









## CASE BASED REVIEW

## Mixed Connective Tissue Disease (MCTD): 6 Case Based Review

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### Abstract

Mixed Connective Tissue Disease is an autoimmune clinical entity with characteristics of overlap syndrome including: Systemic Lupus Erythematosus, Rheumatoid Arthritis, Systemic Sclerosis, Polymyositis and Dermatomyositis. There is an active controversy as to whether it is a variant of Lupus or it is a different entity. The objective of these case reviews is to describe clinical-serological characteristics of 6 patients from AGAR clinic (Guatemalan Association against Rheumatic Diseases) that meet the criteria for this syndrome according to Alarcón-Segovia and Villareal criteria. Although the controversy persists, after an extensive review of the literature we consider that Mixed Connective Tissue Disease is not a variant of lupus, but a different entity with a different course and prognosis.

### Keywords

Alarcón-Segovia and Villareal, Anti-RNP, Mixed connective tissue disease, Gordon sharp

### List of Abbreviations

ACEI: Angiotensin Converting Enzyme Inhibitors; ACPA: Anti-Cyclic Citrullinated Peptide Antibody; AGAR: Guatemalan Association against Rheumatic Diseases; Anti-RNP: Ribonucleoprotein Antibody; ARA II: Angiotensin II Receptor Antagonist; CK: Creatine Kinase; CRP: C-Reactive Protein; DM: Dermatomyositis; DMARDS: Disease Modifying Antirheumatic Drugs; ENA: Extractable Nuclear Antigen; ESR: Erythrocyte Sedimentation Rate; FANA: Fluorescence Anti-Nuclear Antibody; HLA: Human Leukocyte Antigen; ILD: Interstitial Lung Disease; LDH: Lactate Dehydrogenase; MCTD: Mixed Connective Tissue Disease; NSAIDs: Non-steroidal Anti-Inflammatory Drugs; PAH: Pulmonary Arterial Hypertension; PM: Polymyositis; RA: Rheumatoid Arthritis;

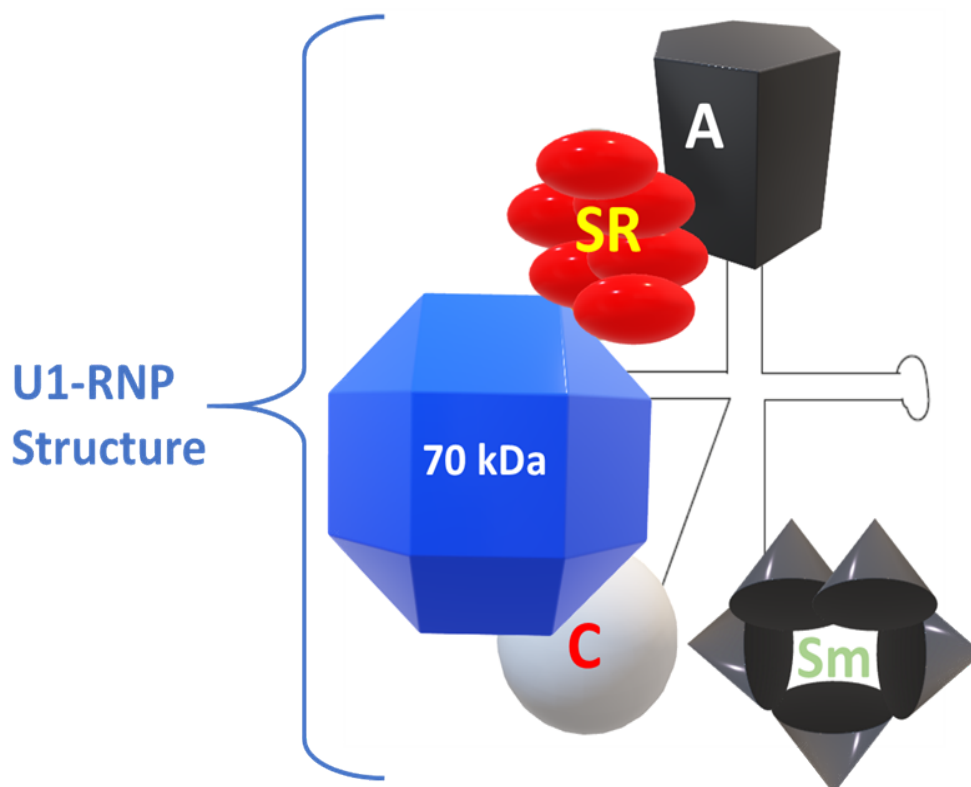
RF: Rheumatoid Factor; RP: Raynaud's Phenomenon; SLE: Systemic Lupus Erythematosus; SS: Sjögren's Syndrome; SSc: Systemic Sclerosis; U1sn-RNP: Uridyl Rich Small Nuclear Ribonucleoprotein

### Introduction

Mixed Connective Tissue Disease (MCTD) was first described in 1972 by Gordon Sharp, et al., conducting a study in 25 patients who had clinical characteristics of several rheumatic diseases: Systemic Lupus Erythematosus (SLE), Systemic sclerosis (SSc) and Polymyositis (PM) [1,2]. Extractable Nuclear Antigen (ENA) now called Ribonucleoproteins (RNP) are a group of cytoplasmic and nuclear antigens of which Anti-U1 RNP (Ribonucleoprotein) is the characteristic of MCTD. The common feature of these patients is that they possessed Anti-U1 RNP in very high titres above 1:1000. All being Anti-Sm negative, and unlike the SLE patients where Anti-RNP positive but with lower values [2,3]. Another finding was that there was no renal involvement. Antinuclear antibodies (FANA) had speckled pattern predominance unlike patients with SLE [1]. The epidemiology shows a clear female predominance 5:1 [4]. The incidence of cases varies in each region from 0.2-0.8 cases per 100,000 people [5-7].

### Genetics

In patients with U1-SnRNP positive it has been found a discrete genetic association with HLA-DR4/Gm (1,3;



**Figure 1:** The structure of U1-RNP. Modified from Greidinger EL, Hoffman RW [11].

5,21) [8], also an increase in anti-U1-snRNP autoantibodies of IgG type [3]. In a Mexican population, an association of HLA-A2 and HLA-B35 was found to be the most frequent MHC class I alleles in patients with MCTD, although they were not statistically significant, the most frequent DQ allele in the patients were DQ1, suggesting a particular genetic association for MCTD [6,9].

### Anti-U1 snRNP

Nucleosomes are compact building blocks of chromatin and consist of an octamer of two copies of histones H2A, H2B, H3 and H4, wrapped around approximately 146 base pairs of DNA [10]. U1-RNP is the target of autoantibodies that meet the classification criteria for anti-RNP antibodies in MCTD. This complex of nuclear macromolecules plays an essential role in splicing pre-mRNA in mRNA. U1-RNP is composed of an RNA skeleton, U1-RNA, three proteins that are highly specific for U1-RNP (proteins U1-A, U1-C and U1-70kD), plus a series of additional proteins. This is common to multiple macromolecules that splice U-RNP and RNA (Figure 1) [11].

### Spliceosome

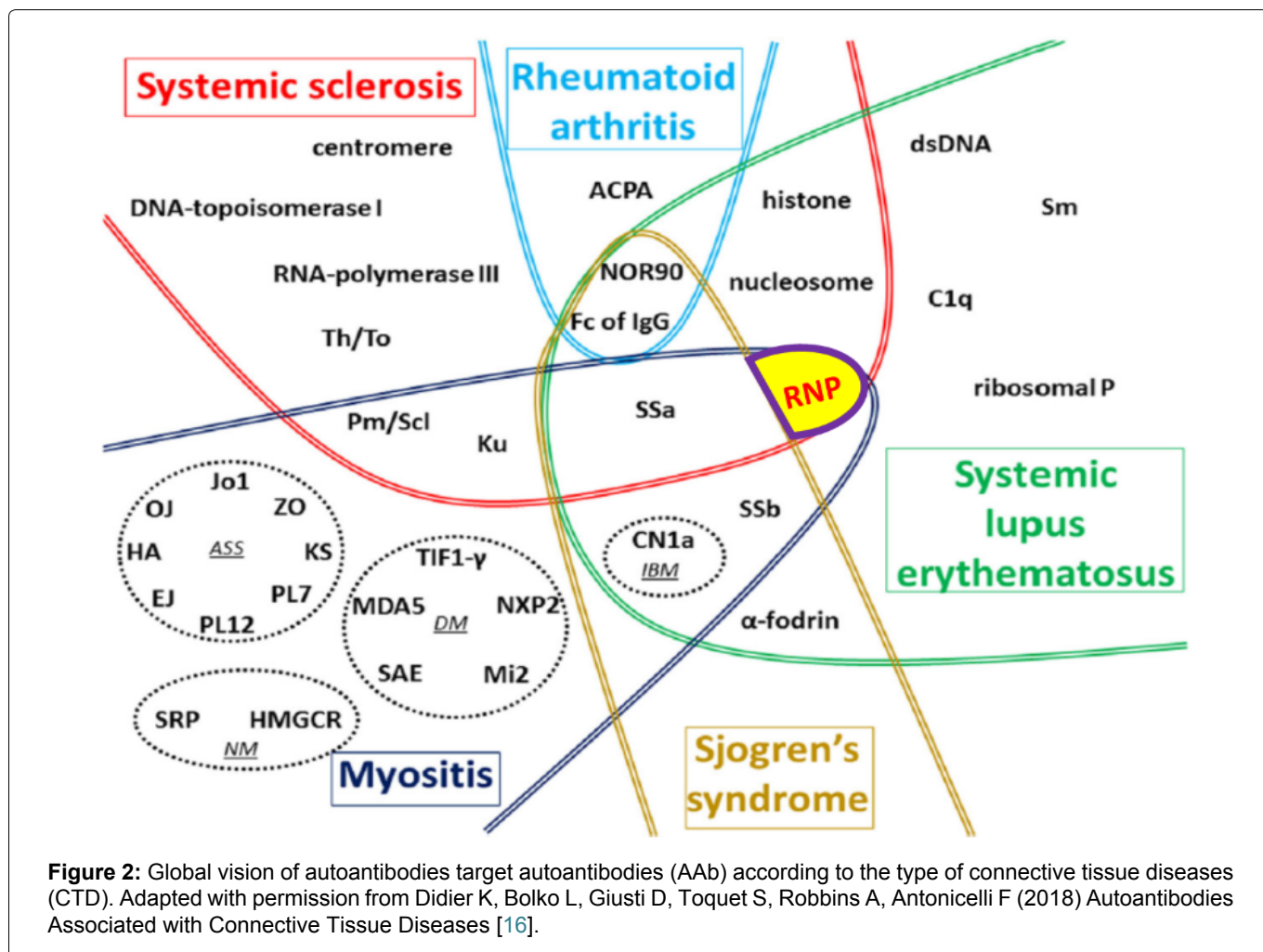
Spliceosome is a large complex consisting of proteins and RNA that catalyzes the removal of introns from pre-messenger RNA (pre-mRNA) and splicing exons coding to produce mature mRNA. U1, U2, U5 and U4/U6 are small nuclear ribonucleoproteic particles (snRNP). These particles are assembled to the pre-mRNA substrate together with another type of protein to form the spli-

ceosome [12,13]. Autoimmunity to specific components of the spliceosome is the immunological characteristic of MCTD.

The snRNPs contain small RNA species that vary in size from 80 to 350 nucleotides that are composed of proteins. These RNAs contain a high uridine content and, therefore, are called U-RNA; five different U-RNAs were defined with the basis of immunoprecipitation (U1, U2, U4, U5 and U6). Autoantibodies to these complexes primarily target the protein components. Anti-Sm antibodies precipitate five proteins with molecular weights of 28,000 (B'B), 16,000 (D), 13,000 (E), 12,000 (F) and 11,000 (G); five of these polypeptides are common to RNAs U1, U2, U4, U5 and U6. Anti-RNP antibodies precipitate three proteins with molecular weights of 68,000 (70K), 33,000 (A') and 22,000 (C); these polypeptides are associated only with U1 RNA. The clinical features considered distinctive of MCTD are associated with the presence of antibodies with specificity of 70 kD, with an immunodominant epitope that encompasses amino acid residue 125 flanked by important conformational residues at positions 119 to 126 [14].

### Epitope spreading

Epitope spreading is the process that occurs when a patient who previously had positivity to a given antibody recognizes another antibody as positive during follow-up; if the new autoantibody recognizes an epitope related to another epitope for which the patient previously had autoantibodies [12,15]. This phenomenon can occur intra-



molecularly, when the propagation of reactivity occurs within a single autoantigen with multiple epitopes in the same protein, or intermolecularly, when the reactivity extends to other polypeptides or proteins within a macromolecular complex. This phenomenon is highly prevalent in patients with MCTD [15-17] (Figure 2).

## Diagnostic Criteria

The diagnosis of MCTD is often a challenge for clinicians, due to the overlap and the number of initial symptoms, with variable courses [18,19]. For this reason, several diagnostic criteria have been proposed, one of the most accepted is Alarcón-Segovia, with a sensitivity and specificity of 62.5% and 86.2%, respectively [20,21] and in this case, we have selected them for their simplicity and easy application.

Several authors do not consider the definition of MCTD a distinctive clinical entity, including Reichlin, et al. [22], LeRoy, et al. [23] and Nimelstein, et al. [24], since some the clinical characteristics of MCTD do not differ from those of SLE patients with positive anti-snRNP antibodies and many MCTD patients progress to SLE or SSc during their evolution [25-27]. However, in Susana Capelli's study [28]; One hundred and sixty-one patients with MCTD were retrospectively evaluated in diagnosis and evolution, they were followed over 7.9 years, concluding that more than half of the patients re-

mained with MCTD [28].

At AGAR clinic (Guatemalan Association against Rheumatic Diseases) in Guatemala City that specializes in the care of rheumatic conditions; we reviewed our cases of MCTD.

## Objective

The objective of this study is to describe the clinical-serological characteristics of patients with MCTD that met criteria of Alarcón-Segovia and Villareal [20].

## Methodology

Case based review registry of 6 patients with MCTD. A review of the cases of the AGAR clinic; 6 cases met criteria for MCTD, over a 10-year period, from 2009 to 2019.

## Inclusion and Exclusion Criteria

The patients included in this review were required to meet the criteria of Alarcón-Segovia listed below:

1. Serological criteria: Positive antibodies to U1 RNP antibodies in a titer  $\geq 1:1600$  dilution
2. Clinical criteria:
  - a) Swelling of the hands (puffy hands)
  - b) Synovitis

- c) Myositis
- d) Raynaud's phenomenon
- e) Sclerodactyly with or without proximal scleroderma

Serological criteria and  $\geq 3$  of the clinical criteria (co-existing edema of the hands, Raynaud's phenomenon and sclerodactyly require additional compliance with criteria 2 or 3) [20].

#### Exclusion criteria:

1. Patients who do not fulfill clinical criteria for MCTD.
2. No confirmatory serological tests.
3. No appropriate follow-up.

## Case Series Results

### Epidemiological characteristics

The patients studied correspond to patients from various areas of the country, of which 50% came from the urban area and the other 50% from the rural area. The ages at the time were in a range between 25 and 71 years old with a mean of 45, the majority being mestizo population and only 1 case of indigenous origin. All the patients studied are female. The time of evolution of clinical features varies from 1 to 10 years since the appearance of the first clinical manifestations; being the time of 1 to 5 years for three cases and the rest of 5 to 10 years of evolution.

Regarding the clinical manifestations for diagnosis of MCTD, using the criteria of Alarcón Segovia and Villareal; It was found that 5 of the 6 patients presented the defining criteria: Hand swelling, synovitis and Raynaud. Only one of the cases presented myositis symptoms. Half of the cases presented sclerodactyly, however; these cases were associated with Raynaud's phenomenon as well. All cases presented positivity for Anti RNP whose titers exceed 1:1600 dilution (Table 1).

Several clinical manifestations were presented that were not limited solely to the criteria of Alarcón-Segovia and Villareal. Therefore, a broader clinical feature stands out from initial manifestations to the present, with signs and symptoms referred to different biophys-

**Table 1:** Alarcón-Segovia and Villareal criteria applied to our clinical cases.

	1	2	3	4	5	6
1. Puffy hands	+		+	+	+	+
2. Sinovitis	+		+	+	+	+
3. Myositis		+				
4. Raynaud	+	+	+	+	+	+
5. Sclerodactyly	+	+	+			
6. AntiRNP $\geq$ 1:1600	+	+	+	+	+	+

**Note:** +: Present in the patient; -: Absent in the patient'.

ical spheres or domains. It is observed in all cases the presence of fever either referred or verified in the clinical history, as well as asthenia in all cases. Signs/symptoms such as: Alopecia, xerophthalmia, weight loss, skin rash and anasarca occurred in 5 of the 6 patients. Symptoms such as anorexia, xerostomia, headache, dyspnea, and photosensitivity in 3 of the 6 cases.

We observed that all the patients had at some time FANA positive 1:80, with a speckled pattern. Anti-DNA antibody positivity was found in three patients, none were positive for Anti-Sm. Rheumatoid Factor (FR) was positive in all patients studied, in high titres; however, none were positive for Anti-cyclic citrullinated peptide Antibody (ACPA). Acute phase reactants were found elevated in only two patients. No patients had hypocomplementemia. There was no positivity for Anti-Scl70 or Anti-Jo. Total creatine kinase was raised only in one case, which was the one that debuted with myositis, although there was no elevation of Aldolase or LDH. An-

**Table 2:** Serological studies performed and most common laboratory findings.

	1	2	3	4	5	6
FANA 1:80	+	+	+	+	+	+
Anti-DNA	178	28	-	-	21	-
Anti-Sm	-	-	-	-	-	-
RF	160	180	256	256	143	96
CRP	24	-	48	-	-	-
C3	-	-	-	-	-	-
C4	-	-	-	-	-	-
ESR	75	-	-	-	-	-
ACPA	-	-	-	-	-	-
Anti Scl70	-	-	-	-	-	-
Anti-Jo	-	-	-	-	-	-
CK Total	-	890	-	-	-	-
Aldolasa	-	-	-	-	-	-
LDH	-	-	-	-	-	-
Anti RNP	1: 1600	1: 1600	1: 1600	1: 1600	1: 1600	1: 1600
Anti SSA/Ro	67	-	-	200	-	-
Anti-SSB/La	-	-	-	-	-	-
Leukopenia	-	+			+	+
Anemia	+	-	-	-	-	
Neutropenia	-	-	-	-	-	+
Lymphopenia	+	-	-	-	-	-
Thrombocytopenia		+				
Proteinuria $\geq$ 500 mg/24 hrs	+	-	-	-	-	-
Hematuria	-	+	-	-	-	-

**Note:** FANA positive 1:80 with Hep-2000 cell substrate'.

+: Present in the patient; -: Absent in the patient'.

The values presented in the various antibodies are given in IU/ml with the exception of Anti RNP shown in dilutions however it corresponds to the values per ELISA given in IU/ml.

ti-RNP was positive in all cases with titres greater than 1:1600. Two patients with positivity for Anti-SSA/Ro were found, however, there was no positivity for Anti-SSB/La (Table 2).

Common laboratory alterations found were hematology and urinalysis in 4 patients, 2 had normal routine clinical laboratory studies. The findings correspond to 3 patients with leukopenia. Clinical case number 1 presented: Anemia, lymphopenia and significant moderate proteinuria. Case number 2 presented thrombocytopenia and macroscopic hematuria; and case number 6 presented neutropenia as the only initial laboratory finding. Clinical case 3 and 4 did not show initial laboratory alterations (Table 2).

## Treatment

Treatment consisted of a combination of a DMARDS, NSAIDs, steroids in some cases: Calcium-antagonists, ARA II, ACEI, phosphodiesterase 5 inhibitors, prokinetic and proton pump inhibitors.

DMARDS were used for arthritis control when there was a predominant clinical SLE like features. Mycophenolate mofetil was used in case 2 because it was the most complicated case due to cardiopulmonary involvement, with the need to use in addition phosphodiesterase 5 inhibitor. Drugs were used for specific clinical situations such as Raynaud's phenomenon, with use of calcium antagonists. In addition, proton pump inhibitors for control of gastroesophageal reflux.

## Complications

Case 2 presents radiological findings, later confirmed with high-resolution computed axial tomography, showed: Reticular opacities and honeycomb pattern of subpleural distribution. In addition, the patient presented echocardiographic findings of tricuspid valve flow regurgitation and increased systolic pressure of the right ventricle as indirect measures of PAH. Case number 6 presented three digital ulcers and one on the right foot.

## Discussion

The clinical cases presented came from both rural and urban areas of Guatemala. All were women, who at the time of diagnosis were between the ages of 25 and 71 with a mean of 45, which is consistent with the review of epidemiological studies, as well as the predominance of the female sex [7,29]. The description of MCTD used in clinical cases was based on the criteria of Alarcón-Segovia and Villareal, patients met both clinical and serological criteria, with Anti-RNP values in high titers without the presence of Anti-Sm.

Regarding the initial clinical presentation, we observed heterogeneous signs and symptoms among the patients studied, although the literature generally refers to the most frequent initial symptom: Raynaud's phenomenon, swelling of the hands and arthralgia; In our

review we found constitutional symptoms in all cases such as fever and asthenia, followed by: Edema, weight loss, alopecia and sicca symptoms.

All patients had Anti-RNP values greater than 1:1600, without the presence of Anti-Sm. It is important to emphasize that three patients had positive Anti-DNA values, but one of them had borderline values, there was no positivity for Anti-Sm that would be considered more defined as SLE [1,18]. The rest of the studies are more heterogeneous, finding positive RF in all patients in high titres [30], in addition to positivity in two cases for anti SSA/Ro, as well as elevation of total CK in a case that was the only one with myositis. These findings are compatible with the positivity of other antibodies to MCTD, we found no antiphospholipid antibodies or ANCA, as described in the literature in 15-20% [18,31,32].

In Sharp's study, a good response to the use of steroids was observed with a gradual reduction and symptom control scheme, and few cases required the use of more than 10 mg/day of prednisone [2,33]. In our cases at AGAR clinic that required steroids all used less than 10 mg/day of prednisone or equivalent.

In relation to long-term complications, only one patient presented PAH data in addition to ILD, which are classified as the most serious complications of MCTD [34-36]. Although all had Raynaud's phenomenon since the beginning of the disease, only one of them was complicated by digital ulcers in hands and feet that had been described previously [37]. However, each patient should be individualized according to the recommendations of most authors.

## Conclusions

MCTD is a well-defined entity, although for many authors it is a preclinical stage for other connective diseases such as SLE, SSc or PM/DM, in our cases the evolution time has even been up to 10 years without modifying the initial diagnosis. The criteria used for the case definition were those of Alarcón-Segovia and Villareal, which are the most simple and specific for diagnosis. The serological profile of the patients is quite heterogeneous, in addition to finding varied constitutional symptoms at the time of diagnosis, as well as hematological alterations. The complications of the disorder have already been described in literature, although only one patient presented complications PAH and ILD, however, it is important to note that both comorbidities worsen patient prognosis. Each case must be individualized, however, because there are no management protocols; management guidelines would be given by the clinical manifestations and the individual profile. Finally it is worth mentioning that in Guatemala there are no previous studies of MCTD.

## Conflicts of Interest

There were no conflicts of interest from authors.

## References

1. Sharp GC, Irvin WS, LaRoque RL, Velez C, Daly V, et al. (1971) Association of autoantibodies to different nuclear antigens with clinical patterns of rheumatic disease and responsiveness to therapy. *J Clin Invest* 50: 350-359.
2. Sharp GC, Irvin WS, Tan EM, Gould RG, Holman HR (1972) Mixed connective tissue disease-an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med* 52: 148-159.
3. Hoffman RW, Sharp GC, Deutscher SL (1995) Analysis of anti-u1 RNA antibodies in patients with connective tissue disease. Association with HLA and clinical manifestations of disease. *Arthritis Rheum* 38: 1837-1844.
4. Ungprasert P, Crowson CS, Chowdhary VR, Ernste FC, Moder KG, et al. (2016) Epidemiology of mixed connective tissue disease, 1985-2014: A population-based study. *Arthritis Care Res (Hoboken)* 68: 1843-1848.
5. Kaipainen-Seppanen O, Aho K (1996) Incidence of rare systemic rheumatic and connective tissue diseases in Finland. *J Intern Med* 240: 81-84.
6. Weckmann AL, Granados J, Cardiel MH, Andrade F, Vargas-Alarcón G, et al. (1999) Immunogenetics of mixed connective tissue disease in a Mexican Mestizo population. *Clin Exp Rheumatol* 17: 91-94.
7. Gunnarsson R, Molberg O, Gilboe IM, Gran JT, PAHNOR1 Study Group (2011) The prevalence and incidence of mixed connective tissue disease: A national multicentre survey of Norwegian patients. *Ann Rheum Dis* 70: 1047-1051.
8. E Genth, H Zarnowski, R Mierau, D Wohltmann, PW Hartl (1987) HLA-DR4 and Gm (1,3;5,21) are associated with UI-nRNP antibody positive connective tissue disease. *Ann Rheum Dis* 46: 189-196.
9. Maddison PJ (2000) Mixed connective tissue disease: Overlap syndromes. *Bailliere's Best Pract Res Clin Rheumatol* 14: 111-124.
10. Radic M, Marion T, Monestier M (2004) Nucleosomes are exposed at the cell surface in apoptosis. *J Immunol* 172: 6692-6700.
11. Greidinger EL, Hoffman RW (2005) Autoantibodies in the pathogenesis of mixed connective tissue disease. *Rheum Dis Clin North Am* 31: 437-450.
12. Monneaux F, Muller S (2002) Epitope spreading in systemic lupus erythematosus: Identification of triggering peptide sequences. *Arthritis Rheum* 46: 1430-1438.
13. Rivett AJ, Hearn AR (2004) Proteasome function in antigen presentation: Immunoproteasome complexes, peptide production, and interactions with viral proteins. *Curr Protein Pept Sci* 5: 153-161.
14. Welin Henriksson E, Wahren-Herlenius M, Lundberg I, Mellquist E, Pettersson I (1999) Key residues revealed in a major conformational epitope of the U1-70K protein. *Proc Natl Acad Sci USA* 96: 14487-14492.
15. Escolà-Vergé L, Pinal-Fernandez I, Fernandez-Codina A, Callejas-Moraga EL, Espinosa J, et al. (2017) Mixed connective tissue disease and epitope spreading: An historical cohort study. *J Clin Rheumatol* 23: 155-159.
16. Didier K, Bolko L, Giusti D, Toquet S, Robbins A, et al. (2018) Autoantibodies associated with connective tissue diseases: What meaning for clinicians? *Front Immunol* 9: 541.
17. Dima A, Jurcut C, Baicus C (2018) The impact of anti-U1-RNP positivity: Systemic lupus erythematosus versus mixed connective tissue disease. *Rheumatol Int* 38: 1169-1178.
18. Vlachoyiannopoulos PG, Guialis A, Tzioufas AG, Moutsopoulos HM (1996) Predominance of IgM anti-U1-RNP antibodies in patients with systemic lupus erythematosus. *Rheumatology* 35: 534-541.
19. Tani C, Carli L, Vagnani S, Talarico R, Baldini C, et al. (2014) The diagnosis and classification of mixed connective tissue disease. *J Autoimmun* 48-49: 46-49.
20. Alarcón-Segovia D, Villarreal M (1987) Classification and diagnostic criteria for mixed connective tissue disease. In: Kasukawa R, Sharp GC, Mixed connective tissue disease and anti-nuclear antibodies. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 33-40.
21. Amigues JM, Cantagrel A, Abbal M, Mazières B (1996) Comparative study of 4 diagnosis criteria sets for mixed connective tissue disease in patients with anti-RNP antibodies. Autoimmunity Group of the Hospitals of Toulouse. *J Rheumatol* 23: 2055-2062.
22. Reichlin M (1976) Problems in differentiating SLE and mixed connective tissue disease. *N Engl J Med* 295: 1194-1195.
23. Le Roy EC, Maricq HR, Kahaleh MB (1980) Undifferentiated connective tissue syndromes. *Arthritis Rheum* 23: 341-343.
24. Nimelstein SH, Brody S, Mc Shane D, Holman HR (1980) Mixed connective tissue disease: A subsequent evaluation of the original 25 patients. *Medicine (Baltimore)* 59: 239-248.
25. Kasukawa R (2011) Mixed Connective Tissue Disease. *Autoimmune Diseases of the Skin*. Springer-Verlag/Wien.
26. Ortega-Hernandez OD, Shoenfeld Y (2012) Mixed connective tissue disease: An overview of clinical manifestations, diagnosis and treatment. *Best Pract Res Clin Rheumatol* 26: 61-72.
27. Benjamin C, Carlo Alberto S, Rosaria T, Tobias A, Zahir A, et al. (2018) Mixed connective tissue disease: State of the art on clinical practice guidelines. *RMD Open* 4: e000783.
28. Cappelli S, Bellando Randone S, Martinović D, Tamas MM, Pasalić K, et al. (2012) "To be or not to be," ten years after: Evidence for mixed connective tissue disease as a distinct entity. *Semin Arthritis Rheum* 41: 589-598.
29. Sullivan WD, Hurst DJ, Harmon CE, Esther JH, Agia GA, et al. (1984) A prospective evaluation emphasizing pulmonary involvement in patients with mixed connective tissue disease. *Medicine (Baltimore)* 63: 92-107.
30. Mimura Y, Ihn H, Jinnin M, Asano Y, Yamane K, et al. (2006) Rheumatoid factor isotypes in mixed connective tissue disease. *Clin Rheumatol* 25: 572-574.
31. Doria A, Ruffatti A, Calligaro A, Del Ross T, Ghirardello A, et al. (1992) Antiphospholipid antibodies in mixed connective tissue disease. *Clin Rheumatol* 11: 48-50.
32. Komatireddy GR, Wang GS, Sharp GC, Hoffman RW (1997) Antiphospholipid antibodies among anti-U1-70 kDa autoantibody positive patients with mixed connective tissue disease. *J Rheumatol* 24: 319-322.
33. Kim P, Grossman JM (2005) Treatment of mixed connective tissue disease. *Rheum Dis Clin North Am* 31: 549-565.
34. Bodolay E, Szekanecz Z, Dévényi K, Galuska L, Csipo I,

- et al. (2005) Evaluation of interstitial lung disease in mixed connective tissue disease (MCTD). *Rheumatology (Oxford)* 44: 656-661.
35. Bull TM, Fagan KA, Badesch DB (2005) Pulmonary vascular manifestations of mixed connective tissue disease. *Rheum Dis Clin North Am* 31: 451-464.
36. Hajas A, Szodoray P, Nakken B, Gaal J, Zold E, et al. (2013) Clinical course, prognosis, and causes of death in mixed connective tissue disease. *J Rheumatol* 40: 1134-1142.
37. Dabiri G, Falanga V (2013) Connective tissue ulcers. *J Tissue Viability* 22: 92-102.