Anti-Ro/SSA antibodies in rheumatoid arthritis: Clinical and immunologic associations

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Abstract

Objective

To assess the prevalence of anti-Ro/SSA in RA and to analyse clinical and serological features of anti-Ro/SSA positive patients with RA.

Methods

195 consecutive patients affected by RA were studied by counterimmunoelectrophoresis and ELISA for the detection of anti-Ro/SSA antibodies. Anti-Ro were found in 12 patients, with a prevalence of 6%. These 12 patients were pooled with other 15 patients known to have anti-Ro/SSA antibodies and RA, in order to evaluate their clinical and laboratory features.

Results

Anti-Ro positive patients showed a common pattern of joint involvement at onset and a comparable progression of disease compared to anti-Ro negative subjects. In addition, extra-articular manifestations (such as xerophthalmia, xerostomia, scleritis, oral ulcers and amyloidosis) and peculiar autoantibody profile (hypergammaglobulinemia, anti-dsDNA and AMA) were found significantly associated to anti-Ro/SSA positivity. Even though DMARDs withdrawals were more frequently detected in anti-Ro/SSA patients, especially when using gold salts, no statistical difference between the two groups was detected. In addition, anti-TNFα treatment did not cause further progression of autoimmunity neither on laboratory nor on clinical ground.

Conclusion

Anti-Ro/SSA can be detected in about 6% of patients affected by RA. These patients presented a peculiar clinical picture characterised by extra-articular manifestations some of which are known to be anti-Ro/SSA correlated, while others are more disease-specific (amyloidosis, episcleritis). Anti-Ro/SSA are significantly associated with other autoantibodies not specific for RA such as anti-dsDNA and AMA. Treatment with anti-TNF drugs did not cause further progression of autoimmunity neither on laboratory nor on clinical ground.

Key words

Anti-Ro, ENA, rheumatoid arthritis, Sjögren's syndrome, SSA.

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Introduction

Anti-Ro/SSA antibodies are frequently described in association to many different autoimmune diseases, such as Sjögren's syndrome (SS), systemic lupus erythematosus (SLE) (1), SLE-like condition with complement factor deficit (2, 3), SCLE (4), and neonatal lupus (5). Although not considered specific markers of disease, anti-Ro/SSA antibodies were found in rheumatoid arthritis (RA) with frequency ranging from 3 to 15% (6, 7). Different reports described anti-Ro positive RA patients with more extra-articular features (sicca, skin vasculitis, leukopenia), wide immunological activation (hypergammaglobulinemia, high titer RF and ANA, activation of complement factors) and different immunogenetic features (6-11). Some of these studies also showed a strong association of anti-Ro/SSA with side effects of D-penicillamine and gold salts treatment (8-10). However, all these studies are carried out on a small number of patients each. In the present study we assessed the prevalence of anti-Ro/SSA in 195 patients using two different methods of detection. Moreover, we tried to evaluate the association between anti-Ro/SSA antibodies and different clinical and laboratory parameters.

Materials and methods

Patients

In the present study 210 patients attending a single rheumatology unit were considered. All of them were affected by RA, according to ACR criteria (12). 195 patients were consecutively enrolled in order to assess the prevalence of anti-Ro/SSA antibodies, while 15 other patients were known to be positive for anti-Ro/SSA: the two groups were pooled in order to better study clinical and serological associations and the fine specificity of the antibody. Clinical and laboratory data were identified from medical records of every patient. Articular and extra-articular features at onset and during follow up were considered. The extra-articular features were divided into vasculitic (rheumatoid nodules, scleritis, interstitial lung disease, skin vasculitis, peripheral neuropathy) and non-vasculitic type (amyloidosis, Felty's syndrome) (13). In addition, clinical manifestations usually associated with anti-Ro/SSA in Sjögren's syndrome (xerophthalmia, xerostomia) and/or in SLE (photosensitivity, malar rash, oral ulcers, serositis) were considered. Patients with subjective xerophtalmia, assessed by Vitali's questionnaire, were submitted to ophtalmologic examination including dacriologic tests (Schirmer's, BUT, Rose Bengal tests). Only 6 patients with xerostomia underwent minor salivary gland biopsy.

Articular features of RA were represented by the pattern of joint involvement at onset (oligo or polyarthritis; symmetric or asymmetric distribution; small and/or large joints) and the trend to develop bone erosions evaluated by Sharp's method (14). Clinical remission of disease was evaluated according to Pinals *et al*. (15). The number and the type of DMARDs given to each patient was considered as well as the withdrawal for major side effects.

Immunological testing

The occurrence of positive tests for rheumatoid factor (RF; Latex, Dade Behring, Marburg, Germany), anti-perinuclear factor (APF; indirect immunofluorescence on epithelial cells from human buccal mucosa); antinuclear antibodies (ANA; indirect immunofluorescence on HEp-2 cells; Kallestad, Chaska, MN, USA), anti-dsDNA antibodies (Farr assay, Kodak Clinical Diagnostics, Amersham, UK), anticardiolipin antibodies (aCL; enzyme immunoassay) and anti-extractable nuclear antigen antibodies (ENA; detected by CIE) were considered at onset and during the clinical follow-up. Anti-mitochondrial antibodies (AMA) were detected by IIF (MeDica, Sacramento, CA, USA) identifying the typical M2/M4 pattern. Anti-β2glycoprotein I antibodies were assessed by ELISA (16). ACL antibodies were measured following the method suggested by the International Standardization Workshop (17) and levels >10 GPL and/or MPL were considered positive. A value ≥ 4.2 UI/ml of dsDNA binding is considered positive in our laboratory. RF, APF and ANA were considered positive at titers ≥40 UI/ml, 1:100 and 1:80, respectively. Anti-CCP antibodies

were detected by enzyme immunoassay (Eurodiagnostica), using a cut-off value >25 Units. Antibodies to ENA were performed in all sera by CIE, according to Bernstein *et al*. (18).
Briefly, 10 µl of heat inactivated sera were loaded into the wells and then electrophorated at constant current of 12 mA for 15 minutes. Freshly rabbit thymus extract (Peel-Freeze, Rogers, AR, USA) was added and electrophoresis continued for a further 40 minutes. Antibodies to Ro/SSA were determined using the same procedure, adding human spleen extract as antigen source, prepared according to Clark *et al*. (19) and Venables *et al*. (20). The plates were then washed overnight in cold PBS and stained in 0.1% Comassie Blue, in order to obtain a permanent record of precipitin lines. Patients' sera were first screened and then characterised, using sera of defined specificity in adjacent walls in order to show lines of immunological identity. In addition, a negative control was added in every CIE test. CIE is the method used for routinely detection of anti-Ro/SSA antibodies in our laboratory. In the present study, the relative sensitivity and specificity of CIE, compared to ELISA, were 77.3% and 97.3% respectively. Anti-Ro/SSA antibodies were confirmed by ELISA in all the sera, using recombinant 52 and 60 kD Ro proteins (Pharmacia): cut-off value was recalculated, as the mean value + 3SD of 115 ANA negative and 62 ENA negative sera. Compared to CIE, relative sensitivity and specificity of ELISA were 77.3% and 97.3%, respectively.
Twenty-seven patients were anti-Ro/SSA positive, while 183 were anti-Ro/SSA negative.

Statistical analysis
All parameters were studied by χ^2 test with Yates' or Fisher's correction, when indicated. Student's t-test was used to perform comparisons between the two groups.

Results

Demographic data
210 patients from a cohort of about 1500 patients with RA attending our outpatient clinic were studied. They were 161 female and 49 male, with a female to male ratio of 3:1, as usually detected in RA. The mean age at onset was 48.8 years (SD: 14 yrs). From the first visit, the patients were followed for a mean of 10.8 years (SD: 8.6 yrs).
Fifteen patients were known to have circulating anti-Ro/SSA, detected by CIE. Twelve out of the other 195 patients were found to have anti-Ro/SSA antibodies either by CIE (7 patients) or by ELISA (5 patients): therefore the prevalence of anti-Ro/SSA in our cohort of patients of 6.1%. These 12 patients were pooled with 15 RA patients, known to have anti-Ro/SSA antibodies, in order to evaluate their clinical and laboratory features. The two anti-Ro/SSA populations did not show any difference in demographic and clinical features (data not shown).

Anti-Ro/SSA patients
The 27 patients with anti-Ro/SSA antibodies were predominantly female (n=25) with a female to male ratio (12.5:1) considerably greater than that of anti-Ro/SSA negative patients (3:1), though not statistically significant. No difference was found within the two groups of patients relatively to mean age at onset (44.9 yrs and 49.4 yrs, respectively) and duration of follow up (13.6 yrs and 10.3 yrs, respectively). Most of anti-Ro/SSA positive patients showed articular features at disease onset (88.9%), namely with polyarthritis or arthralgias in 19 and 5 patients, respectively. The mean interval between inflammatory arthritis and the onset of sicca was 4.5 years (SD: 6.41 yrs). Otherwise, isolate xerophthalmia, as the first disease manifestation, was reported only by 3 subjects (onset 3 months to 3 years before arthritis). Clinical features of the 27 anti-Ro/SSA positive patients are shown in Table I.

Articular features
Polyarthritis was usually reported at onset of RA in 90% of the patients, with symmetrical distribution and prolonged morning stiffness in 98% of cases. Small and large joints were involved in 49%, while only small joints were involved in 48% of cases, without any difference between anti-Ro/SSA positive and anti-Ro/SSA negative groups. Similarly, bone erosions were detected by X-ray during follow-up in both groups, in 78% and 77% respectively. Therefore, no differences regarding RA expression between the two groups were detected.

Extra-articular features
Xerophtalmia, xerostomia, peripheral neuropathies and rheumatoid nodules were the most frequent extra-articular features recorded in the entire cohort, as shown in Table I. Skin vasculitis was observed in 16 patients, featuring livedo reticularis in 11 cases, acral ischae-

Table I. Clinical features in 210 patients affected by RA. The significant clinical features in anti-Ro/SSA positive patients were represented by sicca symptoms, oral ulcers, scleritis and amyloidosis.

	Total 210 (%)	Ro+ 27 (%)	Ro- 183 (%)	p =
Symmetric arthritis	189 (90)	25 (92)	164 (89)	ns
Erosive arthritis	162 (77)	21 (78)	141 (77)	ns
Xerophtalmia	64 (30.5)	20 (74)	44 (24)	0.0000001
Xerostomia	58 (27.6)	15 (55)	43 (23.5)	0.0012
Peripheral neuritis	40 (19)	5 (18.5)	35 (19)	ns
Rheumatoid nodules	37 (17.6)	1 (3.7)	36 (19.7)	ns
Photosensitivity	28 (13.3)	6 (22)	22 (12)	ns
Skin vasculitis	16 (7.6)	3 (11)	13 (7)	ns
Serositis	6 (2.8)	0	6 (3.3)	ns
Malar rash	5 (2.3)	2 (7.4)	3 (1.6)	ns
Oral ulcers	4 (1.9)	3 (11)	1 (0.5)	0.0067*
Scleritis	4 (1.9)	3 (11)	1 (0.5)	0.0067*
Amyloidosis	3 (1.4)	2 (7.4)	1 (0.5)	0.042*

*Corrected Fisher's test.

mic vasculitis in three cases, purpuric lesions in two and recurrent urticaria in one case. Photosensitivity, serositis and malar rash were detected in few cases (13%, 3% and 2%, respectively). No patient developed Felty's syndrome. Anti-Ro/SSA positive patients showed more frequently ocular involvement (xerophtalmia and scleritis with p < 0.000001 and p: 0.0012 respectively), xerostomia (p: 0.0012), oral ulcers (p: 0.0067) and amyloidosis (p: 0.0042), as shown in Table I. A diagnosis of primary Sjögren's syndrome (pSS), according to Vitali's criteria (21), was achieved in 11 anti-Ro/SSA positive subjects. The diagnosis of SS was based on ocular and oral sicca (referred by all 11 subjects) in association with the positivity of Shirmers' or Rose Bengal test (positive in 9 patients) and/or salivary glands histopathology (positive in 4 patients). Clinical data of 11 SS patients are summarized in Table II.

Patients with SS/RA presented primary biliary cirrhosis in one case and tubulointerstitial nephritis in another. In any case, SS in our cohort was underestimated, especially in anti-Ro/SSA negative subjects, because only a few patients underwent salivary gland assessment tests or salivary gland biopsy.

Laboratory data and immunological findings

Anaemia, leucopenia and thrombocytopenia were detected in few cases (23, 7 and 3 patients respectively), without statistical difference between anti-Ro/SSA positive and anti-Ro/SSA negative groups. RA-specific antibodies such as RF, APF and anti-CCP were detected in about 70% of cases (76%, 72% and 68% respectively): no difference was recorded between anti-Ro/ SSA positive and anti-Ro/SSA negative patients regarding APF and/or anti-CCP antibodies. Moreover, anti-Ro/SSA positive patients showed positive rheumatoid factor more frequently than the anti-Ro/SSA negative group (85% vs 75%), without statistically significant difference (Table III).

Anti-Ro/SSA positive patients had a spread autoantibody profile (Table III). Polyclonal hypergammaglobulinemia was detected more frequently in anti-Ro/SSA positive group (54% vs 15%) with a higher mean value of globulins (20.1% vs 16.4% total serum proteins). In addition, ANA was globally detected in 89 patients (43%), usually at low titer, with a speckled pattern, more frequently in anti-Ro/SSA positive sera, as attended. Anti-dsDNA and anti-mitochondrial antibodies (AMA), globally detected in few patients, were reported more frequently in anti-Ro/SSA positive patients (11% and 16% respectively), with statistically significant difference compared to the anti-Ro/SSA negative group (p: 0.0031 and p:0.05, respectively). On the contrary, no difference in anti-CL and anti-β2glicoprotein I antibodies (anti-β2GPI) was detected between the 2 groups. Anti-ENA antibodies, performed by CIE, were positive in 25 cases: anti-Ro/SSA were detected in 22 (associated with anti-La/SSB in 2 sera), while anti-Scl70 and anti-RNP were found in other 2 different sera. Another patient had antibodies to unidentified antigens on rabbit thymus extract (UDA), using common reference monospecific sera.

Each serum was tested by ELISA to detect anti-Ro/SSA antibodies: 17 sera resulted positive and 183 sera negative by both CIE and ELISA, showing a concordance rate between methods of 95%. Five sera, negative by CIE, showed anti-Ro/SSA reactivity by ELISA at low titer: this positivity has been confirmed, performing ELISA, at least 3 times using sera obtained at different times from each patient during clinical follow-up. On the contrary, other five sera, anti-Ro/SSA positive by CIE, re-

Table II. Clinical data on 11 patients affected by SS and RA; they showed erosive symmetric arthritis, mostly characterised by positivity of RF, APF and anti-CCP, as well as the other anti-Ro/SSA negative RA patients.

	n = 11	(%)
Symmetric polyarthritis	10	91
Erosive arthritis	8	72.7
Xerophtalmia	11	100
Xerostomia	11	100
Positive dacriologic tests	9	81.8
Positive salivary gland biopsy	4/6	66.7
Photosensitivity	2	18
Peripheral neuritis	3	27
Oral ulcers	2	18
Rheumatoid Factor	9	81.8
APF	7	63.6
Anti-CCP	6/8	75

Table III. Immunological findings in 210 patients affected by RA. Polyclonal hypergammaglobulinemia, anti-dsDNA and anti-mitochondrial antibodies (AMA) were reported more frequently in anti-Ro/SSA positive patients, with statistically significant difference compared to anti-Ro/SSA negative group.

	Total 210 (%)	Ro+ 27 (%)	Ro- 183 (%)	p <
Hypergammaglobulinemia	42/197 (21.3)	14/26 (53.8)	28/181 (15.4)	0.000001
Mean value gammaglobulins	16.86	20.13	16.4	0.000064*
SD	4.52	5.4	4.19	
RF	160 (76.2)	23 (85)	137 (75)	Ns
APF	149/207 (72)	20/27 (74)	129/180 (71.7)	Ns
Anti-CCP	118/172 (68.6)	16/21 (76.2)	102/151 (67.5)	Ns
ANA	89/207 (43)	24 (88.9)	65/180 (36)	0.0000001
Anti-dsDNA	6/162 (3.7)	3/26 (11.5)	3/136 (2.2)	0.0031
Anti-CL	5/123 (4)	1/18 (5.5)	4/95 (4)	ns
Anti-β2-GPI	6/48 (12.5)	3/9 (33.3)	3/39 (7.6)	ns
AMA	2/59 (3.4)	2/12 (16)	0/47	0.05
ENA by CIE Total	25 (12)	23/27 (85)	2/183 (1)	0.0000001
Ro	20	20	0	
Ro+La	2	2	0	
Scl70	1	1	0	
U1-RNP	1	0	1	
UDA	1	0	1	

*Analysed by Student's t test; UDA: unidentified antigen.

sulted negative by ELISA. The study of the fine specificity of 27 anti-Ro/SSA positive sera was performed by ELISA, using recombinant 52 and 60 kD Ro proteins as substrates. Seventeen sera were anti-Ro/SSA positive by CIE and ELISA: 11 of them showed isolate anti-Ro/SSA 60 kD and 6 sera showed antibodies to 60 kD and 52 kD Ro proteins. Five sera negative by CIE showed reactivity to Ro/SSA 60 kD in three cases and to Ro 52 kD in two.

DMARD treatment

All 210 patients were treated by one or more DMARDs (mean: 3.93), without any difference between the two groups about the number of DMARDs used, as shown in Table IV. Hydroxycloroquine, methotrexate, sulphasalazine and cyclosporin A were given in 97%, 90%, 54% and 51% of cases respectively. Hydroxycloroquine was used more frequently by anti-Ro/SSA negative than anti-Ro/SSA positive patients (99% vs 85%). Infliximab treatment was set out in 68 patients, mostly without anti-Ro/SSA antibodies (35% of anti-Ro/SSA negative vs 11% of anti-Ro/SSA positive groups). On the contrary, D-penicillamine was used more frequently in anti-Ro/SSA positive group mostly at the onset of RA, probably due to a longer duration of the disease compared to anti-Ro/SSA negative group. Of the 5 patients with anti-Ro/SSA antibodies who was given anti-TNFα treatment, only one, with HCV-related hepatitis, had withdrawn etanercept because of elevation of liver enzymes. The other 4 patients (3 on infliximab and one on etanercept) showed a fairly good response and are still on treatment after a mean of 38.9 months of follow-up (range 33 to 52 months).

During follow-up, DMARDs treatment was stopped 52 times due to side effects (52 events on 785 DMARDs globally used in 210 patients). Even though anti-Ro/SSA positive patients reported more frequently DMARDs withdrawals (9.3% vs 6%), especially when using gold salts, no statistical difference between the two groups were detected.

Table IV. DMARD treatments in 210 patients. Hydroxycloroquine and infliximab were used more frequently by anti-Ro/SSA negative than anti-Ro/SSA positive patients In contrast, D-penicillamine was used more frequently in anti-Ro/SSA positive group mostly at the onset of RA.

	Total 210 (%)	Ro+27 (%)	Ro - 183 (%)	p =
No. of DMARDs (mean)	3.93	4.11	3.91	ns
SD	2.10	2.22	2.09	
Hydroxycloroquine	204 (97)	23 (85)	181 (98.9)	0.0007
Methotrexate	189 (90)	23 (85)	166 (91)	ns
Gold salts	113 (53.8)	16 (59.2)	97 (53)	ns
Sulphasalazine	107 (51)	11 (41)	96 (52.4)	ns
Infliximab	68 (32.4)	3 (11)	65 (35.5)	0.02
Cyclosporin A	65 (31)	8 (29.6)	57 (31)	ns
D-penicillamine	24 (11.4)	7 (25.6)	17 (9.3)	0.026
Etanercept	15 (7)	2 (7.4)	13 (7.1)	ns

Discussion

The main points outlined by the present study are the following:

- Assessment of the prevalence of the anti-Ro/SSA antibodies in rheumatoid arthritis using two different assay in combination;
- Delineation of a clinical subgroup of RA with peculiar clinical and immunological features;
- Assessment of the drug side effects.

Testing RA sera for anti-Ro/SSA with two different methods detected the antibody in about 6% of patients. This finding is in line with previous reports using double immunodiffusion, CIE, ELISA with extractive antigen and even immunoprecipitation assays, and outlined both the autoimmune nature of RA and the clinical heterogeneity of the erosive polyarthritis (6, 7). In fact, patients with or without anti-Ro/SSA antibodies did not differ for clinical features strictly related to the onset or the outcome of the articular manifestations. In the present study we have demonstrated a common pattern of joint involvement at onset in the two group of patients and a comparable progression of disease with respect to development of erosions, response to DMARDs and rate of remission. Even the prevalence of autoantibodies considered markers of disease, namely RF, anti-CCP or APF, did not differ in patients with and without anti-Ro/SSA. Given the reported predictive value for these antibodies for the development of more severe disease with erosions, one would expect the same rate of progression of disease independently from other additional autoantibodies.

These findings are important if one considers that arthritis occurs with high frequency in SLE or Sjögren's syndrome and it is, by definition, non-erosive. In addition, we and other (22, 23) have described a strict association between anti-Ro/SSA and anti-La/SSB and the non-erosive, deforming arthropathy of Jaccoud-type in SLE, while other authors reported few patients with an overlap disease between SLE and RA called "rhupus" (24). About 50% of these "rhupus" patients had anti-Ro/SSA antibodies (25). Therefore, anti-Ro/SSA can be detected in patients with arthritis who can or cannot develop an erosive disease but extra-articular manifestations and laboratory features seem to strictly correlate to the presence of such antibody specificity much more than the pattern of articular disease. In fact, extra-articular manifestations and laboratory features are quite different between patients with or without ant-Ro/SSA antibodies. Xerostomia, xerophtalmia (dry eyes) and photosensitivity are significantly associated with anti-Ro/SSA even in RA as already demonstrated in SLE (26) and Sjögren's syndrome (27), and therefore appear to be more antibody associated than disease-specific.

Surprisingly, we also found a significant increase of episcleritis and amyloidosis not previously associated with anti-Ro/SSA in other diseases. The number of patients with such events was very small and therefore our observation need to be confirmed in future studies.

Some authors (8-10) have reported a high incidence of side-effects induced by gold salt and D-penicillamine. In

our study we noted only a slight trend to the development of gold salt side effects in anti-Ro/SSA positive group of patients though not statistically significant. Though anti-TNFα treatment has been reported to induce autoimmunity and, at least, autoantibodies (28-31), in our experience this does not represent a major concern in patients already presenting autoantibody to Ro/SSA before treatment. In fact, only one of our 5 patients receiving either infliximab or etanercept developed clinical manifestations of systemic autoimmune diseases, while none of the patients developed significant increase in autoantibodies' titer or new antibody specificities. It is well known that autoantibodies, developing during anti-TNFα treatment, are prevalently of IgM isotype (32). The assays employed for their detection in the present study were able to detect any isotype of autoantibody. In fact both CIE and Farr assay detect functional activity of antibodies and not Ig isotypes. On the contrary for the anti-52 Ro and anti-60 Ro ELISA we used an anti-IgG antibody conjugate. Noteworthy a patient with anti-Ro/SSA was affected by primary biliary cirrhosis, diagnosed 5 years before anti-TNF-α treatment. She was under ursodesoxycholic drug and the dosage remained unchanged during the follow-up. In addition, a substantial stability of cholestatic enzymes (GGT and ALP) and histopathological features were observed during anti-TNFα treatment.

In conclusion, the present study confirms that anti-Ro/SSA can be detected in about 6% of patients affected by RA. These patients presented a peculiar clinical picture characterised by extra-articular manifestations some of which are known to be anti-Ro/SSA correlated, while others are more disease-specific (amyloidosis, episcleritis). Anti-Ro/SSA are significantly associated with other autoantibodies not specific for RA such as anti-dsDNA and AMA. Treatment with anti-TNFα drugs did not cause further progression of autoimmunity neither on laboratory nor on clinical grounds.

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