Undifferentiated connective tissue disease with antibodies to Ro/SSA: Clinical features and follow-up of 148 patients

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Abstract

Objective

To evaluate the clinical and serologic profile, the rate of progression to well defined CTD and the possible predictors of disease evolution in patients affected by UCTD with antibodies anti-Ro/SSA.

Methods

148 patients diagnosed as UCTD were retrospectively evaluated. Antibodies to SSA/Ro were determined by counter-immunoelectrophoresis and ELISA.

Results

Thirty-six patients (24.3%) developed a well-defined CTD after a mean follow-up of 4.5 years. Most patients developed primary Sjögren's syndrome (SS) (50%) or systemic lupus erythematosus (SLE) (30.5%). Leukopenia and xerophthalmia developed more frequently in the group of patients evolving to defined CTDs ($p < 0.0032$ and $p < 0.0063$). Leukopenia independently predicted the evolution in CTD by multivariate regression analysis ($p < 0.019$). Anti-dsDNA predicted the evolution in SLE ($p < 0.0207$), while the presence of additional anti-ENA specificity to anti-Ro/SSA was not associated with the outcome.

Conclusion

24.3% of patients with UCTD and antibodies to Ro/SSA can progress in a relatively short period of time to well-defined CTDs. The development of primary SS could be predicted by xerophthalmia and SLE by the appearance of anti-dsDNA antibodies.

Key words

UCTD, anti-Ro/SSA antibodies, Sjögren's syndrome, systemic vasculitis, anti-ENA antibodies.

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Introduction

The acronym UCTD, standing for undifferentiated connective tissue disease, has been used since 1980 to define patients with a number of clinical symptoms and signs and/or immunologic abnormalities which could suggest connective tissue disease although not sufficient to allow classification based on the generally accepted criteria (1-7). Whether the UCTDs represent distinct clinical entities or the presentation of well-defined connective tissue disease (CTD) is still a matter of debate.

Antibodies to nuclear antigens (ANA) are detected in the serum of 50-97% of patients affected by UCTD (8-14). Most of them are directed to Ro/SSA or to U1 RNP antigens. Anti-Ro/SSA antibodies are highly represented in many autoimmune diseases such as primary Sjögren's syndrome (primary SS), systemic lupus erythematosus (SLE) and subacute cutaneous lupus erythematosus (SCLE), although they are also not infrequently reported in systemic sclerosis (SSc), polymyositis (PM), mixed connective tissue disease (MCTD) and rheumatoid arthritis (RA). In addition, antibodies to Ro/SSA directed to the alternative isoforms of 52 and 60 kD have been reported in primary SS and SLE (15-16).

Few studies have addressed the outcome in anti-Ro/SSA positive patients (17). Therefore, in order to evaluate the clinical and serologic profile and the rate of progression to well defined CTD, we retrospectively studied a large cohort of patients diagnosed as having UCTD with circulating antibodies anti-Ro/SSA, selected among our patients affected by UCTD. In addition, with the aim of identifying possible predictors of disease evolution, the correlation of outcome with immunological features and clinical signs/symptoms was studied.

Patients and methods

Patients

148 patients diagnosed as UCTD, and with a minimum follow up of 12 months, were retrospectively evaluated and included in the present study. The patients were attending the Clinical Immunology Unit of the Spedali Civili of Brescia and the Rheumatology Unit of the Policlinico S. Matteo of Pavia. The diagnosis of UCTD was made on the basis of anti-Ro/SSA antibody positivity with additional clinical or serologic abnormalities not sufficient for a diagnosis of other connective tissue disease (1-7). The presence of the following clinical features was recorded: systemic symptoms (fatigue, fever), cutaneous manifestations (rash, photosensitivity, teleangectasia, sclerodactyly, livedo reticularis, alopecia, purpura), sicca syndrome, arthralgias/arthritis, myalgias/myositis, Raynaud's phenomenon, acrocyanosis, oral ulcers, serositis.

Clinical and laboratory data were identified from medical records. Immunologic investigations (ANA, ENA, anti-DNA, complement factors) were performed at every visit.

Patients were regularly seen every 6-12 months. In order to reach a final diagnosis every patient, whether symptomatic or not, underwent a complete ophthalmologic evaluation (Schirmer's test, break up time and Rose Bengal test) while minor salivary gland biopsy was performed only in patients with subjective xerostomia and/or positivity for lacrimal investigations.

Methods

Rheumatoid factor (RF) (Bouty Diagnostici, Italy), antinuclear antibodies (ANA) (indirect immunofluorescence on HEp-2 cells, Kallestad, Chaska, MN, USA), anti-dsDNA antibodies (Farr assay, Kodak Clinical Diagnostics LTD, Amersham, UK), anti-cardiolipin antibodies (home made enzyme immunoassay), and anti-ENA antibodies (detected by counterimmunoelectrophoresis) were monitored during the patient follow-up. Anticardiolipin antibodies were measured following the method suggested by the International Standardization Workshop (18). A value of 7 UI/ml or higher of dsDNA binding is considered positive in our laboratory. RF and ANA were considered positive at a titer of 1:40 or higher and 1:80 or higher, respectively.

Antibodies to SSA/Ro were determined by counterimmunoelectrophoresis (CIE) according to Bernstein *et al*. (19)

using a human spleen extract as substrate (20) and by ELISA using 52 and 60 kD recombinant proteins (Eurodiagnostica BV, Arnhem, The Netherlands; Orgentec, Meinz, Germany). Antibodies to other soluble cellular antigens were detected by CIE using a rabbit thymus extract (Peel-Freeze, Rogers, Arkansas, USA). Anti-52 and 60 kD Ro antibodies were detected by ELISA, and sera were judged positive if their optical density values were at least 3 standard deviations above the mean value of 75 CIE defined Ro negative sera (45 from normal controls and 30 antinuclear and anti-ENA negative sera from routine evaluations).

For quantitation, a titration curve was established for the anti-52 kD and 60 kD Ro antibodies. A strongly positive serum for anti-Ro 52 kD or anti-60 kD was titrated until the optical density was equivalent to that given by a normal serum at the same dilution. This point was assigned the value of 1 unit of antibody activity and the other dilutions were assigned values accordingly. The anti-Ro level of the test sample was expressed by comparison with this standard curve run on every plate.

Sera were collected and stored at -70°C for the final determination of anti-Ro antibody titers by ELISA and CIE. Serial dual dilutions of serum were tested against the same lot of extracts. All sera were analyzed at the same time, to avoid inter-assay discrepancies.

Statistical analysis

All the variables were analyzed independently using the chi-squared test with Yates' correction when indicated, or Fisher's exact test for contingency tables, as appropriate. A p value < 0.05 was considered significant. Multivariate linear regression analysis was then used to identify those variables which could jointly represent a predictor of the disease evolution.

Results

Demographic data

148 patients with antibodies to Ro/SSA and a diagnosis of UCTD at the initial evaluation were included in the present study. There was a higher prevalence of females (8.25: 1). The mean age at onset was 38.93 years (SD 13.74), while the mean age at the start of the present study was 43.7 years (SD 14). The patients ranged in age from 12 to 80 years when their UCTD was diagnosed; the total mean follow-up duration was 4.77 years (SD 4.05).

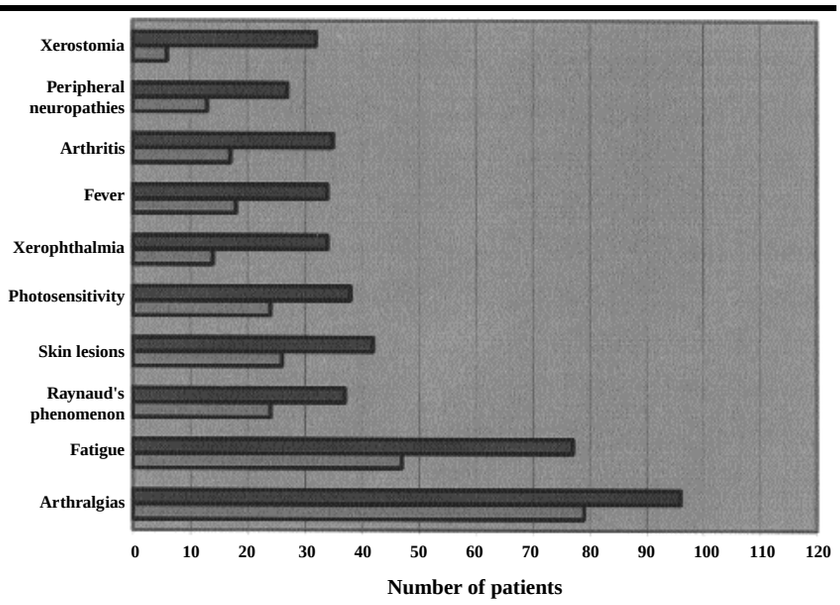

Fig. 1. Arthralgia was the most frequent clinical manifestation (70% and 86% at the onset and during follow-up, respectively), fatigue in 42% and 69%,Raynaud's phenomenon in 21% and 33%,cutaneous manifestations in 23% and 37%, photosensitivity in 21% and 34%, xerophthalmia in 12% and 30%, fever in 16% and 30%,arthritis in 15% and 31%,peripheral neuropaties in 12% and 24%,and xerostomia in 5% and 28%. Light gray bars: Onset (n = 112); dark gray bars: Follow-up (n = 112).

Development of well-defined CTD

Thirty-six out of the original 148 patients (24.3%) developed a well-defined CTD during a mean time follow-up of 4.5 years (ranging from 1 to 15 years), while 112 patients (76%) remained stable. Eighteen patients developed primary SS (50%) and 11 patients SLE (30.5%); others developed RA (2 patients), SSc (2 patients), overlap PM/SSc (1 patient), MCTD (1 patient) and Wegener's granulomatosis (WG) (1 patient). This broad spectrum of evolution is not surprising and reflects the occurrence of anti-Ro/SSA in nearly all the CTD. The mean follow-up before evolution was slightly shorter in patients who developed SLE (4 years,SD 2.96) compared to patients who developed primary SS (4.38 years, SD 4.25); the difference was not statistically significant, however. At the onset, patients who developed SLE were younger than patients with primary SS (29.36 years and 39.55 years, respectively: $p < 0.015$) and younger than the total group of UCTD patients (29.36 years and 38.9 years, respectively: $p < 0.006$).

Clinical data

Figures 1 and 2 illustrate the symptoms more commonly observed in the cohort of 148 UCTD patients studied. At onset, joint involvement was recorded in two-thirds of the patients. Fatigue was a complaint frequently observed (38%), while skin involvement and Raynaud's phenomenon were present in 26% and 24%, respectively. During follow-up, an increasing number of patients (among both those who developed a CTD and those who did not) developed additional symptoms. Arthralgias were again the most commonly reported complaint, particularly in the group of patients with stable UCTD ($p< 0.0041$), while xerophthalmia developed more frequently in the group of patients who developed another defined CTD ($p < 0.0063$), particularly primary SS ($p < 0.002$, RR: 5.64). Furthermore, skin lesions were more frequently seen in patients who developed a CTD ($p < 0.027$), even if acrocyanosis was prominent in the group of patients with stable disease ($p < 0.014$)

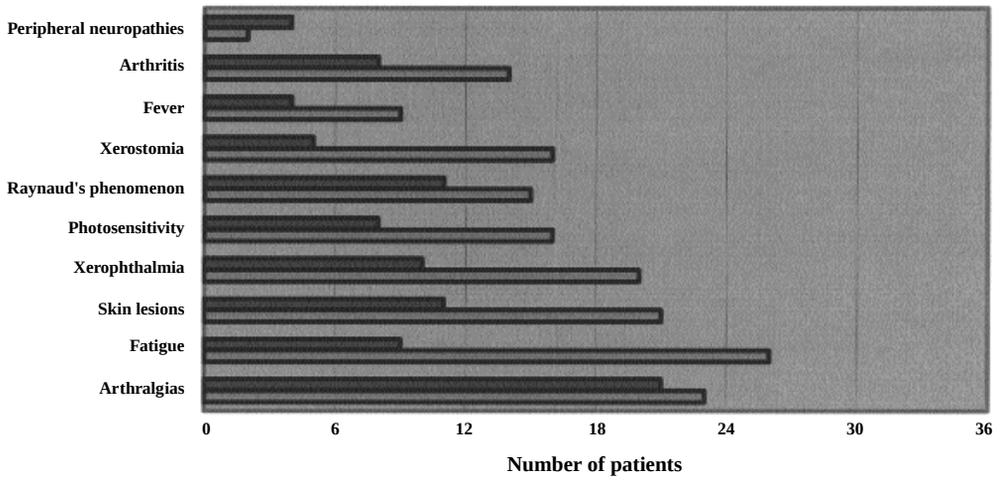

Fig. 2. Arthralgia was present in 58% and in 64% at the onset and during follow-up, respectively, fatigue in 25% and 72%,Raynaud's phenomenon in 30% and 42%,cutaneous manifestations in 33% and 58%, photosensitivity in 22% and 44%, xerophthalmia in 28% and 55%, fever in 11% and 25%, arthritis in 22% and 39%, peripheral neuropathies in 11% and in 5%, and xerostomia in 14% and in 44%. Light gray bars: during follow-up (n = 36); dark gray bars: onset (n = 36).

(Table I). Purpura was observed in 31 out of 84 patients presenting skin lesions (49%), but this manifestation did not predict the outcome of the UCTD.

Laboratory and immunological findings

Laboratory findings showed that only leukopenia appeared to be more frequently detected in patients who developed a CTD ($p < 0.0032$). (Table II). In addition, leukopenia was found to independently predict the outcome of interest by multivariate regression analysis ($p < 0.019$).

Anti-dsDNA antibodies were found in 31 out of the 146 patients tested (21%), but they seemed not to predict the evolution of UCTD into CTD, except for SLE where 6 out of the 11 evolved patients presented anti-ds DNA compared to 21 of the 110 patients with stable disease ($p < 0.0207$; RR: 5.086). No other differences between the two groups with stable or evolving UCTD were found. C3 and C4 fractions were reduced in 15% and 34% of the patients, respectively; while only 11% of patients showed decreased complement activity as measured by CH50. Elevated IgA and C reactive protein were found in 31% and 23% of patients, while cryoglobulins were detected in 5.5%. Anti-thyroglobulin and anti-thyroid peroxydase antibodies were found

Table I. Skin lesions in patients affected by UCTD with antibodies to Ro/SSA.

	Total patients (n = 63)	%	Evolved (n = 21)	%	Stable UCTD (n = 42)	%	P <
Livedo	26	68	5	24	21	50	Ns
Purpura	31	49	10	48	21	50	Ns
Acrocyanosis	10	16	0		10	24	0.014
Sclerodactyly	8	13	2	9.5	6	14	Ns
Teleangectasia	8	13	0		8	19	Ns
Urticaria	6	9.5	2	9.5	4	9.5	Ns
Scleredema	6	9.5	1	5	5	12	Ns
Psoriasis	2	3	1	5	1	2	Ns

Ns: not significant.

Table II. Serological abnormalities in patients affected by UCTD with antibodies to Ro/SSA.

	Total patients (n = 148)	%	Evolved (n = 36)	%	Stable UCTD (n = 112)	%	P <
ANA	139/148	94	34/36	94	105/112	94	Ns
Elevated ESR	98/148	66	27/36	75	71/112	63	Ns
Hypergamma globulinemia	85/147	58	23/36	64	62/111	56	Ns
Elevated IgG	47/83	57	18/25	72	29/58	50	Ns
RA test	53/142	37	17/36	47	36/106	34	Ns
Leukopenia	52/148	35	20/36	55	32/112	28	0.0032
Hyper IgM	16/79	23	8/22	36	8/57	14	Ns
Anemia	36/148	24	9/36	25	27/112	24	Ns
Anti-dsDNA	31/146	21	10/36	28	21/110	19	Ns
Antiphospholipid	9/79	11	2/22	9	7/57	12	Ns
Thrombocytopenia	17/148	11	6/36	17	11/112	10	Ns
Lymphopenia	9/91	10	1/24	4	8/67	12	Ns

Ns: not significant.

Table III. Antibodies to ENA detected by counterimmunoelectrophoresis (CIE).

Antibodies to ENA	Total UCTD (n = 148)	%	Evolved into other CTD (n = 36)	%	Stable UCTD (n = 112)	%
Antibodies to isolated Ro/SSA	95	64	20	55	75	70
Anti-Ro/SSA with other specificities:	53	36	16	44	37	33
La/SSB	48	90	15	94	33	89
RNP	3	6	1	6	2	5
Ku	2	4	1	6	1	3
Sl	1	2	0	0	1	3
Scl 70	1	2	0	0	1	3

Table IV. Fine specificity of anti-Ro/SSA antibodies determined by ELISA.

Anti-Ro/SSA	Total UCTD n = 139		Evolved into other CTD n = 35		Stable UCTD n = 104	
60 kD	6	4%	4	11%	2	2%
52 kD	48	34%	10	29%	38	36%
60+52 kD	77	55%	20	57%	57	55%
Negative	8	6%	1	3%	7	7%

in 20% and 17% of patients, respectively. Only 5% of patients had anti-mitochondrial antibodies (M2).

Autoantibodies directed to isolated Ro/SSA were detected in 95 patients, while they appeared to be associated with other ENA specificities in 53 patients. In 48 out of the 53 patients with additional anti-ENA specificity, anti-La/SSB was detected. The presence of additional anti-ENA specificity was not associated with the different outcome of UCTD. Interestingly, no autoantibodies generally considered to be markers of disease (anti-Sm, aminoacyl-t-RNA sinthetase, anti-centromere) were detected, apart from one case of anti-topoisomerase I in a patient with long-standing stable UCTD (Table III).

The fine specificity of the anti-Ro/SSA response was studied by ELISA in order to see whether a difference in the reactivity to either one of the 2 isoforms or a difference in titer could discriminate between the patients who did and did not develop a CTD (Table IV). Few sera demonstrated reactivity to isolated Ro 60 kD. Most sera showed reactivity to the 52 kD protein either in association or not in association with the 60 kD protein. Surprisingly, 8 sera showed no reactivity against the recombinant proteins.

Discussion

Antibodies to Ro/SSA are the most easily detected autoantibody specificity in autoimmune diseases and in apparently healthy subjects. Furthermore, most patients affected by UCTD with detectable antinuclear specificities have detectable anti-Ro/SSA activity in the serum (9-11). In the present study we analyzed a selected population of patients affected by UCTD in whom circulating antibodies to Ro/SSA were detected by CIE. Our results show that a substantial number of patients developed a well-defined CTD in a relatively short period of time (24.3%). The majority of these patients developed primary SS or SLE, although RA, MCTD, PM/DM, SSc and systemic vasculitis were also diagnosed. The variety of CTDs diagnosed in our study reflects the broad spectrum of diseases associated with Ro/SSA. Even the development of systemic vasculitis in one of our patients, although surprising, is supported by recent reports. Simmons O'Brien *et al.* (17), in a study on 100 patients with anti-Ro/SSA observed 2 cases which evolved into Takayasu's arteritis during a 10-year follow-up. Furthermore, the occurrence of anti-Ro/SSA in Wegener's granulomatosis was observed by Andrassy (21) and confirmed in a recent study of 24 WG patients; 7 of these WG patients had anti-Ro/SSA, but only one was diagnosed with SS while the other 6 had a normal Schirmer's test. Unfortunately, Schmidt *et al*. (22) did not specify the assay used in their study for the detection of anti-Ro.

The progression rate from UCTD to differentiated CTD reported in previously published studies (10-14, 23-25) ranges from 6% to 51%. This broad rate of evolution could be explained by the different patient selection methods used in the different studies and the different disease durations and mean follow-up (reviewed in 14). The progression rate to differentiated CTD found in our study was 24.3% after a mean follow-up period of 4.5 years. Although some studies reported a progression only to SLE (10, 26), others found a wide spectrum of evolution into other CTDs including SSc, primary SS, RA, MCTD, and PM (11-13, 23-25). In contrast to studies in which the most common CTD that developed was SLE (10, 20, 23), RA (14) or SSc (11), our patients progressed to primary SS in the majority of cases. This outcome was not unexpected in a cohort of anti-Ro/SSA positive patients.

Subjective xerophthalmia appearing during follow up represents a predictive factor of progression to a specific CTD, particularly to primary SS. In contrast, Mosca *et al*. (10) found that the presence of sicca symptoms, especially when associated with Raynaud's phenomenon or photosensitivity, showed a high inverse correlation with the development of SLE even if they appeared to be associated with the presence of antibodies to Ro/SSA. In addition, in that study none of the patients complaining of xerostomia and xerophthalmia fulfilled the criteria (the same adopted in the present study) for the diagnosis of Sjögren's syndrome (5, 6). Conversely, another study (11) found that the association of xerostomia and anti-Ro/SSA antibodies at onset can predict the progression to Sjögren's

syndrome. These contrasting results might be explained by the different number and the heterogeneity of the patients included in the different studies. In fact, progression to primary SS was observed in the majority of our patients progressing to CTD (50%), but in only 7 patients in the study by Danieli *et al.* (11) and none in the study by Mosca *et al* (10). In addition, all of our patients had antibodies to Ro/SSA while only 1 and 15 patients respectively had such autoantibodies in the other two studies (10-11).

The presence of anti-dsDNA antibodies is a predictive factor for the progression to SLE in our study. This finding confirmed the observations made by previous studies in UCTD patients by univariate (23) or multivariate analysis (11) and in a cohort of patients with anti-dsDNA antibodies and without a clearcut case of SLE (27). By multivariate regression analysis, however, only leukopenia was found to be an independent, significant predictor of the late occurrence of a well-defined CTD.

Approximately one-third of our patients exhibited a multiple antinuclear specificity. In most patients anti-La/SSB was associated with anti-Ro/SSA, even if some sera contained other specificities such as anti-U1 RNP, anti-Ku and anti-Sl or anti-Scl 70. The frequency of anti-La/SSB found in our cohort of patients was similar to the frequency found in primary SS in our laboratories, and is by far higher than that found in SLE. Nevertheless, we did not find any correlation between the presence of anti-La/SSB and the progression of the UCTD to other CTD. The same is true for the other anti-ENA specificities, at least during our follow-up. The development in UCTD patients of new antinuclear specificities during follow-up is reported by Mosca *et al*. (10) as a possible feature predicting progression to CTD. As we considered the antinuclear specificity occurring at "any point" during the disease follow-up, we cannot confirm such a hypothesis, although we did observe that the anti-ENA profile was firmly stable and only anti-dsDNA appeared at times as an adjunctive antinuclear specificity.

Finally, to analyze the value of the fine specificity of anti-Ro/SSA antibodies, we studied by ELISA the reactivity to 52 and 60 kD recombinant proteins. This approach has been shown in the past to be useful to depict different profiles of reactivity in patients with primary SS and SLE (15-16, 28), as well as in pregnant mothers whose children are at risk of congenital complete heart block (29-30) and in SLE patients with Jaccoud's arthropathy of the hands (31). No association was found between anti-Ro 60 kD or 52 kD and the outcome. Surprisingly, we found 8 sera that were positive for anti-Ro/SSA by CIE and negative by ELISA.

The low frequency of anti-60 kD compared to the 34% anti-52 kD anti-Ro positivity, as well as the high number of negative samples by ELISA, confirmed that immunodiffusion assays (double immunodiffusion and counterimmunoelectrophoresis) are more sensitive in detecting anti-60 kD Ro than ELISA, as has been previously reported (32, 33) and indicates how critical the native structure of the Ro/SSA proteins is for some anti-Ro/SSA sera.

In conclusion, our study shows that approximately one-fourth of patients with UCTD and antibodies to Ro/SSA will progress in a relatively short period of time to a well-defined CTD. Most patients develop primary SS or SLE even if cases of other types of CTDs, including Wegener's granulomatosis, were seen. Multivariate analysis showed the presence of leukopenia to be a predictive factor for CTD evolution. In addition, the development of primary SS could be predicted by xerophthalmia, and the development of SLE by the appearance of anti-dsDNA antibodies. The fine specificity of anti-Ro/SSA detected by ELISA is not adequate to predict the outcome of the UCTD, even though the few patients with isolated anti-60 kD reactivity showed a high tendency to develop a definite CTD.

Taken together, the above findings indicate that patients diagnosed with UCTD and anti-Ro/SSA antibodies should undergo careful, long-term monitoring, so that the appareance of symptoms or laboratory abnormalities indicative of progression to well defined CTD can be detected early and treated appropriately.

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