

MIXED CONNECTIVE TISSUE DISEASE

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I. HISTORICAL LANDMARK ARTICLES

1. Sharp GC, Irvin WS, Gould RG, Holman HR, Tan EM. 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, 1972

Original report of MCTD describing typical clinical and serologic findings in a series of 25 patients.

2. Nimelstein SH, Brody S, McShane D, Holman H. Mixed Connective Tissue Disease: subsequent evaluation of the original 25 patients. *Medicine* 59(4):239-249, 1980
22 of the original 25 patients reported by Sharp et al in 1972 were reassessed clinically and serologically to see how they evolved since the original evaluation.

II. CLINICAL DIAGNOSIS

3. Alarcon-Segovia D, Cardiel MH. Comparison between 3 diagnostic criteria for mixed connective tissue disease. Study of 593 patients. *J Rheum* 16:328-334, 1989
This study compared the sensitivity and specificity of 3 sets of criteria for MCTD in 593 Mexican patients. The Alarcon-Segovia criteria appeared superior to the Japanese and US criteria

4. Pettersson I, Wang G, Smith EI, Wigzell H, Hedfors E, Horn J, Sharp GC. The use of immunoblotting and immuno precipitation of (U) small nuclear ribonucleoproteins in the analysis of sera of patients with mixed connective tissue disease and systemic lupus erythematosus: a cross-sectional, longitudinal study. *Arthritis Rheum* 29:986, 1986
This cross-sectional, longitudinal study showed that antibodies to the 68K (now termed 70KD) protein were associated with the anti-U1-RNP specificity in MCTD and rarely occurred in SLE. Positive snRNP immunoblotting patterns persisted for many years, but finally disappeared in patients in prolonged remission.

5. Hof D, Cheung K, de Rooij DJ, van den Hoogen FH, Pruijn GJ, van Venrooij, WJ, Raats JM. Autoantibodies specific for apoptotic U1-70K are superior serological markers for mixed connective tissue disease. *Arthritis Res Ther.* 7(2):R302-9. Epub 2005 Jan 11.
This study evaluated 25 MCTD patients serologically for the presence of autoantibodies against intact and apoptotic 70k and suggested that these autoantibodies may drive the primary autoimmune response to 70Kd and may precede the appearance of reactivity with intact 70Kd. The authors suggested that 70Kd^{apop} may be important in the early detection of MCTD.

6. Greidinger EL, Casciola-Rosen L, Morris SM, Hoffman RW, Rosen A. Autoantibody

recognition of distinctly modified forms of the U1-70-kd antigen is associated with different clinical disease manifestations. *Arthritis Rheum* 2000 Apr;43(4):881-8.
Sera from 27 rheumatic disease patients with U1-70-kd antibodies were analyzed for recognition of apoptotically and oxidatively modified forms of U1-70-kd autoantigens. Patients with lupus skin disease had higher recognition of apoptotic U1-70-kd than those without skin disease. Patients with Raynaud's phenomenon recognized oxidatively modified U1-70-kd than those without RP.

7. [Mahler M](#), [Stinton LM](#), [Fritzler MJ](#). Improved serological differentiation between systemic lupus erythematosus and mixed connective tissue disease by use of an SmD3 peptide-based immunoassay. *Clinical and Diagnostic Laboratory Immunology*. 12(1);107-113, Jan. 2005

This study evaluated the sera of 15 patients with MCTD, 50 with SLE, 50 with RA, 15 with scleroderma, 11 with polymyositis/dermatomyositis, and 15 patients with other autoimmune diseases for Sm antibodies using various techniques in combination with a variety of different antigens and found the SmD3 peptide-based ELISA (Varelista Sm Antibodies) is 88-100% specific for SLE and may help distinguish SLE from MCTD.

8. [Bodolay E](#), [Szekanecz Z](#), [Devenyi K](#), [Galuska L](#), [Csipo I](#), [Vegh J](#), [Garai I](#), [Szegedi G](#). Evaluation of interstitial lung disease in mixed connective tissue disease (MCTD). *Rheumatology* 44;656-661, 2005

144 patients with MCTD were evaluated using HRCT and ^{99m}Tc-DTPA scintigraphy, PFTs, and immunolaboratory investigations over a one-week period. 66.6% of these patients were found to have ILD by HRCT. 78.8% of patients have evidence of ground glass appearance by HRCT. The ^{99m}Tc-DTPA scintigraphy was abnormal in all 96 patients with active ILD and 78.1% of patients had normalization of ^{99m}Tc-DTPA scintigraphy after therapy. The FEV1 and total lung capacity was reduced in 34% and 41% of patients, respectively. Reduced DLCO was the most sensitive test for predicting the presence of fibrosing alveolitis on HRCT. Pulmonary effects of corticosteroid monotherapy or in combination with cyclophosphamide was also evaluated.

9. [Sullivan WD](#), [Hurst DJ](#), [Harmon CE](#), [Esther JH](#), [Agia GA](#), [Maltby JD](#), [Lillard SB](#), [Held CN](#), [Wolfe JF](#), [Sunderrajan EV](#), [Maricq HR](#), [Sharp GC](#). A prospective evaluation emphasizing pulmonary involvement in patients with mixed connective tissue disease. *Medicine* 63:92, 1984

A prospective, longitudinal study of 34 MCTD patients, emphasizing the high frequency of pulmonary disease (sometimes clinically silent) and pulmonary hypertension as the most frequent, serious complication. Responses to glucocorticoid and cyclophosphamide therapy, prognosis and pathological findings (proliferative vascular lesions without pulmonary fibrosis) are discussed.

10. [Wigley FM](#), [Lima JA](#), [Mayes M](#), [McLain D](#), [Chapin L](#), [Ward-Able C](#). The prevalence of undiagnosed pulmonary arterial hypertension in subjects with connective tissue disease at the secondary health care level of community-based rheumatologists (the UNCOVER study). *Arthritis Rheum* 52(7);2125-2132, July 2005

A multicenter, prospective and retrospective survey of 909 patients with either scleroderma or MCTD seen in 50 community rheumatology practices. 13.3% of patients were found to have PHTN with estimated right ventricular systolic pressure \geq 40mmHg by Doppler echocardiography. Most of these patients had scleroderma (92.1%) the remainder had MCTD (7.9%).

11. Bull TM, Fagan KA, Badesch DB. Pulmonary vascular manifestations of mixed connective tissue disease. *Rheum Dis Clin N Am* 31;451-464, 2005
Review article regarding the pulmonary manifestations of MCTD and the treatments available.

12. Burdt MA, Hoffman RW, Deutscher SL, Wang GS, Johnson JC, Sharp GC. Longterm outcome in mixed connective tissue disease. *Arthritis Rheum* 42:899-909, 1999
A prospective, longitudinal study of over 20 years in a well defined cohort of patients with MCTD. Pulmonary hypertension was the predominant disease-associated cause of death in MCTD.

13. Kitridou RC, Akmal M, Turkel SB, Ehresmann GR, Quismorio FP, Massry SG. Renal involvement in mixed connective tissue disease: a longitudinal clinicopathologic study. *Semin Arthritis Rheum* 16(2);135-145, 1986.
This study evaluated the renal disease in MCTD and noted that most patients had membranous nephropathy or mesangial glomerulitis. 17/30 patients had some degree of proteinuria. The prevalence of nephropathy in this study was 40%. Most of the patient with renal involvement had mutisystem disease.

III. DISEASE PATHOPHYSIOLOGY

14. Prokop J, Jagodzinski PP. Identification of retroviral conserved *pol* sequences in serum of mixed connective tissue disease and systemic sclerosis patients. *Biomedicine & Pharmacotherapy* 58:61-64, 2004
22 patients with MCTD, 69 patients with systemic sclerosis, and 85 healthy controls were evaluated for the presence of HIV-1 conserved pol sequences in DNA debris. 90.9% of patients with MCTD exhibited strong dot blot hybridization reaction with pol probe. 34.1% of the female SSc patients with + ANA, 100% with anti-U1RNP, and 34.1% with anti-Scl-70 antibodies also exhibited the presence of HIV-1 conserved pol sequence. This study suggests that an expression of retroviral components may participate in the development of autoimmune response in MCTD by activating the humoral immune system and production of ANA, anti-U1RNP, and Scl-70 antibodies.

15. Hoffman RW, Rettenmaier LJ, Takeda Y, Hewett JE, Pettersson I, Nyman U, Luger AM, Sharp GC. Human autoantibodies against the 70kD polypeptide of U1 small nuclear ribonucleoprotein are associated with HLA-DR4 among connective tissue disease patients. *Arthritis Rheum* 33:666, 1990
This study showed that there is an association of HLA-DR4 with anti- U1-snRNP 70kD-positive connective tissue disease.

16. [Lundberg I](#), [Hedfors E](#). Clinical course of patients with anti-RNP antibodies. A prospective study of 32 patients. *J Rheum* 18:1511, 1991
In a prospective, long-term study in Sweden, on initial evaluation 17/23 patients with high titer anti-RNP did not fulfill criteria for any connective tissue disease, while at the end of the study 17/23 had developed additional clinical abnormalities and now fulfilled the criteria for MCTD.
17. [Greidinger EL](#), [Zang Y](#), [Jaimes K](#), [Hogenmiller S](#), [Nassiri M](#), [Bejarano P](#), [Barber GN](#), [Hoffman RW](#). A murine model of mixed connective tissue disease induced with U1 small nuclear RNP autoantigen. *Arthritis Rheum* 54(2):661-669, Feb 2006
The C57BL/6-derived mice transgenic for human HLA-DR4 were immunized with 70k and either Freund's complete adjuvant or U1 RNA, which caused the production of anti-70k antibodies in 62% of mice and development of MCTD-like lung disease in 50% of the immunized mice. These anti-70K antibodies strongly correlated with lung disease.
18. [Talken BL](#), [Lee DR](#), [Caldwell CW](#), [Quinn TP](#), [Schäfermeyer KR](#), [Hoffman RW](#). Analysis of T cell receptors specific for U1-70kD Small Nuclear Ribonucleoprotein autoantigen: The Alpha chain complementarity determining region three is highly conservative among connective tissue disease patients. *Human Immunol* 60:200-208, 1999
T cell clones specific for the snRNP 70kD polypeptides were generated using either a 70kD fusion protein or synthetic peptides. The precise peptide epitope recognized by T cells was defined and the TCR receptor, which recognizes these peptides, defined. It was found that the T cell receptor alpha chain complementarity determining region three is highly restricted in MCTD.
19. [Fenning S](#), [Wolff-Vorbeck G](#), [Hacle W](#), [Krawinkel W](#), [Luhmann R](#), [Northemann W](#), [Peter HH](#), [Schlesier M](#). T cell lines recognizing the 70-kD protein of U1 small nuclear ribonucleoprotein (U1snRNP). *Clin Exp Immunol* 101(3):408-413, 1995.
Study looking at MCTD pts and T cell responses to the 70kD U-1 snRNP All T cell lines showed a proliferative response to an N-terminal part (aa 51-195) of recombinant U1-specific 70-kD protein. One CD8+ T cell clone exhibited cytotoxic activity against an autologous B cell line pulsed with snRNP or recombinant fragments (aa 51-95 and aa 51-88)
20. [Lundberg I](#), [Nennesmo I](#), [Hedfors E](#). A clinical, serological, and histopathological study of myositis patients with and without RNP antibodies. *Sem Arth Rheum* 22:127, 1992
In a 10-year study, believed to include most cases of myositis in the south Stockholm region, all 7 patients with anti-RNP antibodies had myositis in association with other connective tissue disease features and fulfilled criteria for MCTD. These anti-RNPpositive patients had less severe myositis and responded well to lower doses of glucocorticoids over a shorter duration compared to the anti-RNP negative myositis groups.

21. [Vianna MA](#), [Borges CT](#), [Borba EF](#), [Caleiro MT](#), [Bonfa E](#), [Marie SK](#). Myositis in mixed connective tissue disease. A unique syndrome characterized by immunohistopathologic elements of both polymyositis and dermatomyositis. *Arq Neuropsiquiatr* 62(4);923-934,2004

14 MCTD patients were followed prospectively and had muscle biopsies that revealed inflammatory cell infiltrate in the perivascular and endomysial regions. Some perimysial vessel wall thickness was noted. This was in contrast to the endomysial and perivascular inflammatory infiltrate noted in polymyositis in moderate intensity. Dermatomyositis was noted to have perivascular inflammatory cell distribution with marked perifascicular atrophy. No proliferation of connective tissue was observed on DM or PM samples. The MCTD patients had significantly less MHC-1, ICAM-1, and VCAM-1 expression on the muscle biopsy as compared to PM and DM patients. The distribution of CD4 and B cells were mainly in the perivascular sites.

22. [Kaneoka H](#), [Hsu K-C](#), [Takeda Y](#), [Sharp GC](#), [Hoffman RW](#). Molecular genetic analysis of HLA-DR and HLA-DQ genes among anti-U1-70kD autoantibody-positive connective tissue disease patients. *Arthritis Rheum* 35:83, 1992

In a comprehensive molecular genetic analysis of HLA-DRB1, HLA-DRB5, HLADQA1, and HLA-DQB1 genes among anti-U1-70kD autoantibody positive patients with connective tissue disease, select molecular subtypes of HLA-DR4 and HLA-DR2 were present among patients. The amino acid residues of this shared epitope identified among anti-U1 70kD autoantibody-positive patients are spatially related, forming a pocket for antigen binding.

23. [Hoffman RW](#), [Cassidy JT](#), [Takeda Y](#), [Smith-Jones EI](#), [Wang GS](#), [Sharp GC](#). U1-70-kd autoantibody-positive mixed connective tissue disease in children: a longitudinal clinical and serologic analysis. *Arthritis Rheum* 36:1599, 1993

Eleven pediatric patients with anti-U1-70kD autoantibodies followed longitudinally demonstrated MCTD clinical manifestations similar to adult patients with anti-U1-70kD autoantibody-positive connective tissue disease. These patients also shared similar immunogenetics with adult patients, with all patients studied possessing HLADRA4 or HLA-DR2.

24. [Mier RJ](#), [Shishov M](#), [Higgins GC](#), [Rennebohm RM](#), [Wortmann DW](#), [Jerath R](#), [Ahumoud E](#). Pediatric-onset mixed connective tissue disease. *Rheum Dis Clin N Am* 31:481-496, 2005

Complete up to date review article regarding pediatric onset MCTD, its clinical features, laboratory findings, and treatment.

25. [Hoffman RW](#), [Sharp GC](#), [Deutscher S](#). Analysis of anti-U1 RNA antibodies in patients with connective tissue disease. *Arthritis Rheum* 38:1837, 1995

Anti-U1 RNA IgM and IgG antibodies were found in 60% of anti-RNP positive patients and were not detected in anti-RNP negative patients. All of the anti-U1 RNA positive patients had anti-70kD, and most had anti-A, U1 snRNP polypeptide antibodies. HLA-DR2/DR4, as well as Raynaud's phenomenon and synovitis, were significantly increased in the anti-U1 RNA positive group.

26. [Skriner K, Sommergruber WH, Tremmel V, Fischer I, Barta A, Smolen JS, Steiner G](#). Anti-A2/RA33 autoantibodies are directed to the RNA binding region of the A2 protein of the heterogeneous nuclear ribonucleoprotein complex. Differential epitope recognition in rheumatoid arthritis, systemic lupus erythematosus, and mixed connective tissue disease. *J Clin Invest* 100:127-135, 1997

The authors describe a new autoantibody system called A2/RA33 which is part of the A2 protein of the heterogenous nuclear ribonucleoprotein complex. They describe differential epitope recognition occurring between mixed connective tissue disease, rheumatoid arthritis and systemic lupus erythematosus. The specific recognition of an epitope by MCTD patients argues in favor of MCTD being a distinct disease entity.

27. [Holyst MM, Hill DL, Hoch SO, Hoffman RW](#). Analysis of human T cell and B cell responses against U small nuclear ribonucleoprotein 70-kd, B, and D polypeptides among patients with systemic lupus erythematosus and mixed connective tissue disease. *Arthritis Rheum* 40:1493-1503.

The work is an extension of earlier studies showing that MCTD and SLE patients have circulating T cells which react with small nuclear ribonucleoprotein (snRNP) polypeptides including 70kD. These T cells have T helper cell surface markers and produce cytokines known to influence B cell help and differentiation. Importantly, it was found that the presence of snRNP-reactive T cells in peripheral blood parallel the specificity of snRNP-reactive antibodies detectable in patient's sera, showing that T and B cell responses were linked in vivo.

28. [Aringer M, Steiner G, Smolen J](#). Does mixed connective tissue disease exist? Yes. *Rheum Dis Clin N Am* 31;411-420, 2005

The authors provide a critical review of the topic of whether MCTD is a unique clinical entity deserving of a distinctive title or merely a subset of other well defined rheumatic diseases.

29. [Grader-Beck T, Wigley FM](#). Raynaud's phenomenon in mixed connective tissue disease. *Rheum Dis Clin North Am* 31;465-481, 2005

This article reviews Raynaud's phenomenon seen in pts with MCTD and its association with autoantibodies, pathogenesis, and differences from Raynaud's phenomenon seen in other CTD.

30. [Kitridou RC](#). Pregnancy in mixed connective tissue disease. *Rheum Dis Clin N Am* 31;497-508, 2005

This review article looks at outcomes of pregnancy in MCTD.

IV. CLINICAL TREATMENTS

31. [Oudiz RJ, Schilz RJ, Barst RJ, Galie N, Rich S, Rubin LJ, Simonneau G](#).

Treprostinil, a prostacyclin analogue, in pulmonary arterial hypertension associated with connective tissue disease. *Chest* 126;420-427, 2004

Multicenter, randomized prospective trial looking at treprostinil vs. placebo in 470 patients with PAH. A subset of 90 patients with CTD and PAH were evaluated. The change in cardiac index from baseline after 12 weeks of treatment was statistically significant. There was improvement in exercise capacity, symptoms of PAH, and hemodynamics.

32. [Kim P](#), [Grossman JM](#). Treatment of mixed connective tissue disease. *Rheum Dis Clin N AM* 31;549-565, 2005

A review of treatment strategies in patients with MCTD

33. [Rubin LJ](#), [Badesch DB](#), [Barst RJ](#), [Galie N](#), [Black C](#), [Keogh A](#), [Pulido T](#), [Frost A](#), [Roux S](#), [Leconte I](#), [Lanszberg M](#), [Simonneau G](#). Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med* 346;896-903, 2002.

Multicenter trial looking at 213 patients with either primary pulmonary hypertension, or pulmonary hypertension secondary to scleroderma or SLE randomized to receive either bosentan or placebo initially at a dose of 62.5mg BID for four weeks then increased to 125 or 250mg BID for twelve weeks. Period 1 of the trial was completed in sixteen weeks. Some patients went on to period 2 of the trial looking at efficacy and safety prospectively for another twelve weeks. This study showed that bosentan improved exercise capacity and cardiopulmonary hemodynamics. At sixteen weeks the six minute walking distance improved by 36 m in the treatment group and 38% of the patients receiving 125mg BID bosentan and 34% of patients receiving 250mg of Bosentan had improved from a WHO functional classification III to II.

34. [Sitbon O](#), [Badesch DB](#), [Channick RN](#), [Frost A](#), [Robbins IM](#), [Simonneau G](#), [Tapson VF](#), [Rubin LJ](#). Effects of the dual endothelin receptor antagonist bosentan in patients with pulmonary arterial hypertension- A 1-year follow-up study. *Chest* 124;247-254, 2003.

This multicenter open label extension study, after a twelve week double-blind, placebo controlled trial looking at bosanten in 32 patients with PPH or associated with scleroderma, looked at long term safety and tolerability of bosentan treatment.

35. [Rich S](#), [Kaufmann E](#), [Levy PS](#). The effect of high doses of calcium-channel blockers on survival in primary pulmonary hypertension. *N Engl J Med* 9;327:117-119, 1992.

Landmark article evaluating high dose calcium channel blockers in patients with primary pulmonary hypertension. 26% of patients responded to either nifedipine 172mg +/-41mg daily or diltiazem 720mg +/- 208mg daily. The patients who responded had a drop in the PAP by 39% and pulmonary vascular resistance index by 53%. After 5 years 94% of patients who had responded were alive compared to 55% of patients who had not responded.

36. [Korn JH](#), [Mayes M](#), [Cerinic M](#), [Rainisio M](#), [Pope J](#), [Hachulla E](#), [Rich E](#), [Carpentier P](#), [Molitor J](#), [Seibold JR](#), [Hsu V](#), [Guillevin L](#), [Chatterjee S](#), [Peter HH](#), [Coppock J](#), [Herrick A](#), [Merkel PA](#), [Simms R](#), [Denton CP](#), [Furst D](#), [Nguyen N](#), [Gaitonde M](#), and [Carol Black](#), for the RAPIDs-1 study group. Digital ulcers in systemic sclerosis. Prevention by treatment

with bosentan, an oral endothelin receptor antagonist. *Arthritis Rheum* 50(12):3985-3993, 2004.

A randomized, prospective, placebo-controlled, double-blind study of 122 at 17 centers in Europe and North America looking at effects of bosentan and development of new ulcers. During the 16 week study period there was a 48% reduction in the mean number of new ulcers, improvement in hand functionality, but no improvement in existing ulcers were noted.