of 161 patients with MCTD showed that 58% required aggressive immunosuppression and only 3% achieved disease control using symptomatic therapies[11].

The use of anti-TNF $\alpha$  in MCTD with refractory erosive arthritis, was assessed in a prospective study of 280 patients. 44(17.5%) were diagnosed with erosive arthritis, all except two were treated with methotrexate plus anti-TNF $\alpha$  drug[13]. No adverse effects were reported. However, the experience with its use is limited and there are case reports that anti-TNF $\alpha$  was associated with development of an SLE like syndrome[62].

The treatment of PAH associated with MCTD is complex. General measures (oxygen supply, diuretic etc.) are usually insufficient and invariably more aggressive treatment with prostanoids, endothelin receptor antagonists, and phosphodiesterase-5 inhibitors are needed to improve pulmonary functional status[13].

Surgical options are the alternative for those patients who continue to progress despite aggressive therapy. They include procedures like atrial septostomy and lung transplantation[26].

## **Prognosis**

The initial notion of MCTD being a benign disease was abandoned after many studies showing that most patients did not have favourable outcome.

Lundberg and colleagues reported that 1/3 of patients with MCTD had a benign course, 1/3 had an aggressive course and 1/3 improved with immunosuppressive therapy but required it for several years[43]. However, the small number of long term outcome studies limits our knowledge in morbidity and mortality in MCTD patients.

The principal causes of morbidity in first years of MCTD were RP and esophageal hypomotility. Sclerodactyly, diffuse sclerosis, PAH and nervous system disease are major causes of morbidity in long-term MCTD patients[43].

In a recent study, 12 of the 147 (8.2%) patients died after followed up for a mean of 5.6 years. Three of the five patients having PH died from right ventricular failure. In the other 9 deceased patients, the causes of death were ILD (n=2); coronary heart disease (n=2), cancer (n=4) and unknown (n=1)[17].

A prospective study of 280 patients found a survival of 98% at 5 years; 96% at 10 years and 88% at 15 years[13]. Twenty two (7.8%) patients died. In 12 out of 22, the causes of death were directly related to MCTD manifestations. Ten patients died as a result of complications from MCTD.

The presence of cardiovascular events, esophageal hypomotility, serositis, secondary antiphospholipid syndrome and malignancy was significantly higher in the deceased patients. Also, the presence of ACL, anti- $\beta$ 2-GPI and AECA increased the risk of mortality[13].

## **Conclusion**

In Table 3 a comparison is shown of the original features said to have distinguished MCTD and the features currently claimed to constitute the condition. It shows that a major evolution in the concept has occurred.

Some evidence implying that MCTD does ‘exist’ as an entity has emerged from genetic studies, but the genetic association is actually not very consolidated. There are data showing that components of U1-snRNP are important for triggering immune responses and that anti-RNP has a central pathogenic role and may contribute to disease manifestations.

Nevertheless there is no evidence that >40% of patients given a diagnosis of SLE/ RA/ Sjogren’s change their clinical features to become recognized as another ARD analogous to MCTD. We postulate that different forms of antigen modification and epitope spreading together with B and T-cell interactions may have implications in disease evolution to other ARDs. Thus we continue to question whether the term MCTD is appropriately retained, preferring to use the term “undifferentiated autoimmune rheumatic disease”.

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**Table 1: New Findings in Clinical Features of MCTD in Recent 10 Years**

<b>Clinical Features of Mixed Connective Tissue Disease (MCTD)</b>		
<b>References</b>	<b>Observations</b>	<b>Comments</b>
<p><b><u>General</u></b></p> <p>- Cappelli S et al.[11] 2012</p>	<p><b><u>General</u></b></p> <p>-After a mean of 7.9 years, 57.9% patients still satisfied Kasukawa's criteria</p> <p>-Patients &gt;5 years of disease more frequently evolved into other ARDs.</p> <p>- Kasukawa's criteria were more sensitive than Alarcón-Segovia's and Sharp's criteria.</p> <p>-Anti-DNA was associated with evolution into SLE; esophageal abnormality and sclerodactyly with evolution into SSc.</p> <p>-Clinical features of MCTD may evolve from inflammatory phase to sclerotic phase.</p>	<p><b><u>General</u></b></p> <p>-Retrospective review of 161 MCTD patients at time of diagnosis and in 2008 for evolution.</p>
<p>- Hajas A et al.[13] 2013</p>	<p>-During FU new symptoms developed; but patients didn't show progression to other ARDs.</p>	<p>-Prospective FU of 280 MCTD patients diagnosed between 1979 &amp; 2011.</p>
<p>- Gunnarsson R et al.[12] 2011</p>	<p>-Prevalence of living adult MCTD patients in Norway = 3.8 (95% CI 3.2-4.4) per 100 000.</p> <p>-Incidence of adult-onset MCTD in Norway from 1996-2005 = 2.1 (95% CI 1.7-2.5) per million per year.</p> <p>-The point prevalence showed no statistical difference between the 3 classification criteria.</p>	<p>-Nationwide cross-sectional retrospective study of 147 patients investigating the prevalence and incidence of MCTD.</p>
<p><b><u>Pulmonary hypertension (PH):</u></b></p> <p>-Hajas A et al.[13] 2013</p>	<p><b><u>Pulmonary hypertension:</u></b></p> <p>-50 of 280 (17.9%) patients developed PAH 14.5±3.71 yrs after MCTD diagnosis.</p>	<p><b><u>Pulmonary hypertension:</u></b></p> <p>-Prospective FU of 280 MCTD patients diagnosed between 1979 &amp; 2011.</p>
<p>- Gunnarsson R et al.[17] 2013</p>	<p>-PH frequency was 3.4% in MCTD (5/147). 2 had isolated PAH &amp; 3 had PH associated with ILD.</p>	<p>-Nationwide multicentre cohort of 147 adult MCTD patients screened for PH with mean FU of 5.6 years.</p>
<p>- Vegh J et al.[18] 2006</p>	<p>-25 of 179 MCTD patients (14%) developed PAH. Interval between MCTD diagnosis and PAH was 8.4+/-4.1 yrs</p> <p>-5-year survival was lower in 25 MCTD-PAH than in 154 MCTD-non-PAH patients (73% vs 96%;P&lt;0.01).</p> <p>-AECA were more frequently present in MCTD-PAH than in MCTD-non-PAH patients (P&lt;0.001).</p> <p>-Levels of anti-U1RNP were higher in MCTD-PAH than in MCTD-non-PAH patients (56.2+/-</p>	<p>-FU of 179 MCTD patients &amp; compared those who developed PAH with those who didn't.</p>

	<p>19.4 U/ml vs 26.08+/-18.4 U/ml; P&lt;0.001).</p> <p>-Accumulated damage index was higher in MCTD-PAH than in MCTD-non-PAH patients (30.6+/-6.8 vs 27.6+/-4.4;P&lt;0.001).</p>	
- Jeon CH et al.[23] 2012	<p>-In 321 ARD-PAH patients, SLE accounted for 35.3%, SSc 28.3%, RA 7.8%, overlap syndrome 9.0%, and MCTD 5.9%.</p> <p>-No significant differences in hemodynamics, functional class &amp; diffusing capacity between the disease subgroups. Mean sPAP level was higher in SLE-PAH than in SSc-PAH or MCTD-PAH.</p> <p>-PAH in MCTD is more commonly associated with Raynaud's syndrome, abnormal capillaroscopy.</p>	-Cohort of 321 ARD patients who had WHO group I PAH diagnosed from 2008-2010.
- Hajas A et al.[13] 2013 - Szodoray P et al.[33] 2012 - Hasegawa E et al.[20] 2009	<p>-Anti-U1RNP, AECA &amp; ACL were associated with PAH. Anti-U1RNP increased surface expression of adhesion molecules on pulmonary artery endothelial cell <i>in vitro</i>.</p> <p>-Mean IgG anti-β2-GPI was higher among MCTD patients with PAH than in those without (34.2±46.8 vs 12.3±9.1, P=0.018).</p>	<p>-See above</p> <p>-Cluster analysis of 201 MCTD patients followed up longitudinally</p> <p>-Prospective analysis of antiphospholipid antibodies in 39 MCTD patients and 21 controls.</p>
<b><u>Interstitial lung disease (ILD):</u></b> - Hajas A et al.[13] 2013	<p><b><u>Interstitial lung disease (ILD):</u></b></p> <p>-132/280 (47.1%) MCTD patients had ILD.</p> <p>-Most frequent symptoms were polyarthritis (89.6%), Raynaud's (57.5%), ILD (47.1%) &amp; esophageal dysmotility (49.6%).</p>	<b><u>Interstitial lung disease (ILD):</u></b> -Prospective FU of 280 MCTD patients diagnosed in 1979-2011.
- Gunnarsson R et al.[29] 2012	<p>-52% of 126 MCTD patients had abnormal HRCT consistent with lung fibrosis (35%). Lung fibrosis was quantified as minor in 7%, moderate in 9% and severe in 19%.</p> <p>-Patients with severe lung fibrosis had poorer lung functions, shorter 6MWT and higher mean NYHA functional class. After a mean 4.2yrs, overall mortality was 7.9%. Mortality with normal HRCT was 3.3% vs 20.8% with severe fibrosis (p&lt;0.01)</p> <p>-24 patients with severe lung fibrosis were older, had shorter mean disease duration (6.4 years) than minor or moderate fibrosis (13.2 years)</p>	-Systemic examination of 126 MCTD patients for ILD.
- Szodoray P et al.[33] 2012	<p>-3 different sub-phenotypes of MCTD: 1<sup>st</sup> subgroup: AECA and antiphospholipid antibodies in association with PAH, Raynaud's, livedo reticularis and vascular thrombosis. 2<sup>nd</sup> subgroup: ILD, esophageal dysmotility and myositis. 3<sup>rd</sup> subgroup: higher prevalence of anti-CCP and erosive arthritis</p> <p>-In cluster 2, the incidences of ILD (98.7%), myositis (77.2%), and esophageal dysmotility</p>	-Longitudinal FU and cluster analysis of 201 MCTD patients.

	<p>(89.8%) were significantly greater than in cluster 1 and 3 (<math>p&lt;0.001</math>). No patients with ILD had positive anti-Jo1.</p> <p>-Immune complex formation and complement were associated with ILD and myositis.</p> <p>-Worst overall survival probability was in cluster 1 (vascular damage), followed by cluster 2 (ILD, myositis), the best survival was in cluster 3 (arthritis)</p>	
- Fagundes MN et al.[30] 2009	<p>-HRCT abnormalities were present in 39/50 patients. The presence of ILD was significantly higher among patients with esophageal dilatation (92% vs. 45%;<math>p&lt;0.01</math>) and esophageal motor dysfunction (90% vs. 35%;<math>p&lt;0.001</math>).</p> <p>-No statistically significant differences in ILD between patients with normal and abnormal acid reflux.</p> <p>-Correlation between diffusing capacity and ILD, but not between total lung capacity and ILD.</p>	-Evaluation of the relationship between esophageal dysfunction and ILD in 50 MCTD patients
- Hof D et al.[31] 2005	-Anti-U1RNP may have pathogenic role by interacting with lung tissue.	-Cross sectional & longitudinal study of 53 MCTD patients tested positive for anti-U1snRNP and anti-70K.
<b><u>Esophageal involvement</u></b>	<b><u>Esophageal involvement</u></b>	<b><u>Esophageal involvement</u></b>
- Fagundes MN et al.[30] 2009	<p>-Esophageal dilatation, gastroesophageal reflux, and esophageal motor impairment were very prevalent (present in 28/50, 18/36, and 30/36 patients, respectively).</p> <p>-36 patients underwent esophageal manometry: normal (n=6/36;16.7%); moderate dysfunction (n=7/36;19.4%); and severe dysfunction (n=23/36;63.9%).</p> <p>-36 patients had 24-hour pH measurements: 18 (50%) had distal abnormal acid reflux, 6 (16%) had proximal acid reflux. Severe esophageal dysfunction (aperistalsis) on manometry was not significantly related to proximal (<math>p=0.38</math>) or distal reflux (<math>p=0.16</math>)</p>	-Evaluation of relationship between esophageal dysfunction and ILD in 50 MCTD patients
- Caleiro MTC et al.[34] 2006	-Association between abnormalities in pulmonary function & esophageal dysfunction in radionucleotide scintigraphy showing significant delay in the clearance of the nucleotide.	-Assessment of esophageal motility disorder in 24 MCTD patients vs 11 healthy individuals.
<b><u>Arthritis</u></b>	<b><u>Arthritis</u></b>	<b><u>Arthritis</u></b>
- Mimura Y et al.[35] 2006	<p>-Serum IgM-, IgG- and IgA-RF in MCTD were significantly higher than controls. (mean<math>\pm</math>SD: 3.61<math>\pm</math>4.71 vs 0.75<math>\pm</math>0.54, <math>p&lt;0.05</math>; 1.35<math>\pm</math>0.96 vs 0.52<math>\pm</math>0.39, <math>p&lt;0.001</math>; and 2.26<math>\pm</math>2.83 vs 0.62<math>\pm</math>1.24, <math>p&lt;0.0005</math>).</p> <p>-Frequency of IgM-RF in MCTD patients was 48%, lower than in RA patients (77%). Frequency of IgG-RF in MCTD was 38%, compared to RA (25%). The frequency of IgA-RF in MCTD was 33%, lower than in RA (53%).</p>	-Longitudinal analysis of levels of RF isotypes in 21 MCTD patients and 14 controls.

<p><u>Cardiovascular involvement</u></p> <p>- Végh J et al.[24] 2006</p>	<p><u>Cardiovascular involvement</u></p> <p>-Diastolic dysfunction of left ventricle was detected in MCTD. Diastolic Ee/Aa velocity quotient was lower (<math>p&lt;0.01</math>), mean deceleration time was longer (<math>p&lt;0.001</math>) than control group. Tei index demonstrated damage of the global ventricle function.</p> <p>-Tei index of the right ventricle indicated global failure of the right ventricle in cases of MCTD with PAH (Tei index <math>0.36\pm0.07</math> in MCTD with PAH vs. <math>0.28\pm0.04</math> without, <math>p&lt;0.001</math>). Right ventricle function of MCTD patients without PAH was no different from controls.</p>	<p><u>Cardiovascular involvement</u></p> <p>-Cross-sectional study of right and left ventricle functions in 51 MCTD patients &amp; 30 healthy controls.</p>
<p>- Soltesz P et al.[42] 2010</p>	<p>-Flow-mediated dilation (FMD) was significantly impaired in MCTD vs controls (%FMD: <math>4.7\pm4.2\%</math> vs. <math>8.7\pm5.0\%</math>; <math>P&lt;0.001</math>).</p> <p>-FMD negatively correlated with disease duration, apolipoprotein A1 levels, paraoxonase-1 activity, and systolic blood pressure in MCTD.</p> <p>-% FMD was significantly lower in MCTD with cardiovascular diseases (CVD), than in those without (%FMD: <math>3.5\pm2.9</math> vs. <math>5.8\pm4.8</math>, <math>P&lt;0.0002</math>).</p> <p>-% nitrate-mediated dilation (NMD) did not differ between MCTD vs controls (<math>14.3\pm6.6\%</math> vs. <math>17.1\pm6.7\%</math>; <math>P=0.073</math>), and patients with or without CVDs.</p> <p>-Mean carotid intima-media thickness was higher in MCTD than in controls (<math>0.64\pm0.13</math> mm vs. <math>0.53\pm0.14</math> mm; <math>P&lt;0.001</math>).</p> <p>-Anti-U1RNP, AECA and ACL were significantly higher in MCTD and differed between MCTD with and without CVD.</p> <p>-Endothelial cell markers such as soluble thrombomodulin (<math>12.2\pm8.1</math> ng/ml vs. <math>3.2\pm1.3</math> ng/ml; <math>P&lt;0.001</math>) &amp; vWFAg (<math>224.1\pm115\%</math> vs. <math>89.4\pm27.1\%</math>; <math>P&lt;0.001</math>) were the highest in MCTD with CVD.</p>	<p>-FU study of 50 MCTD patients and 38 controls to investigate association between cardiovascular risk factors &amp; endothelial dysfunction.</p>
<p><u>Renal involvement</u></p> <p>- Pope JE.[36] 2005</p>	<p><u>Renal involvement</u></p> <p>-40% of MCTD patients had renal involvement including glomerulonephritis, nephrotic syndrome, scleroderma renal crisis</p> <p>-MCTD patients with renal disease had more systemic manifestations than those without. 72% of the nephropathy patients had resolution or improvement with steroid.</p> <p>-Electron microscopy revealed immune complex deposition in the glomeruli.</p>	<p><u>Renal involvement</u></p> <p>-Review of other manifestations in MCTD.</p>
<p>- Lundberg IE.[43] 2005</p>	<p>-Renal involvement occurred with frequency between 5% and 36%.</p>	<p>-Review of MCTD.</p>

<p>- Greidinger EL et al.[46] 2006</p>	<p>-Immunization of mice with RNP antigen induced anti-RNP and MCTD manifestations, (ILD but not renal disease); but for mice deficient in Toll-like receptor 3, RNP antigen exposure induced SLE-like nephritis</p>	<p>-Case series and immunization study of murine models</p>
<p><u>RP &amp; capillaroscopy</u></p> <p>- Pavlov-Dolijanovic S et al.[48] 2012</p>	<p><u>Raynaud's phenomenon (RP) &amp; capillaroscopy</u></p> <p>-At the end of FU, 1,660 (54,8%) patients still had primary RP, 246 (8,1%) had suspected secondary RP, and 1,123 (37,1%) developed ARDs (363 UCTD, 263 SSc, 143 SLE, 24 MCTD)</p> <p>-Mean time interval from RP to diag of ARD =6.2 yrs</p> <p>-Scleroderma (SD) pattern in capillaroscopy were significantly associated with development of SSc(<math>P=0.0001</math>), dermatomyositis(<math>P=0.0004</math>), overlap syndrome with signs of SSc(<math>P=0.0001</math>), and MCTD(<math>P=0.007</math>).</p> <p>-Half of MCTD patients had normal capillaries, other half had nonspecific or SD type capillary changes. No patients with SSc or dermatomyositis showed normal capillaries.</p> <p>-SD pattern was present in 246/263 (94%) patients with SSc, in 5/7 (71%) with dermatomyositis, in 28/61 (46%) with overlap syndrome (all patients with SD pattern had signs of SSc), and in 9/24 (37%) with MCTD.</p> <p>-All patients with primary RP and most patients with RA (88%), UCTD (82%), Sjögren's syndrome (82%), SLE (75% )had normal capillaries.</p> <p>-SD type nailfold capillary changes had better negative than positive predictive value for development of ARD. SD changes can predict future development of ARD. OR ratio for development of SSc in those with SD type capillary abnormality was 163, dermatomyositis (OR 13.67), overlap syndrome with signs of SSc (OR 4.83), and MCTD (OR 3.30).</p>	<p><u>RP &amp; capillaroscopy</u></p> <p>-Prospective outcome study of 3,029 patients with diagnosis of primary RP FU for a mean of 4.8 yrs.</p>
<p>- Meli M et al.[49] 2006</p>	<p>-Presence of SD type capillary changes with RP is indicative of future development of ARD, even in the absence of other disease symptoms.</p>	<p>-Capillaroscopy alone or combined with fluorescence videomicroscopy study of 1024 patients with RP.</p>
<p>- Hajas A et al.[13] 2013</p>	<p>-Capillaroscopy showed gradual progression of vascular abnormalities. "Scleroderma pattern" at the time of diagnosis of MCTD was noted in 31.4% of patients, after 13.1 years' FU there was a weak progression (40.3%; <math>p&lt;0.03</math>). 99/258 (38.3%) living patients vs. 14/22 (63.3%) deceased patients had SD-pattern (<math>p&lt;0.02</math>).</p>	<p>-Prospective FU of 280 MCTD patients diagnosed in 1979-2011 in Hungarian population.</p>

<p>- de Holanda Mafaldo Diogenes A et al.[50] 2007</p>	<p>-SD-pattern was observed in 41 patients at entry (65%) and in 45 at previous capillaroscopy (71.5%), P=0.20. 10 patients (16%) had changed capillaroscopy. Disease duration, number and frequency of organ involvement were similar in patients with and without SD-pattern.</p> <p>-Reduced capillary density was more frequently observed in patients taking immunosuppressants than those without medication (66.7 vs 33.3%, P=0.001).</p> <p>-Nailfold capillaroscopy in MCTD is a dynamic process. Analysis of SD-pattern parameter may be good indicator of disease severity.</p>	<p>-Nailfold capillaroscopy was performed 5 years preceding inclusion &amp; at least 18 months after 1<sup>st</sup> study evaluation of 63 MCTD patients.</p>
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**Table 2: New Findings in Immunology & Genetic Aspects of MCTD in Recent 10 Years**

Serology & Immunology of Mixed Connective Tissue Disease (MCTD)		
References	Observations	Comments
<p>- Cappelli S et al.[11] 2012</p> <p>- Mesa A et al.[53] 2013</p> <p>-Salmhofer W et al.[54] 2007</p>	<p>-Anti-U1snRNP are not confined to MCTD. 20%-40% of SLE patients, 2-14% with SSc, and 6%-9% with myositis are anti-U1snRNP positive. RA patients are generally negative.</p> <p>-IgM anti-U1snRNP titers were significantly higher in SLE than in MCTD or healthy individuals(<math>p \leq 0.05</math>). IgG anti-U1snRNP were significantly higher both in SLE and MCTD than in healthy group; but IgG reactivity does not differ between two ARDs.</p> <p>-Combining IgM anti-U1C and anti-U1A is capable of classifying SLE and MCTD patients with an accuracy of 71.3%.</p> <p>-MCTD showed higher levels of anti-RNP-70kD than SLE. Levels of anti-SmBB' or anti-SmD were not significantly different between SLE and MCTD. High anti-RNP-70kD is useful for diagnosing MCTD.</p>	<p>-Retrospective study of 161 MCTD patients at time of diagnosis and in 2008 for evolution.</p> <p>-Study of IgM- and IgG-mediated responses against U1snRNP subunits in 81 SLE, 41 MCTD patients &amp; 31 healthy individuals.</p> <p>-Cross sectional comparative analysis of immunological markers in 51 SLE and 10 MCTD patients and 59 controls.</p>
<p>- Hajas A et al.[56] 2013</p>	<p>-Anti-U1RNP levels decreased after treatment (<math>p &lt; 0.0467</math>). The most effective drug was cyclophosphamide. Methotrexate and methylprednisolone decreased antibody levels, but not significantly.</p> <p>-Proportion of transitional B cells, naive B cells &amp; double negative (DN) B lymphocytes was higher in MCTD than in controls. The memory B cells level showed close correlation with disease activity. The number of plasma cells was increased; their number was associated with anti-U1RNP levels. Cyclophosphamide, methotrexate, and corticosteroid decreased the number of DN and CD27 high B cells.</p>	<p>-Study of B cell subsets in 46 MCTD vs 20 healthy individuals.</p>
<p>- Maldonado ME et al.[60] 2008</p> <p>- Baráth S et al.[61] 2006</p>	<p>-In MCTD, CD4+CD25high cells decreased with increasing levels of disease activity measured by SLEDAI (<math>r^2=0.11</math>).</p> <p>-The % and absolute number of CD4+CD25+high Treg cells were lower in MCTD patients than in healthy controls (<math>p &lt; 0.04</math>), and were further decreased in active MCTD compared with inactive stage (<math>p &lt; 0.01</math>). There's an increase in % and absolute number of CD4+IL-10+Treg cells in MCTD patients compared to controls (<math>p &lt; 0.02</math>). Their % was higher in active than in inactive stage (<math>p &lt; 0.005</math>).</p>	<p>-Cross-sectional study of 21 MCTD &amp; 39 SLE patients from Miami and Missouri Caucasian cohorts.</p> <p>-Cross sectional study of % and absolute number of CD4+ regulatory T-cells (Treg) in 48 MCTD patients.</p>
<p>- Nagai K et al.[57] 2012</p>	<p>-MCTD and SLE are characterized by increase of low phosphorylated U1-68k. Post-translational modifications on autoantigens are involved in the production of autoantibodies.</p>	<p>-Study of antigen modification on U1snRNP 68k subunit, a major antigen of anti-RNP in 4 MCTD, 4 SLE, 4 RA patients and 3 healthy individuals.</p>

- Hof D et al.[31] 2005	-29/53 (54%) MCTD sera preferentially recognize apoptotic form of 70K over intact 70K. Antibodies directed to apoptosis-specific epitope on 70K are more specifically associated with MCTD.	-Cross sectional & longitudinal study of 53 MCTD patients positive for anti-U1snRNP and anti-70K.
- Somarelli J et al.[59] 2011	-From epitope mapping, most of the U1snRNP antigenic sites reside on the surface of the complex.  -Most of the peptides from U1snRNP are mildly to moderately antigenic. The 2 most antigenic peptides are from U1C protein while the least antigenic is from U1A protein.  - U1-70K is the best at segregating SLE from healthy samples and the second best at separating MCTD from healthy samples (p=0.0001).  - Peptides 1 and 13 from U1A protein, are the 2 best predictors of SLE vs. MCTD (p=0.167 and p=0.206). Though they're not capable of significantly segregating SLE from MCTD.	-Cohort study of 15 peptides on U1snRNP structure in 68 SLE, 29 MCTD patients and 26 healthy individuals.
- Hasegawa E et al.[20] 2009	-Medium to high titres of ACL & anti-β2-GPI were found in 4/39 (10.2%) MCTD patients. High to moderate titres of anti-β2-GPI & APS were rare in MCTD.	-Prospective analysis of antiphospholipid antibodies in 39 MCTD patients and 21 controls.

### Genetic Aspects of MCTD

References	Observations	Comments
- Hoffman RW et al.[55] 2008	-Novel genetic associations within the MHC on chromosome 6 and select regions on chromosome 3 were identified for MCTD.  -Frequency of HLA-DR4 is increased in MCTD compared to healthy controls. In MCTD, no association was found with MHC haplotypes associated with SLE (HLA-DR3) or SSc (HLA-DR5).	-Review of immune pathogenesis of MCTD.
- Aringer M et al.[22] 2007	-Significant association of U1RNP-associated disease with HLA-DR4 and DR154–61, which is different from SLE or SSc. HLA class II molecules might influence clinical phenotype as carrying HLA-DR3 leads to higher risk for lung fibrosis.	-Review of MCTD.
- Aringer M et al.[25] 2005	-HLA evidence in favour of MCTD as distinct disease from SLE, SSc, or PM/DM, and as disease that is T-cell dependent, given the HLA class II association.	-Review of MCTD.