Rheumatology 1999;38:1228–1233

Recurrent pregnancy loss and autoantibody profile in autoimmune diseases

C. P. Mavragani, J. P. A. Ioannidis[1], A. G. Tzioufas, I. E. Hantoumi and H. M. Moutsopoulos

Department of Pathophysiology, School of Medicine, National University of Athens and [1]Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece

Abstract

Objective. To explore the association of non-organ-specific autoimmune responses against three distinct Ro antigen-related reactivities (Ro52, Ro60, p57) with a history of pregnancy loss in women with autoimmune disorders.

Materials and methods. Seventy unselected anti-Ro/SSA-positive women were studied in a retrospective cohort study. Forty anti-Ro/SSA-positive women were age matched to an equal number of women with autoimmune disorders who were anti-Ro/SSA negative in a case–control study. The association of reactivities against three distinct antigen specificities (Ro52, Ro60, p57) with recurrent pregnancy loss was investigated. Independence and modification of these associations from the effect of antithyroglobulin, antithyroid peroxidase and anticardiolipin antibodies were also examined.

Results. In the cohort study, reactivity against each of the three antigen specificities (Ro52, Ro60, p57) was independently associated with a history of recurrent pregnancy loss. In the case–control study, the effects were still independent and were not modified when other autoantibodies were considered. In particular, the number of reactivities against Ro52, Ro60 and p57 peptides, and the presence of antithyroglobulin antibodies, were independent predictors of recurrent pregnancy loss (odds ratios 3.35 per each additional reactivity and 5.54 in the presence of antithyroglobulin; $P = 0.002$ and 0.025, respectively).

Conclusions. In women with autoimmune disorders, a history of recurrent pregnancy loss is independently associated with reactivity against each of the three antigen specificities (Ro52, Ro60, p57) and also with the presence of antithyroglobulin antibodies, suggesting that cumulative autoimmune responses against these non-organ-specific and organ-specific antigens correlate with the risk of stillbirth and spontaneous abortion.

KEY WORDS: Recurrent pregnancy loss, Anti-Ro/SSA, Antithyroid antibodies, Autoimmune diseases, Anti-p57.

Among the multiple factors implicated in the pathogenesis of adverse pregnancy outcome, autoimmune disorders appear to play a prominent role [1–3]. Anti-Ro/SSA antibody (the main causative factor of congenital heart block) has been considered recently in the pathogenesis of pregnancy loss in women with connective tissue disorders, including both systemic lupus erythematosus (SLE) [4–7] and other autoimmune disorders such as Sjögren's syndrome (SS) and rheumatoid arthritis (RA) [7]. It is well known that anti-Ro/SSA-positive sera may contain either of two sets of antibodies recognizing either a 60 kDa (Ro60) or a 52 kDa (Ro52) polypeptidic component of the Ro molecule [8]. Moreover, IgG antibodies against a 57 kDa protein (p57) were recently proposed as an additional risk factor in the pathogenesis of neonatal lupus syndrome. These antibodies are found in 10% of SLE patients, always in association with anti-Ro/SSA antibodies [9]. The extent to which antibodies against each of the three peptides described may interact or contribute independently to recurrent pregnancy loss in patients with SLE and other autoimmune diseases is not known.

In the present work, we evaluated whether reactivity against each of the three determined antigenic specificities is independently associated with the risk of recurrent pregnancy loss in women with a variety of autoimmune disorders, and whether these associations may be confounded by other potentially important autoimmune risk factors, in particular the presence of antithyroid antibodies, often detected in these patients [10, 11]. To achieve this aim, we first analysed data on Ro52, Ro60 and p57 peptides and pregnancy outcomes from a cohort

Submitted 18 August 1998; revised version accepted 6 July 1999.

Correspondence to: H. M. Moutsopoulos, 75, M.Asias, 115 27, Athens, Greece.

© 1999 British Society for Rheumatology

study of 70 women known to have anti-Ro/SSA responses. Furthermore, in order to evaluate better whether the association of the above responses was independent of other autoimmunity risk factors which have been linked to recurrent pregnancy loss, such as antithyroid antibodies [12–14] and antiphospholipid antibodies [15–17], we performed a case–control study including 40 Ro/SSA-positive women and 40 age-matched Ro/SSA-negative women with autoimmune disorders. This design allowed us to evaluate how the composite profile of anti-Ro/SSA non-organ-specific responses and organ-specific autoimmune responses (such as those against thyroid antigens) correlate with pregnancy loss.

Materials and methods

Patients

In the retrospective cohort study, serum samples from 70 women with various autoimmune rheumatic disorders were selected on the basis of anti-Ro/SSA antibody in serum as determined by counterimmunoelectrophoresis (CIE) during routine evaluation in the Laboratory of Immunology, Department of Pathophysiology, University of Athens, from January 1995 to January 1996. All participants had a history of at least one pregnancy. All these sera were tested by Western blotting for the detection of reactivities against the 60, 52 and 57 kDa proteins. In addition, information on antinuclear antibodies (ANA) (detected by indirect immunofluorescence using Hep2 epithelial cells as substrate, positive ANA titre 1/80) and on other precipitin antibodies was also recorded. These patients had been evaluated at the out-patient rheumatology clinic in the Department of Pathophysiology, University of Athens, and included 30 patients with SLE, 26 with SS, four with systemic sclerosis (SScl), three with RA and seven with an undifferentiated connective tissue disorder. All diagnoses were based on previously established criteria [18–21].

In the case–control study, 40 of the above anti-Ro/SSA-positive sera and an equal number of age-matched anti-Ro/SSA-negative sera (based on CIE results) from autoimmune women with a history of at least one pregnancy were tested for the presence of thyroid peroxidase (TPO) antibodies and thyroglobulin antibodies by a competitive radioimmunoassay [22]. Serum aliquots from patients and controls were stored at -20°C until assayed.

Data regarding the obstetric history of all study participants were extracted from a previous study investigating the association of anti-Ro/SSA with recurrent pregnancy loss [7]. Recurrent pregnancy loss was defined as the incidence of at least two stillbirths or spontaneous abortions of first or second trimester. Spontaneous abortion was defined as the spontaneous loss of a fetus before the end of the 22nd week of gestation. Stillbirth was defined as intrauterine fetal death occurring after 22 weeks of gestation. No infants were reported to have neonatal lupus, but information was not collected systematically.

Since in our previous study [7] possible confounders (presence of anti-La/SSB, anti-DNA or anticardiolipin in the serum, age at anti-Ro/SSA diagnosis, number of pregnancies, indicator whether outcome occurred before or after diagnosis) did not affect the risk of recurrent pregnancy loss, it was not considered necessary to study and analyse the same parameters in the present report. We nevertheless considered the presence of anticardiolipin antibodies as an adjusting factor in the multivariate analyses, since this has been proposed to be strongly related to pregnancy loss in other studies [15–17]. The effect of anticardiolipin antibodies was not significant in our study and it did not affect the magnitude of the observed associations, therefore it is not reported.

Laboratory methods

Counterimmunoelectrophoresis. Antibodies to Ro/SSA antigen were assayed by CIE using calf thymus extract as an antigen source, as described by Bunn *et al.* [23]. Precipitin lines formed were stained with Coomassie brilliant blue R and antibody specificity was determined by direct comparison with reference sera.

Western blot with HeLa cell extract. Cytoplasmic extract was prepared from cultured HeLa cells as described previously. Samples of cytoplasmic HeLa extracts corresponding to 40×10^6 cells were loaded onto 15% acrylamide, 0.085% bisacrylamide gels. In order to facilitate the electrophoretic separation of the 52 kDa Ro/SSA antigen from the 48 kDa La/SSB antigen, the final ratio of acrylamide to bisacrylamide was 172.4. Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis, electrophoretic transfer and immunoblotting were subsequently performed as described previously [24, 25].

Radioimmunoassays for the detection of antithyroid antibodies. Thyroid peroxidase antibodies and thyroglobulin antibodies were assayed with radioimmunoassay kits (DYNO test®). The TPO assay is a single-antibody radioimmunoassay in which endogenous thyroid peroxidase antibody is reacted with enzymatic active native TPO from human thyroids. During incubation, solid-phase monoclonal anti-TPO antibodies and enzymatic active native TPO from human thyroids form a complex with another iodine- 125-labelled monoclonal anti-TPO antibody. Reaction tubes are then centrifuged to separate tracer bound to antibody from the non-reacted, free portion in the supernatant, which is decanted. The radioactivity bound in the pellet is directly proportional to the concentration of TPO antibody present in the serum. Thyroglobulin antibody is detected in a system identical to that described for TPO antibody, except that iodinated thyroglobulin is used in place of thyroid perodixase. A positive result in TPO and thyroglobulin antibodies was defined as >60 and >50 IU/ml, respectively.

Statistical methods

Associations of reactivities against Ro52, Ro60, p57 and antithyroid antibodies with recurrent pregnancy losses

were evaluated with logistic regression. Both univariate and multivariate regressions were performed. Recurrent pregnancy loss was the dependent variable and each patient was used as an observation. A different set of logistic regression models considered each pregnancy as a separate observation, excluding induced first trimester abortions. The dependent variable in these models is the pregnancy outcome (live born *vs* stillborn or spontaneous abortion). Induced elective abortions of the first trimester were excluded from this analysis, since these pregnancies may be considered to be censored (in the large majority, it is unknown whether they would have resulted in stillbirth, spontaneous abortion or a live birth). Multiple gestations were counted as single events in all analyses.

In addition to considering each of the three reactivities separately, we also considered a score equal to the number of different reactivities in each patient. The score took values between 0 and 3. The relationship of the score with recurrent pregnancy loss and with adverse pregnancy outcomes was investigated with the χ^2 test.

Statistical analyses were implemented in Advanced SPSS [26]. All P values are two-tailed.

Results

Retrospective cohort study of Ro/SSA-positive women

A total of 289 pregnancies were recorded in 70 anti-Ro/SSA-positive women, 13 of whom were classified as having had recurrent pregnancy loss. The mean age at the time of determination of Ro status was 44.9 yr (S.D. 11.9) and the mean age at last pregnancy was 30.4 yr (S.D. 6.3). Of the 289 pregnancies, 117 had been electively interrupted in the first trimester, 123 had resulted in live births, and there were eight stillbirths, 34 first trimester spontaneous abortions and seven second trimester spontaneous abortions. There were seven women with one pregnancy, 14 with two pregnancies, 14 with three pregnancies and 35 with more than three pregnancies.

Reactivity against Ro52, Ro60 and p57 was observed in 35/70, 34/70 and 15/70 sera, respectively, and there was no correlation between the presence of any two reactivities ($P > 0.6$ for all three pairs). Reactivity against each of the three antigenic peptides was associated both with the risk of a woman having had recurrent pregnancy loss and with the risk of adverse outcome for a specific pregnancy in a woman (Table 1). Multivariate regressions suggested that the effects of each of three responses were independent and their magnitude of effect remained unchanged in the multivariate models. When the sum score of the three reactivities was considered, the odds ratio for recurrent pregnancy loss and for adverse pregnancy outcome was 3.70 ($P = 0.004$) and 2.30 ($P = 0.0001$), respectively, for each additional antigen reactivity.

The proportion of women with recurrent pregnancy loss was 0% (0/14), 15.6% (5/32), 25% (5/20) and 75% (3/4) in women with 0, 1, 2 and 3 peptide reactivities, respectively ($P = 0.005$). The proportion of adverse pregnancy outcomes was 3.4% (1/29), 26.9% (18/67), 33.9% (20/59) and 58.8% (10/17) in pregnancies of women with 0, 1, 2 and 3 peptide reactivities, respectively ($P < 0.001$). These percentages show a clear gradation of risk.

Case–control study

The cases and controls were well matched both for the age at Ro determination [mean 44.7 (S.D. 12.5) and 44.6 (S.D. 12.0) yr] and age at last pregnancy [mean 29.8 (S.D. 6.0) and 31.7 (S.D. 6.0) yr]. Twelve women were classified as having had recurrent pregnancy loss. There were a total of 314 pregnancies, including 115 elective induced abortions, seven stillbirths, 33 spontaneous abortions of the first trimester, eight spontaneous abortions of the second trimester and 151 pregnancies which resulted in live births. Among the 40 Ro-positive women, six had one pregnancy, seven had two, six had three, and 21 had more than three pregnancies. Among the 40 Ro-negative women, seven had one pregnancy, eight had two, eight had three, and 17 had more than three pregnancies.

Reactivity against each of the three antigenic peptides was associated both with the risk of a woman having had recurrent pregnancy loss and with the risk of adverse outcome for a specific pregnancy in a woman (Table 2). The same was true for the presence of antibodies against thyroglobulin, but was less evident for antibodies against thyroid peroxidase. Multivariate regressions suggested that the effects of each of three antigenic responses were independent and their magnitude of effect remained unchanged in the multivariate models, although the estimates were not formally statistically significant when all three peptides were considered. When the sum score of peptide reactivities was considered, the odds ratio for recurrent pregnancy loss and for adverse pregnancy outcome was 3.00 ($P = 0.002$) and 1.85 ($P = 0.002$), respectively, for each additional reactivity.

When this was adjusted for the presence of antibodies against thyroglobulin, the odds ratio for recurrent pregnancy loss and for adverse pregnancy outcome was 3.35 ($P = 0.002$) and 1.90 ($P = 0.001$), respectively, for each additional peptide reactivity. The odds ratios for antithyroglobulin reactivity in these multivariate models were 5.54 ($P = 0.025$) and 2.30 ($P = 0.024$), respectively. The strength of the association with the sum score of Ro52, Ro60 and p57 proteins was stronger than the association with Ro/SSA, as determined by CIE, which was a marginal statistical significance [odds ratios 3.58 ($P = 0.07$) and 2.17 ($P = 0.024$), respectively, in the two logistic models].

The proportion of women with recurrent pregnancy loss was 6.8% (3/44), 13.6% (3/22), 30% (3/10) and 75% (3/4) in women with 0, 1, 2 and 3 peptide reactivities, respectively ($P = 0.002$). The proportion of adverse pregnancy outcomes was 15.7% (17/108), 25% (11/44), 33.3% (10/30) and 58.8% (10/17) in pregnancies of women with 0, 1, 2 and 3 peptide reactivities, respectively ($P < 0.001$). These percentages also show a clear gradation of risk, as in the cohort study.

TABLE 1. Associations of antibodies against Ro52, Ro60 and p57 peptides with pregnancy loss among women with Ro/SSA antibodies by CIE

	Outcome: recurrent pregnancy loss Odds ratio (*P*)		Outcome: pregnancy loss Odds ratio (*P*)	
Antibodies	Univariate	Multivariate	Univariate	Multivariate
Ro52	4.27 (0.04)	4.68 (0.04)	2.54 (0.01)	2.32 (0.03)
Ro60	2.88 (0.11)	2.99 (0.12)	2.03 (0.04)	1.94 (0.07)
p57	2.94 (0.11)	3.70 (0.08)	2.70 (0.01)	2.79 (0.01)

For definitions of outcomes, see Materials and methods.

TABLE 2. Associations of antibodies against three Ro/SSA specificities, thyroid peroxidase and thyroglobulin with pregnancy loss in the case–control study

	Outcome: recurrent pregnancy loss Odds ratio (*P*)		Outcome: pregnancy loss Odds ratio (*P*)	
Antibodies	Univariate	Multivariate[a]	Univariate	Multivariate[a]
Ro52	6.00 (0.01)	3.52 (0.09)	2.97 (0.001)	1.85 (0.14)
Ro60	5.40 (0.01)	3.01 (0.13)	2.97 (0.002)	1.86 (0.14)
p57	4.20 (0.08)	2.24 (0.38)	3.16 (0.01)	1.82 (0.23)
Thyroglobulin	4.08 (0.03)	NI	2.00 (0.04)	NI
Peroxidase	2.58 (0.14)	NI	1.18 (0.63)	NI
Any thyroid	2.57 (0.14)	NI	1.29 (0.45)	NI

For definitions of outcomes, see Materials and methods.

[a]Multivariate models considering all three peptides; the magnitude of effects is not different, when also adjusting for antithyroid antibodies in multivariate analyses. NI, not included in this presented model; for both outcomes, multivariate models using back-elimination of variables according to log-likelihood ratio criteria (with $P > 0.10$ for variable removal and $P < 0.05$ for variable entry) selected Ro52 and thyroglobulin antibodies as the most important independent variables. A better fit was achieved when the sum score of Ro peptide responses was considered (instead of each peptide separately) along with antithyroglobulin antibodies (see the text).

Discussion

Among the possible factors implicated in the pathogenesis of adverse fetal outcome in autoimmune patients, antibodies against Ro/SSA antigens may play a significant role. These antibodies are strongly associated with congenital heart block through transplacental passage during pregnancy, independent of maternal disease activity or classification [27]. However, their presence in maternal serum does not always lead to fetal disease and the presence of reactivities against specific antigenic peptides may be important in this regard. Indeed, Buyon *et al.* [28] reported that mothers who have only anti-Ro/SSA antibodies in low titres that do not recognize either 52 or 60 kDa components on SDS immunoblot appear to be at lower risk for giving birth to a child with neonatal lupus. Furthermore, Dorner *et al.* [29] support the view that the coincidence of anti-52 kDa, 60 kDa and La/SSB antibodies is strongly associated with complete congenital heart block.

In a previous study [7] based on the hypothesis that unexplained pregnancy loss in autoimmune patients may represent an intrauterine feature of congenital heart block, the role of anti-Ro/SSA in the pathogenesis of recurrent pregnancy loss was investigated. The heterogeneous involvement of this antibody in different clinical groups revealed in this study urged us to investigate the role of other parameters in the pathogenesis of recurrent pregnancy loss in these patients. Thus, in the present study, the fine specificity patterns of maternal anti-Ro/SSA antibodies were analysed and antibodies against three distinct peptides Ro60, Ro52 and the recently recognized p57 [9] were also considered. Furthermore, the possible involvement of antithyroid antibodies was explored.

Our results suggest that there is a clear gradation of risk in the association between recurrent pregnancy loss and the number of distinct Ro/SSA peptides that are recognized by the sera of autoimmune women. In the retrospective cohort study, the rates of recurrent pregnancy loss varied from 0% among women whose sera did not recognize any peptides to 75% when all three peptides were recognized. The results of the case–control study were also consistent with this clear gradation of risk. The case–control study further revealed that the association of these proteins with adverse pregnancy outcomes was not modified when other autoimmune parameters such as thyroid and cardiolipin autoantibodies were considered. In particular, the presence of antithyroglobulin antibodies seemed to have an independent association with a history of recurrent pregnancy loss. Overall, our data suggest that the cumulative number of autoantibody responses against the non-organ-specific Ro antigen specificities, and possibly also the organ-specific thyroid antigens, may correlate with a history of recurrent pregnancy loss.

Until recently, recurrent pregnancy loss in women with autoimmune diseases was almost exclusively associated with antibodies against phospholipids [15–17]. In the present study, distinct anti-Ro/SSA patterns and antithyroid antibodies, especially against thyroglobulin, seemed to be more strongly associated with a history of

pregnancy loss. Our findings should not be interpreted as proof that these autoantibodies are directly linked to the pathogenesis of pregnancy loss. Determination of the autoimmune profile in our patients was performed several years, on average more than a decade, after the last pregnancy. It is unknown whether the profile would have been similar at the time these patients suffered adverse pregnancy outcomes. Therefore, a cautious interpretation of the observed associations is warranted and prospective studies should evaluate whether the cumulative reactivity against these peptides similarly determines future pregnancy outcomes. What our current data suggest is that the extent and variety of organ-specific and non-organ-specific autoantibody responses in a population of women with autoimmune disorders correlate with their past obstetric history. It may be hypothesized that the presence of autoantibodies in autoimmune disorders may represent an epiphenomenon of an underlying immune disturbance leading possibly to immune rejection of the 'allogeneic fetus', clinically conceived as spontaneous abortion. These autoantibodies may be markers of a polyclonal B-cell activation or of an underlying T-cell defect causally related to infertility and pregnancy loss. The more generalized this dysregulation is, i.e. the more peptides and distinct antigens are recognized, the stronger the risk of pregnancy loss may become.

Finally, a feature differentiating our study compared with most previous studies in the field is the fact that it was performed in a population of patients with autoimmune disorders. For example, several studies have examined the relationship between thyroid abnormalities and reproductive failure, with conflicting results [12–14, 30–34]. All these studies evaluated unselected women with recurrent pregnancy loss rather than autoimmune patients. Study designs targeting selected populations of autoimmune women may be particularly useful to delineate multiple independent associations of peptide-specific reactivities against Ro and other antigens targeted by autoimmune responses.

References

1. Friedman EA, Rutherford JW. Pregnancy and lupus erythematosus. Obstet Gynecol 1956;8:601–10.
2. Siamopoulou-Mavridou A, Manoussakis MN, Mavridis AK, Moutsopoulos HM. The outcome of pregnancy in autoimmune rheumatic disease patients before the disease onset. Ann Rheum Dis 1988;47:982–7.
3. Petri M. Systemic lupus erythematosus and pregnancy. Rheum Dis Clin North Am 1994;20:87–118.
4. Hull RG, Harris EN, Morgan SH *et al.* Anti-Ro antibodies and abortions in women with SLE. Lancet 1983;2:1138.
5. Watson RM, Braunstein BL, Watson AJ, Hochberg MC, Provost TT. Fetal wastage in women with anti-Ro (SSA) antibody. J Rheumatol 1986;13:90–4.
6. Ramsey-Goldman R, Hom D, Deng JS *et al.* Anti-SSA antibodies and fetal outcome in maternal systemic lupus erythematosus. Arthritis Rheum 1986;29:1269–73.
7. Mavragani CP, Dafni UG, Tzioufas AG, Moutsopoulos HM. Fetal outcome and anti-Ro/SSA antibodies in autoimmune diseases: a retrospective cohort study. Br J Rheumatol 1998;37:740–5.
8. Ben-Chetrit E, Fox RI, Tan EM. Dissociation of immune responses to the SS-A (Ro) 52-KD and 60-KD polypeptides in systemic lupus erythematosus and Sjögren's syndrome. Arthritis Rheum 1990;33:349–55.
9. Maddison PJ, Lee L, Reichlein M, Sinclair A, Wasson C, Schemmer G *et al.* Anti-p57: a novel association with neonatal lupus. Clin Exp Immunol 1995;99:42–8.
10. Karsh J, Pavlidis N, Weintraub BD, Moutsopoulos HM. Thyroid disease in Sjögren's syndrome. Arthritis Rheum 1980;23:1326–9.
11. Miller FW, Moore GF, Weintraub BD, Steinberg AD. Prevalence of thyroid disease and abnormal thyroid function test results in patients with systemic lupus erythematosus. Arthritis Rheum 1987;30:1124–31.
12. Stagnaro-Green A, Roman SH, Cobin RH, el-Harazy E, Alvarez-Marfany M, Davies TF. Detection of at-risk pregnancy by means of highly sensitive assays for thyroid autoantibodies. J Am Med Assoc 1990;19:1422–5.
13. Glinoer D, Soto MF, Bourdoux P, Lejeune B, Delange F, Lemone M *et al.* Pregnancy in patients with mild thyroid abnormalities: maternal and neonatal repercussions. J Clin Endocrinol Metab 1991;73:421–7.
14. Wilson R, Ling H, MacLean MA, Mooney J, Kinnane D, McKillop JH *et al.* Thyroid antibody titer and avidity in patients with recurrent miscarriage. Fertil Steril 1999;71:558–61.
15. Lockshin MD, Druzin ML, Goei S, Qamar T, Magid MS, Jovanovic L *et al.* Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Engl J Med 1985;313:152–6.
16. Lynch A, Marlar R, Murphy J, Davila G, Santos M, Rutledge J *et al.* Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study. Ann Intern Med 1994;120:470–5.
17. Branch DW, Scott JR, Kochenour NK, Hershgold E. Obstetric complications associated with the lupus anticoagulant. N Engl J Med 1985;313:1322–6.
18. Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.
19. Arnett FC, Edsworthy SM, Block DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
20. Vitali C, Bombardieri S, Moutsopoulos HM *et al.* Preliminary criteria for the classification of Sjögren's syndrome. Arthritis Rheum 1993;36:340–7.
21. Masi AT, Rodnan GP, Medsger TA Jr *et al.* Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980;23:581–90.
22. Beever K, Bradbury J, Phillips D, McLachlan SM, Pegg C, Goral A *et al.* Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. Clin Chem 1989;35:1949–54.
23. Bunn CC, Gharavi AE, Hughes GRV. Antibodies to extractable nuclear antigens in 173 patients with DNA-binding positive SLE: an association between antibodies to RNP and Sm antigens observed by counter immunoelectrophoresis. J Clin Lab Immunol 1982;8:13–8.
24. Habets WJ, De Rooij DJ, Hoet MH, Van de Putte LB, Van Venrooij WJ. Quantitation of anti-RNP and anti-Sm antibodies in MCTD and SLE patients by immunoblotting. Clin Exp Immunol 1985;59:457–66.
25. Buyon JP, Slade SG, Chan EKL, Tan EM, Winchester R.

Effective separation of the 52KDa SSA/Ro polypeptide from the 48 KDa SSB/La polypeptide by altering conditions of polyacrylamide gel electrophoresis. J Immunol Methods 1990;129:207–10.
26. SPSS Inc. Advanced SPSS 6.1. Chicago: SPSS, 1994.
27. Waltuck J, Buyon JP. Autoantibody-associated congenital heart block: outcome in mothers and children. Ann Intern Med 1994;120:544–51.
28. Buyon JP, Winchester RJ, Slade SG *et al*. Identification of mothers at risk for congenital heart block and other neonatal lupus syndromes in their children. Arthritis Rheum 1993;36:1263–73.
29. Dorner T, Chaoui R, Feist E, Goldner B, Yamamoto K, Hiepe F. Significantly increased maternal and fetal IgG autoantibody levels to 52KD Ro/SSA and La/SSB in complete congenital heart block. J Autoimmun 1995; 8:675–84.
30. Pratt D, Novotny M, Kaberlein G, Dudkiewicz A, Gleicher N. Antithyroid antibodies and the association with non-organ-specific antibodies in recurrent pregnancy loss. Am J Obstet Gynecol 1993;168:837–84.
31. Pratt DE, Kaberlein G, Dudkiewicz A, Karande V, Gleicher N. The association of antithyroid antibodies in euthyroid non pregnant women with recurrent first-trimester abortions in the next pregnancy. Fertil Steril 1993;60:1001–5.
32. Bussen SS, Steck T. Thyroid antibodies and their relation to antithrombin antibodies, anticardiolipin antibodies and lupus anticoagulant in women with recurrent spontaneous abortions (antithyroid, anticardiolipin and antithrombin autoantibodies and lupus anticoagulant in habitual aborters). Eur J Obstet Gynecol Reprod Biol 1997;74:139–43.
33. Gleicher N, el-Roeiy A, Confino E, Friberg J. Reproductive failure because of autoantibodies: unexplained infertility and pregnancy wastage. Am J Obstet Gynecol 1989;160:1376–80; discussion: 1380–5.
34. Esplin MS, Branch DW, Silver R, Stagnaro-Green A. Thyroid autoantibodies are not associated with recurrent pregnancy loss. Am J Obstet Gynecol 1998;179:1583–6.