

Fluctuation of anti-Ro/SS-A antibody levels in patients with systemic lupus erythematosus and Sjögren's syndrome: A prospective study

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Abstract

Objective

To determine whether the titers of anti-Ro/SS-A (Ro) antibodies fluctuate during the course of SLE and Sjögren's syndrome (SS) in parallel with disease activity, and if such fluctuations could be used to predict disease flares. We also evaluated whether the anti-Ro profile (anti-Ro 52, anti-Ro 60) changes over time, since such information could provide new insights into the induction and regulation of anti-Ro autoimmunity.

Methods

Sixteen patients with SLE and 15 patients with SS, all anti-Ro/SS-A antibody positive, were followed up for two years at three-month intervals. Clinical and laboratory parameters of disease activity were examined. Determination of the anti-Ro/SS-A titer was performed by counterimmunoelectrophoresis and the fine anti-Ro antibody specificity was determined by immunoblotting.

Results

The titers of anti-Ro antibodies fluctuated during the course of the illness in both SLE and SS patients. In SLE patients these changes were not (except in one case) associated with disease activity nor were they predictive of disease flares. The same was true for the SS patients, with the exception of two patients with skin vasculitis in whom anti-Ro antibody titers fluctuated in parallel with the disease activity. The anti-Ro antibody (anti-Ro 60 kD, anti-Ro 52 kD) specificity did not change in any of the patients during the follow-up period.

Conclusion

Anti-Ro antibodies could represent a valuable indicator of disease activity in SS patients with cutaneous disorders. They do not, on the other hand, reflect disease activity in patients with SLE. The stable antibody profile in both SLE and SS patients supports the hypothesis that autoantibody production is predominantly genetically regulated.

Key words

Systemic lupus erythematosus, Sjögren's syndrome, Ro/SS-A antibodies, prospective study.

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Introduction

Anti-Ro/SS-A (Ro) antibodies occur in 40-50% of patients with systemic lupus erythematosus (SLE), in 60-75% of patients with primary Sjögren's syndrome (SS) and in a high proportion of patients with secondary SS (1). These antibodies have diagnostic value for some of the clinical subsets of SLE, including subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, homozygous C2 and C4 deficiency, and ANA negative lupus (2, 3). Certain clinical manifestations of SLE such as photosensitive rash and interstitial lung disease are apparently associated with the presence of anti-Ro antibodies (4). Their presence in SS is associated with serologic hyperreactivity, vasculitis and nervous system involvement (5, 6). Furthermore, there is evidence that the immune complexes of Ro/anti-Ro antibody may be directly involved in the pathogenesis of SLE in humans, especially in the heart disease of neonatal lupus (7).

Anti-Ro antibodies bind to several intracellular proteins, predominantly the 52 and 60 kD Ro antigens, which differ in terms of their antigenicity and structure. Patients suffering from SLE and SS have been found to have various types of anti-Ro reactivity. Some have antibodies against both the 52 kD and 60 kD proteins, while others have antibodies against only one of them. So far, no SLE or SS specific anti-Ro antibodies have been found (8, 9). Anti-La/SS-B (La) antibodies are frequently present in the sera containing anti-Ro antibodies. This might be, at least in part, explained by the coexistence of La and 60 kD Ro proteins in the same ribonucleoprotein complex (10). It is, however, uncertain whether the 52 kD Ro protein is also a component of this complex (8, 11). The mechanisms underlying both the induction and regulation of the immune response against the various Ro polypeptides are still unclear. In most instances, an individual patient's autoantibody profile does not change significantly over time (12, 13). On the other hand, there is some evidence of intermolecular-intrastructural spreading of the autoantibody response to Ro particles (11, 14).

Here we report the results of a two-year prospective study of anti-Ro positive

SLE and SS patients. More specifically, we studied the possible association between anti-Ro antibody titers and the clinical course of the disease. We also investigated whether the anti-Ro antibody profiles, as evaluated by SDS-immunoblotting, remained constant or if they changed over time, and whether such findings were related to the disease course.

Patients and methods

Patients

Thirty-one consecutive SLE and SS patients were selected for our prospective study based on the presence of anti-Ro antibodies as determined by counter-immunoelectrophoresis (CIE). All were Caucasians. Twelve patients (11 females, 1 male) fulfilled the ACR criteria (15) for SLE, 15 patients (all females) fulfilled Fox's criteria for primary SS (16), and 4 patients (all females) had SLE associated with SS. The average age of the patients was 31.5 years (range 18 to 54 years). Patients were followed up for two years. The clinical and laboratory parameters of disease activity were examined every 3 months. Serum samples were collected and stored at -70°C for the final determination of anti-Ro antibody titers. An additional evaluation was performed in cases of a disease flare occurring between two regular evaluations. All patients gave their informed consent before entering the study, and the study protocol was approved by the National Ethics Committee.

Disease activity criteria

SLE activity was assessed using the ECLAM scoring system (17). SS activity was evaluated based on the presence of extraglandular manifestations. Flares were classified as either present or absent and were also divided into subgroups such as vasculitic, central nervous system, serosal, hematological and musculoskeletal.

Counterimmunoelectrophoresis (CIE)

The titers of anti-Ro autoantibody were determined by CIE, which was performed as reported by Bunn and Kveder (18). Human spleen extract was used as a source of antigen (18, 19). Serial double dilutions of serum were tested against

the same lot of extract in sets. All sera were analyzed simultaneously to avoid inter-assay discrepancies.

Immunoblotting (IB)

A cytoplasmic extract of HeLa cells was prepared according to the method of Ben-Chetrit *et al.* (20) and was separated on a 10% SDS-polyacrylamide gel with a modified acrylamide: bisacrylamide ratio to allow an efficient separation of the Ro 52 and Ro 60 proteins, as described elsewhere (21-23).

Statistics

Serial changes in the level of anti-Ro antibody were analyzed in relation to the SLE disease activity index (ECLAM). Spearman's rank correlation with ties was calculated. Regression analysis was used to test the possible correlation between disease flares and anti-Ro antibody titers at 3 and 6 months before the flares appeared. The correlation between the changes in anti-Ro antibody titers and the presence or absence of extra-glandular manifestations in SS patients was not calculated due to the small number of changes recorded in the titer and/or disease flare.

Results

Anti-Ro antibody levels in relation to SLE disease activity

The changes in anti-Ro antibody titers in the 16 SLE patients are shown in Table I. A four-fold change in titer was considered significant.

Stable levels of anti-Ro antibodies were found in the sera of 6 patients. Five of these had stable or inactive disease and were on low or tapering doses of steroids; no statistical analysis was performed in these patients. The sixth patient with a stable anti-Ro antibody titer (no. 12) experienced a disease flare with malar rash, lupus glomerulonephritis and high fever in the ninth month of follow-up. She was treated with pulse cyclophosphamide and steroids.

The anti-Ro antibody titers fluctuated in the sera of the remaining 10 patients, 4 of whom had associated SS. All 10 patients experienced either major or minor disease flares. However, the changes in disease activity were not, except in one case, associated with the fluctuations in anti-Ro antibody titers. In patient no. 2 the antibody titer increased coincidentally with the occurrence of glomerulonephritis and pleuritis. She was treated

with high dose steroids and pulse cyclophosphamide and her ECLAM disease activity score fell from 8 to 2 within one month. At the same time her anti-Ro antibody titer, which was 8 at the peak of disease activity, dropped to undetectable levels. Patient no. 3 experienced a disease flare (pneumonitis, pleuritis) one month after a regular visit, but her antibody level did not predict the disease flare. Patients no. 10 and 12 were tested again 15 and 40 days, respectively, after their regular visit. In both cases their disease progressed to lupus glomerulonephritis. In neither case, however, did the antibody levels have a predictive value for the disease exacerbation.

Anti-Ro antibody level in relation to SS disease activity

Constant levels of anti-Ro antibodies were found in 11 patients with primary SS (Table II). Nine of them had stable or inactive disease and none were on immunomodulatory treatment. Of the remaining two, one had two episodes of disease flare involving the joints and pleura, and the other had one episode manifesting as arthritis and leucopenia. The titers of anti-Ro autoantibody fluctuated

Table I. Correlation between anti-Ro antibody titers and disease activity in SLE patients in three-months intervals. In the case of stable disease and/or anti-Ro antibody titer, the correlation was not calculated.

Pt. no.	Anti-Ro titers by CIE (dilutions)	Anti-Ro specificity by IB	Disease activity by ECLAM score	r_s	Correlation p
1	2-0-0-0-2-2-2-2	52 kD	5-2-2-2-3-2-2-2	0.04	NS
2	4-2-8-0-0-0-0-0	60 kD	4-4-8-2-2-0-0-0	0.88	< 0.05
3#	8-0-2-2-2-0-4-0-8-4	52 kD	8-3-3-1-1-4-1-1-0-0	-0.31	NS
4	8-8-8-8-4-2-0-2-2	52, 60 kD	6-4-2-2-2-4-4-3-2	-0.08	NS
5	4 (no changes)	neg	3 (no changes)	ND	
6	8-8-8-8-0-8-8-8-8	52, 60 kD	6-2-5-2-2-5-2-2-2	0.25	NS
7	8 (no changes)	60 kD	0 (no changes)	ND	
8	8 (no changes)	60 kD	2 (no changes)	ND	
9	8 (no changes)	60 kD	3 (no changes)	ND	
10#	0-256-0-0-0-0-0-1-0	52, 60 kD	8-4-3-4-6-4-3-4-3-3	-0.12	NS
11	64 (no changes)	neg	3 (no changes)	ND	
12#	128 (no changes)	52 kD	3-3-4-8-6-4-2-2-2-2	ND	
13*	2048-1024-2048-512-2048-2048-2048-2048	52, 60 kD	7-2-1-1-1-1-1-1-1	-0.22	NS
14*	1024-2048-512-1024-1024-1024-1024-1024	60 kD	3-3-4-3-3-3-2-2-2	-0.31	NS
15*	512-256-512-256-128-256-256-128-256	52, 60 kD	3-3-4-4-3-3-3-2-2	0.50	NS
16*	512-128-512-512-256-512-256-512-256	52, 60 kD	4-3-3-4-3-3-3-2-1	0.33	NS

*SLE patients with associated Sjögren's syndrome; # additional visits had been made due to disease flare.

CIE: counterimmunoelectrophoresis, IB: immunoblot, neg: negative, r_s : Spearman's rank coefficient with ties, ND: not done, NS: not significant, p: probability at the critical values for r_s .

Table II. Association between changes in anti-Ro antibody titers and disease activity (classified as the presence or absence of extraglandular manifestations) in primary Sjögren's syndrome patients in three-month intervals.

Pt.no.	Anti-Ro titer by CIE (dilutions)	Anti-Ro specificity by IB	Disease flare (extragland. manif.)
1	8 (no changes)	neg	- (no changes)
2	8 (no changes)	60 kD	- (no changes)
3	8-8-8-16-32-16-8-8-8	60 kD	- - - - + - - -
4	32 (no changes)	52 kD	- (no changes)
5	64 (no changes)	52, 60 kD	- + - - + - - -
6	256-128-128-128-128-128-64-128-32	52, 60 kD	- - - + + - - -
7	128 (no changes)	60 kD	- (no changes)
8	512-256-512-1024-512-256-512-512-512	52, 60 kD	- (no changes)
9	512 (no changes)	52, 60 kD	- (no changes)
10	512 (no changes)	60 kD	- (no changes)
11	2048-2048-64-512-512-512-512-512	60 kD	+ + - - - - - -
12	1024 (no changes)	neg	- - - - - + - -
13	2048 (no changes)	60 kD	- (no changes)
14	2048 (no changes)	60 kD	- (no changes)
15	2048 (no changes)	60 kD	- (no changes)

CIE: counterimmuno-electrophoresis, IB: immunoblot, (-): negative.

tuated in 4 patients. In one of them (no. 8) anti-Ro autoantibody titers changed in the absence of active disease. One patient (no. 6) experienced arthritis and leucopenia, which were not concomitant with the changes in her anti-Ro antibody titer. The remaining two patients (nos. 3 and 11) had vasculitic skin changes whose appearance coincided with their peak levels of anti-Ro antibodies. Patient no. 11 was a 35-year-old woman who had been suffering from primary SS

for 3 years. At the time of her inclusion in the study she presented with fever and cutaneous vasculitis manifesting as both palpable and non-palpable purpura over the arms and legs. Histopathologic examination of the skin showed a leukocytoclastic type of vasculitis. Laboratory analysis revealed hypergammaglobulinemia, hypocomplementemia and a high titer of anti-Ro antibodies (Fig. 1). Treatment with prednisolone (0.5 mg/kg body weight) resulted in regression of

the skin changes within one month. The anti-Ro antibody titer concomitantly decreased to 64. During the next 3 months the titers rose to 512; prednisolone therapy was stopped after this increase. The patient remained well thereafter and her anti-Ro antibodies did not change.

Patient no. 3 was a 26-year-old woman with a 2-year history of SS. Initially her predominant symptoms were dry mouth and dry eyes. Her anti-Ro antibody titer was 8 at that time and no clinical signs of disease activity were apparent (Fig. 2). During the following 9 months, her anti-Ro antibody titer gradually increased. This trend continued, and after 12 months she developed a maculopapular rash on the face and arms. Histological analysis of a skin specimen revealed small vessel vasculitis with mononuclear cell infiltration. While her complement activity and platelet count were within normal ranges, her gammaglobulin levels were significantly increased. The cutaneous vasculitis gradually disappeared over a period of three months without treatment, and her anti-Ro antibody titers decreased concomitantly.

Anti-Ro antibody profiles

The fine specificity of anti-Ro autoantibodies in the SLE and SS patients is summarized in Tables I and II. Two patients from each disease group tested positive for anti-Ro antibodies on CIE,

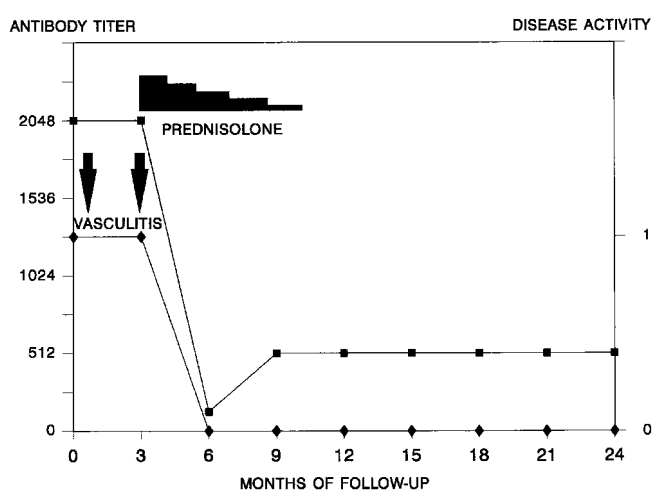


Fig. 1. Changes in the titer of antibodies to Ro (■) and disease activity (skin vasculitis) (●) in patient no. 11 with primary Sjögren syndrome. Disease activity was determined as the presence (1) or absence (0) of extraglandular symptoms.

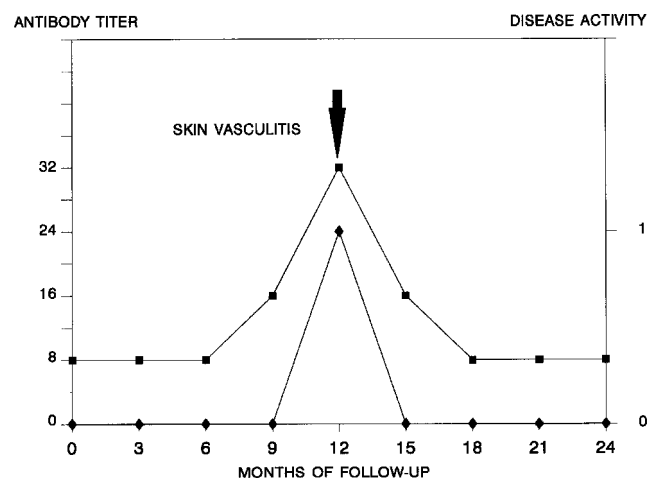


Fig. 2. Changes in the titer of antibodies to Ro (■) and disease activity (skin vasculitis) (●) in patient no. 3 with primary Sjögren syndrome. Disease activity was determined as the presence (1) or absence (0) of extraglandular symptoms.

but were negative on IB. In none of the patients did the autoantibody pattern found at the beginning of the study change during the two-year follow-up period.

Discussion

The immunopathogenesis of SLE and related diseases is not completely understood. There is some evidence that the immune complexes of Ro/anti-Ro antibodies play a pathogenic role. The levels of antibodies to Ro proteins were found to be raised in acid eluates from the affected organs of SLE and SS patients. These included two cases of lupus nephritis, a parotid gland from a patient with SS, and the heart of a child who died of complete congenital heart block (7, 24, 25). Anti-Ro antibodies have been shown to induce repolarization abnormalities in neonatal rabbit hearts (26). More recently, it has been demonstrated that following ultraviolet exposure, keratinocytes from some patients with SLE produced up to a 2000-fold greater amount of Ro protein than keratinocytes from normals (27). These increases in the expression of Ro antigen on the cell surface may enhance the possibility of direct injury to skin cells by anti-Ro antibodies. If anti-Ro antibodies do indeed reflect disease activity at the tissue level, we would expect at least some fluctuations in the sera. Scopelitis *et al.* (28) retrospectively demonstrated that the titers of anti-Ro antibody changed in relation to certain types of SLE disease activity, in particular serositis. However, it was concluded that the duration of the increases and decreases in anti-Ro antibody titers appeared to be longer compared to other antibodies. No prospective studies on the correlation between anti-Ro antibody titers and disease activity in SLE and related diseases have yet been published.

Our primary goal was to study the fluctuation in titers of the overall population of antibodies against native Ro proteins, and not of the antibodies against particular anti-Ro subsets (Ro 52, Ro 60). We therefore chose CIE as the technique to qualitatively and quantitatively detect the whole population of anti-Ro antibodies. It represents one of the most reliable methods based on human extracts as na-

tive antigen sources (29), exhibiting 100% specificity and 85-90% sensitivity (1).

We found that the onset of skin vasculitis in two SS patients was accompanied by a rise in anti-Ro antibody titers. According to the IB results, the anti-Ro antibody response was directed toward the Ro 60 protein in both cases. The titers decreased concomitantly with the improvement in vasculitis. These fluctuations were not likely to be a consequence of the therapy. One patient had not received any immunomodulatory treatment and in the other patient discontinuation of prednisolone was not followed by any change in anti-Ro antibody titers. Since only these two patients with SS developed vasculitis, one could speculate that this manifestation could have been a consequence of the damage caused by anti-Ro antibodies.

It has been well documented that some of the cutaneous disorders that arise in SS are associated with the presence of anti-Ro antibodies (5, 30-33). Alexander *et al.* (32) reported a significant increase in the frequency of vasculitis in anti-Ro positive primary and secondary SS compared to anti-Ro negative SS patients. He detected anti-Ro antibodies in 86% of the SS patients with vasculitis, compared to only 19% of the patients without vasculitis. The rise in anti-Ro antibody titers associated with skin vasculitis in our patients could represent even clearer evidence of this conjunction. Provost *et al.* (34) reported two types of vasculitis in SS patients: a leucocytoclastic type that was associated with high titers of anti-Ro antibodies, rheumatoid factor, hyper-gammaglobulinemia and hypocomplementemia; and a mononuclear inflammatory type found in association with a low titer of anti-Ro antibodies, normal complement activity and gammaglobulins (34). Each of our patients fell into one of these two groups.

On the other hand, we were not able to find similar associations in SLE patients. In the SLE patients with unstable disease activity, the anti-Ro titers fluctuated in all but one patient. However, with the exception of one case, these changes were not associated with the disease activity. In one SLE patient the antibody titer increased simultaneously with the

occurrence of glomerulonephritis, arthritis and pleuritis. This did not occur in any of the other patients who suffered an exacerbation of either glomerulonephritis or pleuritis. Furthermore, one patient with a glomerulonephritis flare had stable levels of anti-Ro antibodies. Anti-Ro antibody levels did not change with other types of disease activity as well in SLE patients. None of the SLE patients developed skin changes during the follow-up period, except for the occurrence of malar rash in one patient. We therefore could not confirm the presence of an association similar to that found in SS patients. It can be concluded that serial measurements of anti-Ro antibody levels have only limited clinical value in predicting SLE activity or exacerbations.

The fine specificity of anti-Ro antibodies in both groups of patients remained stable over the follow-up period. Similar results were reported by Tseng *et al.* (35): sera from 23 mothers whose children had neonatal lupus were evaluated by SDS-immunoblot. The anti-Ro antibody profile was stable in all of the tested cases.

The induction and regulation of immune response against the various Ro polypeptides are still unclear. However, recently Scofield *et al.* (14) provided further evidence for the intermolecular-intrastructural spreading of the autoantibody response to Ro particles. They immunized NZB rabbits with 60 kD Ro-derived synthetic peptides. The animals not only produced antibodies to the injected peptide, but also mounted an immune response against the entire 60 kD protein. In addition, 20% of the rabbits also produced anti-La antibodies. Similarly, Topfer *et al.* (11) demonstrated that the immunization of healthy non-autoimmune mice with recombinant mouse or human La proteins elicited specific anti-Ro 60 kD IgG antibodies in all of the strains tested. Mice immunized with 60 kD Ro protein produced a high titer anti-Ro antibody response, which was also associated with intermolecular spreading, resulting in the specific appearance of anti-La antibodies.

It could be speculated that we did not find evidence for autoimmunity spreading between Ro 52 kD and Ro 60 kD in

our patients because these two proteins may not be components of the same complex (8, 11, 14). We also did not find any new immunity against the La protein during the follow-up period (data not shown). It seems that the antibody profile against the Ro ribonucleoprotein particle does not change over time in humans. One possible explanation could be that genetic factors represent a major determinant for the expression of various antibody populations (36). In summary, fluctuations in the titers of anti-Ro antibodies only occasionally correlate with clinical disease expression, at least in SLE patients. On the other hand they could represent an indicator of the disease activity in SS patients with cutaneous disorders.

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