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ORIGINAL PAPER

Anti-U1-RNP antibodies and mixed connective tissue disease: A real-world diagnostic phenotype

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Abstract

Background. The study aims to observe real-life diagnostic outcome of patients testing positive for anti-U1-RNP antibodies and to identify clinical features useful for distinguishing mixed connective tissue disease (MCTD).

Methods. Data of all patients who were admitted to a tertiary university rheumatology hospital within the last 5 years (Oct 15th 2019 – Nov 18th 2024) and who were tested quantitatively for anti-U1-RNP antibodies were retrospectively collected. All diagnoses and clinical manifestations were according to each attending senior rheumatologist.

Results. Only 36 patients (5.4%) had positive anti-U1-RNP antibodies. These patients were in most cases women (88.9%), with a median age of 44 years. The median titers of positive anti-U1-RNP antibodies were 200 U/mL. Only 66.7% were diagnosed with MCTD. Compared to non-MCTD patients, MCTD patients were significantly older, with higher median anti-U1-RNP antibody titers, and higher prevalence of Raynaud's phenomena, puffy hands/fingers, and rheumatoid factor positivity, lower prevalence of thrombocytopenia, positive anti-dsDNA antibodies, positive anti-Sm antibodies and hypocomplementemia. Only 38.9% of positive anti-U1-RNP antibody patients fulfilled the Alarcón-Segovia and Villareal criteria (ASVC). Only 54.2% of patients diagnosed with MCTD fulfilled the ASVC.

Conclusion. The predominant phenotype of patients with positive anti-U1-RNP antibodies and a clinical diagnosis of MTCT includes female sex, fifth decade of life, arthritis, hand edema, Raynaud's phenomena, esophageal involvement and inflammatory syndrome. Clinical judgment is more inclusive than the ASVC for MCTD and it seems to be oriented towards MCTD by higher titers of anti-U1-RNP antibodies and toward SLE by positive anti-dsDNA and anti-Sm antibodies.

Keywords: mixed connective tissue disease, anti-U1-RNP antibodies, phenotype.

Introduction

Mixed connective tissue disease (MCTD) is an autoimmune disorder with overlapping features of systemic lupus erythematosus (SLE), systemic sclerosis (SS), rheumatoid arthritis (RA) and polymyositis. However, its independent nosology status [1] is still challenged [2] since it rests on the specificity of a single biomarker (anti-U1-nuclear ribonucleoprotein - anti-U1-RNP antibodies [3]), and since a proportion of MCTD patients migrate phenotypically to other differentiated connective tissue diseases [1]. Symptoms and signs include Raynaud's phenomenon, swollen hands, arthritis/arthralgia, muscle weakness, skin rashes, and pulmonary hypertension, which may accrue in time [4]. The exact cause of the disease is unknown, but genetic and environmental factors contribute. Diagnosis is based on clinical presentation, serology, and imaging, while treatment includes glucocorticoids, immunosuppressants, and symptom management. Prognosis varies, with some cases remaining mild and others progressing to severe organ involvement. Regular monitoring is essential for managing complications and improving outcomes. MCTD is characterized by high levels of anti-U1 ribonucleoprotein (RNP) antibodies, which are useful to distinguish MCTD from other autoimmune diseases and from its components.

Anti-U1-RNP antibodies target the U1 small nuclear ribonucleoprotein complex, involved in RNA splicing, and can be tested in the serum or cerebrospinal fluid [5]. While they are strongly associated with MCTD, they can also be found in SLE (up to 30% in some cohorts [6]), SS and other rheumatic diseases, in which they tend to categorize subgroups of patients regarding clinical manifestations and outcomes [6, 7]. Although not exclusive to MCTD, high titers of anti-U1-RNP antibodies support its diagnosis and may be involved in the pathogenesis of target-tissue manifestations [8], since they contribute to immune complex formation and subsequent inflammation. Their clinical significance includes associations with Raynaud's phenomenon, arthritis, myositis, and pulmonary hypertension. Other biomarkers of MCT have been investigated, such as

anti-survival motor neuron complex (SMN) antibodies [9], which have been associated specifically with lung involvement [10]. In the pathogenesis of MCTD, dysregulated B and T cell responses lead not only to autoantibody production, but also to cytokine release, promoting chronic inflammation and tissue damage. Key cytokines involved include interferon-α, tumor necrosis factor-alpha (TNF-α), and interleukins, which drive fibrosis, vascular damage, and immune-mediated myopathy. Endothelial dysfunction and fibrosis play central roles, particularly in pulmonary hypertension and skin thickening. Additionally, impaired apoptotic clearance of cellular debris enhances autoantigen presentation, perpetuating autoimmunity. Overall, MCTD pathogenesis involves a combination of autoantibody production, immune activation, and progressive fibrotic and vascular changes, resulting in the characteristic multisystem involvement [11, 12].

In this context, the current study aims to observe real-life diagnostic outcome of patients testing positive for anti-U1-RNP antibodies and to identify clinical features useful for distinguishing MCTD.

Materials and Methods

Patients

Data of all patients who were admitted to the hospital within the last 5 years (Oct 15th 2019 – Nov 18 2024) and who were tested quantitatively for anti-U1-RNP antibodies were retrieved from the electronic database of a tertiary university rheumatology hospital, along their sex and age at the time of the anti-U1-RNP antibody test. All patients gave written informed consent for medical evaluations and scientific use of data.

Laboratory tests

All laboratory tests were done by the same local laboratory, using commercially available kits. Anti-U1-RNP antibodies were tested using fluorimetric enzyme-linked immunoassay, with negative titers below 25 U/mL and unquantifiable titers above 200 U/mL. Anti-U1-RNP antibody titers were further categorized as either low positive (25-40 U/mL), moderately positive (41-80 U/mL) and high positive (> 80 U/mL). Other laboratory tests included auto-antibodies (antinuclear antibodies, anti-dsDNA, anti-Sm, anti-ScL70, anti-Ro, anti-La, anti-centromer, anti-SCL70, anti-CCP, rheumatoid factor, anti-phospholipid syndrome-associated antibodies), which were quantitatively evaluated using enzyme-linked immunosorbent assays, inflammatory markers (C-reactive protein, erythrocyte sedimentation rate, serum complement C3 and C4), total creatin-kinase and complete blood count (noting: anemia – hemoglobin < 13.5 g/dL in men and < 11.0 g/dL in women; leukopenia – withe blood count < 4000/μL; thrombocytopenia – platelet count < 150000/ μL). All laboratory data were coded as dichotomic nominal variables (positive/negative or yes/no).

Clinical manifestations and diagnoses

All diagnoses and clinical manifestations were recorded according to each attending senior rheumatologist who confirmed the diagnoses and were retrieved from the hospital medical electronic database, including: clinical manifestations (puffy hands/fingers, arthralgia/arthritis, myalgia, Raynaud's phenomena, sclerodactyly, acrosclerosis, sicca syndrome, scleroderma, facial erythema, Gottron's sign, neuro-psychiatric manifestations), functional investigations and imaging results (video-capillaroscopy, gastritis/acid reflux disease, pleurisy/pericarditis, heart block, interstitial lung disease, pulmonary hypertension, kidney involvement), coded as dichotomic nominal variables (yes/no). The Alarcón-Segovia and Villareal criteria for the diagnosis of MCTD [13] were retrospectively applied to patients with positive anti-U1-RNP antibodies.

Statistics

Continuous variables are reported as "mean ± standard deviation" if normally distributed, or as "median (interquartile range)" if non-normally distributed. Differences of continuous variables among categories of nominal variables were tested with Mann Whitney U tests, while the association of nominal variables was tested with $\chi 2$ tests or Fisher's exact tests, considered significant if $p < 0.05$. All statistical tests were done with SPSS (IBM Corp. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp; 2019).

Results

Anti-U1-RNP antibody tests

Within the mentioned time frame, there were 804 tests for anti-U1-RNP antibodies, of which only 80 were positive (10%). The tested population consisted of 665 unique individuals, of whom 566 (85.1%) were women and who had an average age of 50.8 ± 14.9 years. Of note, among the unique tested population, there were 10 (1.5%) patients of pediatric age (< 18 years), none of whom had positive anti-U1-RNP antibody titers. Among the unique tested population, only 36 patients (5.4%) had positive anti-U1-RNP antibody titers.

These positive unique patients were preponderately women (88.9%), with a median age of 44 (25) years. The median age and anti-U1-RNP antibody titer did not differ significantly among sexes ($p = 0.827$ respectively $p = 0.248$), nor any of the nominal variables. Pathological capillaroscopy finding were more frequent in men (75% compared to 25%, $p = 0.076$).

Anti-U1-RNP antibody titers

The median titers of positive anti-U1-RNP antibodies in unique patients (n = 36) were 200 (106) U/mL: 29 patients (80.6%) had high positive titers, 4 patients (11.1%) had medium positive titers and 3

patients (8.3%) had low positive titers. Compared to patients with either low or medium positive titers, patients with high positive titers did not differ significantly in median age (p = 0.345), nor in any of the nominal variables. Titers clustered around the upper end of the distribution, with 24 patients (66.7%) having positive anti-U1-RNP antibodies with unquantifiable titers (> 200 U/mL). Compared to patients with quantifiable anti-U1-RNP antibody titers, patients with unquantifiable titers did not differ significantly in median age (p = 0.540), nor in any of the nominal variables. Despite this, those unquantifiable anti-U1-RNP antibody titers more frequently displayed arthritis (95.8%% versus 75.0%, p = 0.061) and leukopenia (25.0% versus 0%, p = 0.058), and less frequently heart block and facial erythema (both 4.2% versus 25.0%, both p = 0.061).

Clinical and laboratory manifestations

Among patients with positive anti-U1-RNP antibodies (n = 36), the most common clinical manifestations were arthritis (88.9%), Raynaud's phenomena (80.6%) and esophageal involvement (41.7%; Figure 1A), while the most frequent laboratory findings were positive antinuclear antibodies (91.7%) and elevated acute phase reactants (Figure 1B).

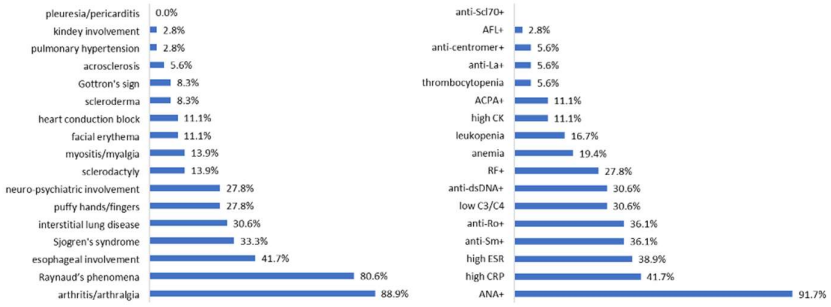

Figure 1. *Clinical (upper panel) and laboratory (lower panel) manifestation among patients with positive anti-U1-RNP antibodies (n = 36). Abbreviations: ACPA - anti-citrullinated protein antibodies; AFL – antiphospholipid; ANA – antinuclear antibodies; CK – creatin-kinase; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; RF – rheumatoid factor; + positive.*

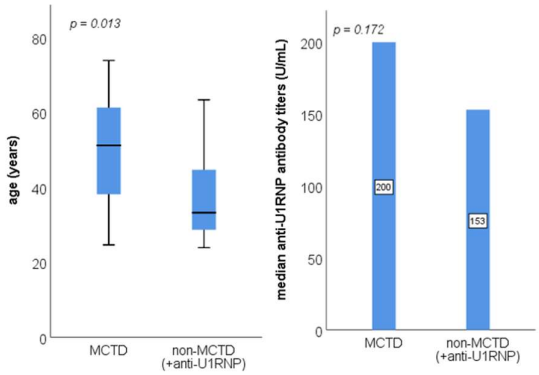

Figure 2. *Median age (left) and anti-U1-RNP antibody titers among patients with positive and anti-U1-RNP antibodies diagnosed or not with MCTD.*

Clinical diagnoses

Regarding the diagnoses of patients with positive anti-U1-RNP antibodies (n = 36), 66.7% were diagnosed with MCTD, while the rest were diagnosed either with SLE (27.8%), SS (8.3%, all of whom were overlapped with SLE) and RA (8.3%, one of whom overlapped with SLE).

Compared to non-MCTD patients (n = 12), patients diagnosed with MCTD (n = 24) were significantly older (51 (22) versus 33 (11) years, p = 0.013, Figure 2), with higher median anti-U1-RNP antibody titers (200 (197) versus 153 (36) U/mL, p = 0.172, Figure 2) and higher prevalence of Raynaud's phenomena (91.7% versus 58.3%, p = 0.017, Figure 3), puffy hands/fingers (41.7% versus 0%, p = 0.009, Figure 3), and rheumatoid factor positivity (37.5% versus 8.3%, p = 0.066, Figure 3), but lower prevalence of thrombocytopenia (0% versus 16.7%, p = 0.040), positive anti-dsDNA antibodies (12.5% versus 66.7%, p = 0.001), positive anti-Sm antibodies (20.8% versus 66.7%, p = 0.007) and hypocomplementemia (20.8% versus 50.0%, p = 0.073).

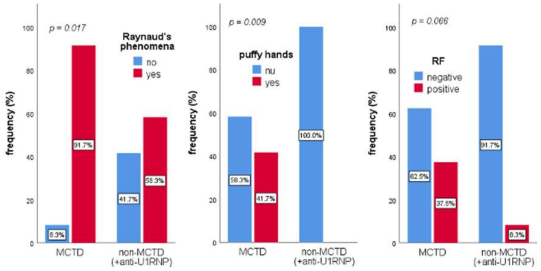

Figure 2. *The higher frequency of Raynaud's phenomena, puffy hands and positive RF in patients with positive anti-U1-RNP antibodies diagnosed with MCTD (n = 26), compared to those not diagnosed with MCTD (n = 12).*

Among patients with positive anti-U1-RNP antibodies (n = 36), only 14 (38.9%) patients fulfilled the Alarcón-Segovia and Villareal criteria for MCTD: patients fulfilling the Alarcón-Segovia and Villareal criteria (n = 14), compared to those who did not (n = 22), had higher frequencies of puffy hands (71.4% versus 0%, p = 0.000), Raynaud's phenomena (100% versus to 68.2%, p = 0.029), sclerodactyly (35.7% versus 0%, p = 0.005), interstitial lung disease (50.0% versus 18.2%, p = 0.067) and lower frequency of positive anti-SM antibodies (7.1% versus 54.5%, p = 0.005).

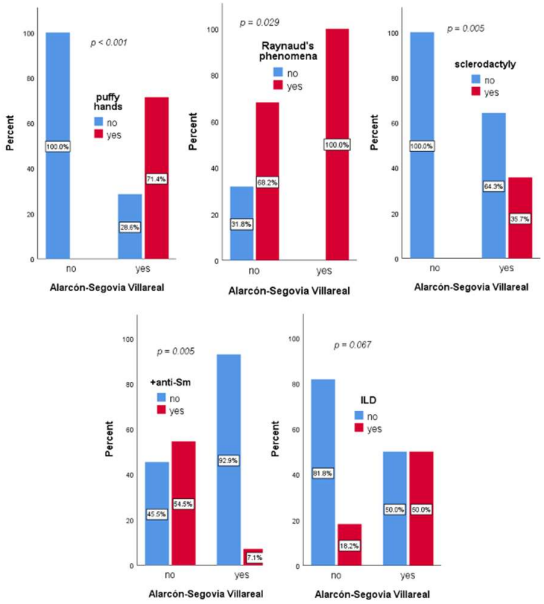

Figure 3. *The frequency of puffy hands, Raynaud's phenomena, sclerodactyly, interstitial lung disease (ILD) and positive anti-Sm antibodies among patients fulfilling the Alarcón-Segovia and Villareal criteria for MCTD (n = 14) and those who did not (n = 22).*

Of those diagnosed with MCTD (n = 24), only 54.2% fulfilled the Alarcón-Segovia and Villareal criteria for MCTD, and 8.3% of those diagnosed with other autoimmune diseases fulfilled these criteria.

Discussions

Only 10% of clinically-indicated anti-U1-RNP antibody tests are positive, summing to 5.4% of positive unique patients. Women are predominant both in clinical indications of the test and in its positivity. This female predominance is a common feature of many autoimmune diseases and can be attributed to an incompletely known combination of genetic, hormonal, and immunological factors. Of note, none of the tested pediatric patients had positive anti-U1-RNP antibodies, which is consistent with the rarity of MCTD in children [14]. High (80.6%) and unquantifiable positive (66.7%) titers are the most frequent results of the test. High titers of anti-U1-RNP antibodies seem to be associated with arthritis, Raynaud's phenomena and leukopenia, but their association with disease activity and specific manifestations remains insufficiently characterized [15]. Particularly, Raynaud's phenomena have been associated with anti-U1-RNP antibody positivity and with MCTD diagnoses [16-18], but its prevalence in other connective tissue diseases still makes it non-specific. Specific manifestations of the disease were used to cluster MCTD patients, revealing different prognoses [18], but larger samples are need to refine clustering, as well as differentiated anti-U1-RNP testing (by immunoglobulin class and by molecular spliceosome target [6]) – a hypothesis that has been tested and yielded unuseful results [19].

Remarkably, only 66.7% of patients with positive anti-U1-RNP antibodies were diagnosed by their rheumatologists with MCTD, an observation that has been reported by other authors in different settings [15, 20, 21]. Even though anti-U1-RNP antibodies are considered a hallmark of MCTD and are included in classification criteria, clinical reality consistently reports anti-U1-RNP-negative MCTD (one would label it as "seronegative" MCTD). The lack of anti-U1-RNP antibodies in these patients does not entail the lack of autoimmunity, since these patients can exhibit positive titers or anti-Ro, anti-La, anti-Sm or anti-centromere antibodies, suggesting a different/varying immunopathogenic mechanism of the disease. Observing the evolution of the disease is also mandatory, since follow up of these patients could actually reveal an early or atypical form of another yet undifferentiated connective tissue disease (a "false" MCTD). Regardless of anti-U1-RNP status, treatment is still generally guided by organ involvement rather than serological status. In this study, patients diagnosed with MCTD were older, they had higher anti-U1-RNP antibody titers and higher prevalence of Raynaud's phenomena, puffy hands/fingers, and rheumatoid factor positivity.

Similarly, only 38.9% of patients with positive anti-U1-RNP antibodies fulfilled the Alarcón-Segovia and Villareal criteria for MCTD. Again, clinical reality challenges the understanding of MCTD, since it consistently reports MCTD diagnoses which do not fulfill any know criteria set [13, 22, 23] (one would name it "unclassifiable" MCTD). Patients who do not

fulfill diagnostic criteria may lack anti-U1-RNP antibodies, exhibit incomplete clinical manifestations, or present with features that overlap with undifferentiated connective tissue disease or other autoimmune disorders. These cases either represent a subset of MCTD with a distinct pathophysiology, a "false" MCTD diagnosis or the failure of the existing criteria sets to model the clinical reality of MCTD. In this study, patients fulfilling the Alarcón-Segovia and Villareal criteria for MCTD had higher prevalence of puffy hands, Raynaud's phenomena and interstitial lung disease [24]. Again, only 54.2% of patients diagnosed with MCTD fulfilled the Alarcón-Segovia and Villareal criteria for MCTD.

The results of the study should be interpreted by taking into consideration several limitations: the low number of patients; selection bias due to the requirement to be admitted, the included patients may not be representative of the general population and may underrepresent the spectrum of disease manifestations and their severity); information bias caused by the fact that data quality depends on the accuracy and completeness of medical records and that clinical evaluations and diagnoses are unstandardized and physician-based; the effect of unmeasured or unknown confounders that may influence associations between the observed variables.

Conclusions

Anti-U1-RNP antibody testing displays an adequate balance between testing capacity and MCTD prevalence. The predominant phenotype of patients with positive anti-U1-RNP antibodies and a clinical diagnosis of MTCT includes female sex, fifth decade of life, arthritis, hand edema, Raynaud's phenomena, esophageal involvement and inflammatory syndrome. Clinical judgment is more inclusive than the Alarcón-Segovia and Villareal criteria for MCTD and it seems to be oriented towards MCTD by higher titers of anti-U1-RNP antibodies and toward SLE by positive anti-dsDNA and anti-Sm antibodies.

Conflicts of Interest: The authors declare no conflicts of interest.

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