

Anti-52 kDa Ro, Anti-60 kDa Ro, and Anti-La Antibody Profiles in Neonatal Lupus

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ABSTRACT. *Objective.* Studies suggest that anti-52 kDa Ro antibodies are more sensitive and specific than anti-60 kDa Ro antibodies for neonatal lupus. However, these studies mainly used immunoblot or ELISA using recombinant protein, which have poor sensitivity for anti-60 kDa Ro antibodies. In addition, the control patients were not disease matched. We reassessed the sensitivity and specificity of anti-52 kDa Ro, anti-60 kDa Ro, and anti-La, addressing these limitations.

Methods and Results. To assess sensitivity, 125 mothers of children with neonatal lupus (NLM) were recruited. All maternal sera were assessed using a commercial line immunoassay that uses natural 60 kDa Ro protein (Inno-Lia™ ANA Update, Innogenetics NV, Gent, Belgium). By this method, 96% of the sera had antibodies to 60 kDa Ro, 86% to 52 kDa Ro, and 78% to 48 kDa La. Immunoblot of 65 NLM showed significantly fewer positive results for anti-60 kDa Ro ($p < 0.001$) and anti-52 kDa Ro ($p < 0.05$). Sensitivity of the 3 antibodies was assessed in the symptomatic mothers of children with congenital heart block (CHB) (78 women) and disease matched controls with unaffected children (65 women) using Inno-Lia™ ANA Update. The sensitivity of each antibody was compared by multiple logistic regression to adjust for maternal disease. There was no significant difference between the groups for 60 kDa Ro or for anti-52 kDa Ro antibody. However, there was a significant difference for the anti-La antibody ($p = 0.001$), with an odds ratio of 3.59. This translates to an increase in risk from a published 2% for CHB in an anti-Ro-positive mother to 3.1% if the woman is also anti-La antibody-positive, and to a decrease in risk to 0.9% if anti-La-negative.

Conclusion. Contrary to previous reports, 52 kDa Ro as detected by Inno-Lia™ ANA Update is not more specific for or frequent in CHB than 60 kDa Ro. However, the presence of anti-La antibodies significantly increases the risk for CHB. (J Rheumatol 2004;31:2480-7)

Key Indexing Terms:

CONGENITAL HEART BLOCK

NEONATAL LUPUS RASH

Neonatal lupus (NL) is a disease of passive immunity highly associated with anti-52 kDa Ro, anti-60 kDa Ro, and La antibodies. The 2 dominant manifestations are the neonatal lupus rash and congenital heart block (CHB). CHB occurs in roughly 2% of pregnancies of anti-Ro antibody-positive mothers, is associated with high infant mortality, and persists throughout life¹. The neonatal rash normally resolves by 9 months of age.

Studies assessing 52 kDa and 60 kDa Ro antibodies have

generally been relatively small²⁻⁸, with only one study assessing more than 33 cases of NL⁹. Despite the small numbers, the majority of these studies suggest that anti-52 kDa Ro antibodies confer a higher risk of NL than anti-60 kDa Ro antibodies^{2-5,8,9}. However, these studies had several limitations that led to debate concerning the results^{9,10}. First, the majority used immunoblot^{2,5,9} or ELISA using recombinant protein^{3,4,8,11} to assess the anti-52 kDa Ro and anti-60 kDa Ro antibodies. Antibodies to the 60 kDa Ro antigen are

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frequently to conformational epitopes and may therefore not bind to the denatured protein used in immunoblot¹² or recombinant protein. It has been calculated that as much as 23% of anti-60 kDa Ro sera will only bind to the native antigen¹³. In contrast, anti-52 kDa Ro antibodies are mainly directed against the denatured protein¹³. Immunoblot or ELISA utilizing recombinant antigen therefore has a tendency to underestimate anti-60 kDa Ro antibody titers compared to anti-52 kDa Ro antibodies. The previous studies would therefore have had a tendency to overestimate the incidence of anti-52 kDa antibodies compared to anti-60 kDa antibodies. This possibility was fully acknowledged in the original study by Buyon, *et al*⁹ and is supported by the fact that all mothers of children with CHB were positive by ELISA using bovine antigen for anti-Ro antibodies (bovine Ro antigen is essentially completely 60 kDa Ro)⁹.

Second, the control and CHB groups were not specifically matched for maternal disease. When assessed by immunoblot, anti-Ro-positive patients with systemic lupus erythematosus (SLE) had a higher incidence of anti-60 kDa Ro antibody and a lower incidence of anti-52 kDa Ro antibodies than patients with Sjögren's syndrome (SS)¹⁴. For example, in Buyon's study⁹ 84% of the control group mothers had SLE compared to 25% of the mothers of children with CHB. Thus the greater sensitivity of anti-52 kDa Ro antibodies for the identification of CHB mothers in the previous study could potentially be secondary to the differing maternal disease profiles in the control and disease groups.

Accordingly, our aim was to reassess the sensitivity and specificity of anti-52 kDa Ro, anti-60 kDa Ro, and anti-La antibodies in mothers of children with NL (NLM) using methodology that addressed the 2 limitations of the previous studies. The sensitivity of each of the 3 antibodies to correctly identify NLM was assessed utilizing a line immunoassay (Inno-LiaTM ANA Update; Innogenetics NV, Gent, Belgium; denoted LIA), which uses natural 60 kDa Ro antigen. The NLM were unselected and thus included symptomatic and asymptomatic mothers, mothers of children with rash, or heart block, or both. The specificity of each of the 3 antibodies for symptomatic mothers with children with CHB was assessed separately. Only symptomatic mothers were assessed, as asymptomatic anti-Ro-antibody-positive controls were not available. The control group was recruited from anti-Ro-antibody-positive symptomatic mothers with unaffected children. The statistical analysis was designed to take maternal disease into account.

MATERIALS AND METHODS

NLM were recruited from the following centers: St. Thomas' and Guy's Hospitals, London, England; Hospital for Joint Diseases, New York University School of Medicine, New York, USA; and Ospedale Niguarda, Milan, and the Clinical Immunology Unit, Milan, Italy. The health status of the mother is based on review of available medical records, as described¹⁵. Mothers were classified as having SLE if they met at least 4 of the American College of Rheumatology criteria¹⁶. Mothers were categorized

as having primary SS if they had: (1) both dry eyes and dry mouth; or (2) either dry eyes or dry mouth along with objective documentation of salivary or lacrimal gland hypofunction or lymphocytic infiltration of these glands¹⁵. Undifferentiated autoimmune syndrome (UAS) was diagnosed in those patients with features of a rheumatic disease who did not have prominent sicca complaints and did not meet criteria for SLE¹⁵. Asymptomatic mothers were those with no clinical evidence of a rheumatic illness.

A serum sample from each mother was tested for anti-52 kDa Ro, anti-60 kDa Ro, anti-La, and anti-RNP antibodies by LIA using the method described by Meheus, *et al*¹⁷. The LIA uses recombinant 52 kDa Ro and 48 kDa La antigens and purified human 60 kDa Ro protein. Briefly, test strips consisted of a plastic backing covered with a nylon membrane, on which the antigens and a positive control line (human IgG) had been applied as parallel lines. Each strip was incubated 1 h in a plastic trough containing 2 ml of sample diluent to which 10 μ l of serum sample had been added. Binding of the antibodies was detected using goat anti-human IgG alkaline phosphatase conjugate and developed with the chromogenic enzyme substrate NBT/BCIP (nitroblue tetrazolium/5-bromo-4-chloro-3-indolylphosphate). Intensity of positive antigen lines on the strips was compared to the corresponding intensity of a cutoff control. The cutoff value was based on receiver operating characteristic (ROC) curves in full analysis.

In addition, 65 samples from one center were tested by immunoblot, which uses denatured human protein. Sodium dodecyl sulfate immunoblots to optimally identify antibody reactivity to 52 kDa SSA/Ro were performed as described⁹ with minor modifications of the detection system. In brief, the MOLT-4 cell line was used with a running gel containing 15% acrylamide and 0.0855% bis-acrylamide. This running gel formula allows efficient separation of the 48 kDa La and 52 kDa Ro antigens. Gels were electrophoresed at 22°C at 8 mA overnight. The proteins were electrotransferred to a nitrocellulose sheet for 3 h at a constant voltage of 60 V. The nitrocellulose strips were incubated 1 h in phosphate buffered saline (PBS) containing 0.05% Tween 20 and 3% nonfat milk to block nonspecific sites, followed by incubation with a 1:100 dilution of sera, and washed for 1 h in the PBS-Tween solution. In place of Protein A-1²⁵ to detect human antibodies, membranes were incubated 1 h in blocking buffer containing 1:5000 dilution of horseradish peroxidase (HRP)-linked anti-human IgG or 1:3000 dilution of HRP-linked anti-rabbit IgG (New England Bio-Labs, Beverly, MA, USA). Filters were washed again in PBS-Tween and detection was accomplished using the Phototope-HRP Western Blot detection kit (New England Bio-Labs) as per manufacturer's instructions. Membranes were wrapped in Saran Wrap and exposed to x-ray film.

Cases in which none of the 3 antibodies or anti-RNP antibodies were detected in the maternal serum by any method were excluded from the study, as such cases may represent different pathologies. The sensitivity of the 3 antibodies for neonatal lupus, NL rash, and for CHB, as assessed by LIA, was calculated. In addition, the sensitivity of the LIA for the 3 antibodies was compared to that of immunoblot using the McNemar test.

The second aspect of the study was to assess the specificity of anti-52 kDa Ro, anti-60 kDa Ro, and anti-La antibodies for CHB mothers. Only CHB mothers who were symptomatic were included in this branch of the study, as there were no available asymptomatic controls. A control group of anti-Ro-antibody-positive patients with SLE, UAS, or SS or both SLE and SS who had given birth to children without neonatal lupus were recruited from 3 centers (St. Thomas' and Guy's Hospitals, London; Ospedale Niguarda, Milan; and the Clinical Immunology Unit, Istituto Auxologico Italiano, Milan). Their sera were also analyzed for anti-52 kDa Ro, anti-60 kDa Ro, and anti-La antibodies by LIA. The results were compared to the anti-Ro antibody-positive CHB mothers with SLE, UAS, or SS or both SLE and SS. This analysis was performed by multiple logistic regression with standard robust errors using the Stata statistical package to take into account the different maternal diagnoses.

RESULTS

In total, 125 NLM were included in the study, 68 from the Hospital for Joint Diseases, New York, 44 from St. Thomas'

and Guy's Hospitals, London, and 13 from the Ospedale Niguarda and the Clinical Immunology Unit, Milan. Sixteen had children with rash only, 99 had children with CHB only, and 10 had children with both. At the time blood was taken, 30 mothers were asymptomatic, 30 had UAS, 28 had SS, 25 had SLE, and 8 had SLE with secondary SS, in 3 the diagnosis was unknown, and one had mixed connective tissue disease (Figure 1).

Sensitivity of anti-52 kDa Ro, 60 kDa Ro, and La antibodies in neonatal lupus. On LIA, 96% of the sera from the 125 NLM had antibodies to 60 kDa Ro, 86% to 52 kDa Ro, and 78% to 48 kDa La. Antibodies to 60 kDa Ro were significantly more sensitive than antibodies to 52 kDa Ro ($p < 0.05$) and antibodies to 48 kDa La ($p < 0.001$) in NLM. Using the LIA, at least one of the antibodies was detected in 98% of the NLM. The distribution of the 3 antibodies was similar for CHB and rash. When NLM with children with rash alone or CHB alone were compared, there was no significant difference in the prevalence of any of the 3 antibodies between the 2 groups. On LIA, sensitivity to detect the 16 NL rash-only mothers was 87.5% for anti-60 kDa Ro, compared to 75% for anti-52 kDa Ro and 75% for anti-La-antibodies. One mother of a child with NL rash was positive for anti-52 kDa Ro antibody only, and another was positive for anti-RNP antibody only.

On LIA, sensitivity to detect the 109 CHB mothers was 97% for anti-60 kDa Ro, compared to 87% for anti-52 kDa Ro and 78% for anti-La antibodies. An illustration of the different permutations of the 3 antibodies in CHB mothers is shown in Figure 2. The majority of mothers were positive for all 3 antibodies (76/109, 70%). No CHB mother was

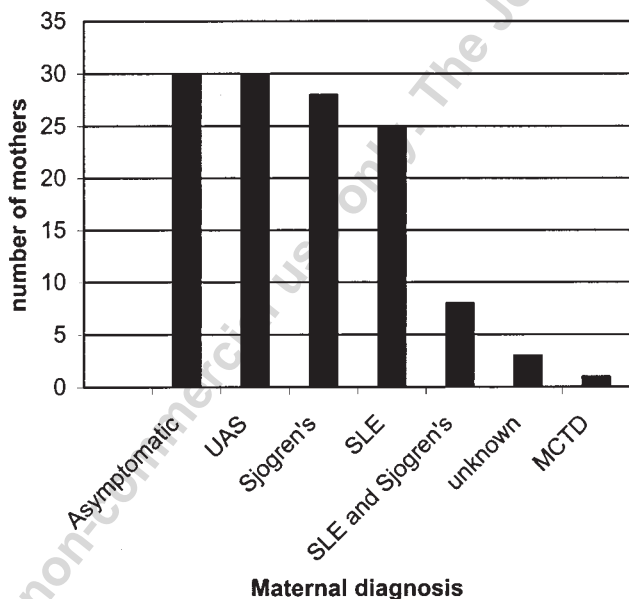


Figure 1. Diagnosis of all mothers of children with neonatal lupus. UAS: undifferentiated autoimmune syndrome, SLE: systemic lupus erythematosus, MCTD: mixed connective tissue disease.

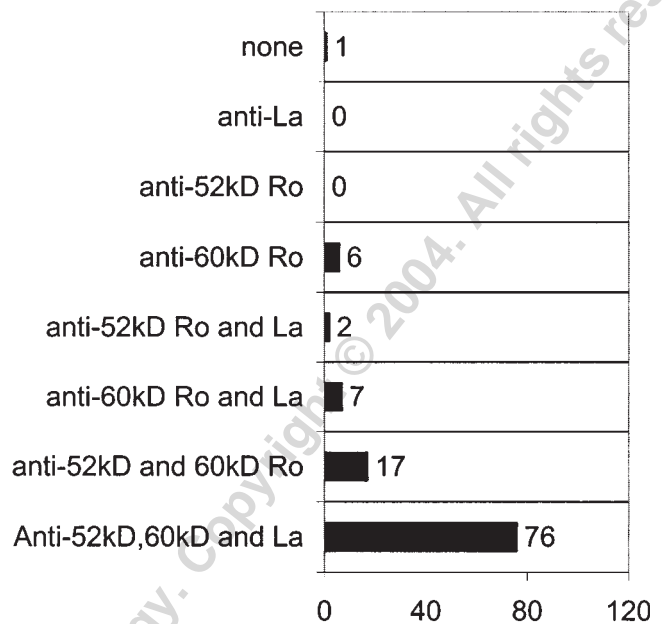


Figure 2. Number of CHB mothers with each permutation of the 3 antibodies as tested by Inno-Lia™ ANA Update (total 113).

positive for anti-La or anti-52 kDa Ro antibodies alone when tested by LIA. One of the CHB mothers had none of the 3 antibodies when tested by LIA; this mother had UAS and anti-Ro antibodies detectable by counterimmunoelectrophoresis and was positive for 52 kDa Ro antibody by immunoblot.

A subset of the NLM were also assessed by immunoblot to compare the results with those of the LIA. Immunoblot of 65 NLM showed significantly fewer positive results than by LIA for anti-60 kDa Ro ($p < 0.001$) and anti-52 kDa Ro antibodies ($p < 0.05$; Figure 3). By immunoblot, sensitivity to detect the NLM by anti-La was 84.6%, compared to 81.5% for anti-52 kDa Ro (p nonsignificant) and 53.8% for anti-60 kDa Ro antibodies ($p < 0.001$ vs anti-La). The sensitivity of any combination of the 3 antibodies for NLM was 98.4% by LIA and 96.9% by immunoblot in this group.

Specificity of anti-52 kDa Ro, 60 kDa Ro, and La antibodies in CHB mothers. In total, 77 of the 109 (71%) CHB mothers were symptomatic and anti-Ro-positive: 41 were from the Hospital for Joint Diseases, New York; 28 from St. Thomas' and Guy's Hospitals, London; and 8 from the Ospedale Niguarda and the Clinical Immunology Unit, Milan, and the Spedali Civili, Brescia. One patient with mixed connective tissue disease (St. Thomas' and Guy's Hospitals) was excluded from the analysis, as there was no suitable disease matched control. The remaining 76 women had a diagnosis of SLE, SS, SLE with secondary SS, or UAS. In this group, 26 had an UAS, 25 SS, 19 SLE, and 6 SLE with secondary SS (Figure 4).

The symptomatic CHB mothers were compared to a control group of 65 anti-Ro-antibody-positive women with chil-

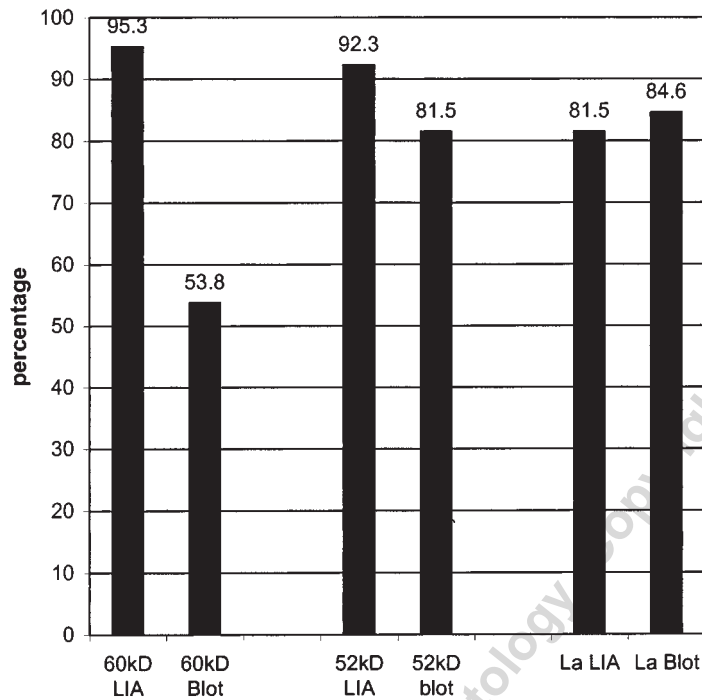


Figure 3. Percentages of samples positive for each antibody tested comparing Inno-Lia™ ANA Update (LIA) and immunoblot results in 65 mothers of children with neonatal lupus (NLM). Immunoblot of 65 NLM samples showed significantly fewer positive results than LIA for anti-60 kDa Ro ($p < 0.001$) and anti-52 kDa Ro ($p < 0.05$).

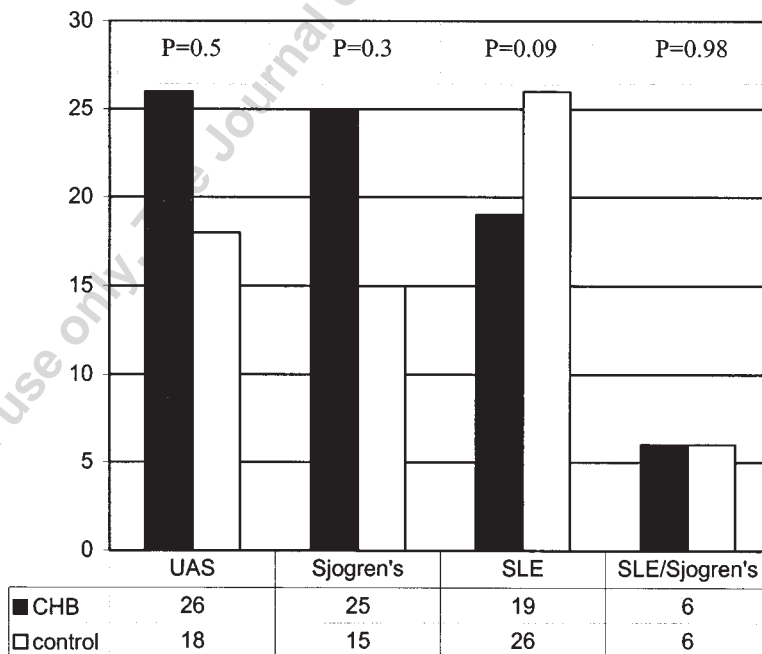


Figure 4. Diagnoses of symptomatic mothers included in the arm of the study to calculate the specificity of anti-52 kDa Ro, anti-60 kDa Ro, and anti-48 kDa La for CHB. Only anti-Ro or La antibody-positive women were included. Asymptomatic mothers and one mother with mixed connective tissue disease were excluded due to lack of suitable disease matched controls. In total, 76 mothers with CHB and 65 controls (anti-Ro-positive mothers with unaffected children) were included. UAS: undifferentiated autoimmune syndrome.

dren who did not have manifestations of NL. These patients were from St. Thomas' and Guy's Hospitals (43 patients) and the Ospedale Niguarda and the Clinical Immunology Unit, Milan (22 patients). Fifty-eight of these women had their pregnancies managed at connective tissue disease pregnancy clinics at the participating centers. The remaining 7 patients had pregnancies managed elsewhere but subsequent to the development of their symptoms. In this group, 18 had an UAS, 15 SS, 26 SLE, and 6 SLE with secondary SS (Figure 4). Comparisons were made between the groups for anti-60 kDa Ro, 52 kDa Ro, and La antibodies using multiple logistic regression analysis to take into account the different maternal diagnoses (Tables 1 and 2). There were no significant differences between the CHB mothers and controls for anti-60 kDa Ro antibody ($p = 0.98$, with a corrected odds ratio of 1.02, 95% confidence interval 0.19–5.53). For anti-52 kDa Ro antibody, the corrected odds ratio for having a child with CHB was 1.54 (95% CI 0.61–3.93), but this was not significant ($p = 0.36$). However, there was a significant difference between the 2 groups for the anti-La antibody ($p = 0.001$, OR 3.59, 95% CI 1.68–7.67) for having a child with CHB.

Therefore, based on Bayes's theorem, if the risk of the fetus developing CHB is assumed to be 2% in a pregnancy of a woman who is anti-Ro antibody-positive, this risk is increased to 3.1% if the woman is also anti-La antibody-positive. Similarly, if a woman is anti-Ro antibody-positive but anti-La antibody-negative, the chance that the fetus will not develop CHB is increased from 98% to 99.1%. These figures were based on a 2% prevalence rate of CHB in the group (assumed), sensitivity of 60/76 (observed), and specificity of 32/65 (observed) for anti-La antibody as an additional predictor. Positive and negative predictive values were then calculated using standard methods, based on Bayes's theorem, as follows:

Positive predictive value of anti-La antibody assuming 2% prevalence = $2 \times 60/76 / (2 \times 60/76 + 98 \times 33/65) = 0.03075 = 3.1\%$
 Negative predictive value of anti-La antibody assuming 2% prevalence = $98 \times 32/65 / (2 \times 16/76 + 98 \times 32/65) = 0.99134833 = 99.1\%$

DISCUSSION

A few studies have evaluated the antibody profiles for 52 kDa, 60 kDa Ro, and La in mothers of children with neona-

Table 1. Diagnosis and antibody frequencies of mothers included in the arm of the study to calculate the specificity of anti-52 kDa Ro, anti-60 kDa Ro, and 48 kDa La for CHB. Only anti-Ro or La antibody-positive women were included. Asymptomatic mothers and one mother with mixed connective tissue disease were excluded due to lack of suitable disease matched controls. In total, 76 mothers with CHB and 65 controls (anti-Ro-positive mothers with unaffected children) were included. Data represent the number of mothers in each category and the figure in parentheses the percentage.

	UAS		Sjögren's		SLE		SLE + Sjögren's	
	CHB	Control	CHB	Control	CHB	Control	CHB	Control
Anti-La	18 (69)	9 (50)	18 (72)	13 (87)	17 (89)	7 (27)*	6 (100)	4 (67)
Anti-52 kDa Ro	24 (92)	13 (72)	22 (88)	15 (100)	14 (74)	17 (65)	5 (83)	6 (100)
Anti-60 kDa Ro	26 (100)	16 (89)	25 (100)	15 (100)	18 (95)	25 (96)	5 (83)	6 (100)
Total	26	18	25	15	19	26	6	6

* $p < 0.001$, comparisons in other cells were not significantly different. UAS: undifferentiated autoimmune syndrome, CHB: congenital heart block.

Table 2. Diagnoses and antibody profiles of mothers included in the arm of the study to calculate the specificity of anti-52 kDa Ro, anti-60 kDa Ro, and 48 kDa La for CHB. Only anti-Ro or La antibody-positive women were included. Asymptomatic mothers and one mother with mixed connective tissue disease were excluded due to lack of suitable disease matched controls. In total, 76 mothers with CHB and 65 controls (anti-Ro-positive mothers with unaffected children) were included. Data represent the number of mothers in each category and the figure in parentheses the percentage.

	UAS		Sjögren's		SLE		SLE + Sjögren's	
	CHB	Control	CHB	Control	CHB	Control	CHB	Control
Anti-La	0	1 (5.5)	0	0	0	0	0	0
Anti-52 kDa Ro	0	1 (5.5)	0	0	0	1 (4)	0	0
Anti-60 kDa Ro	2 (8)	3 (16.5)	1 (4)	0	1 (5)	6 (23)	0	0
Anti-52 kDa Ro and La	0	0	0	0	1 (5)	0	1 (17)	0
Anti-60 kDa Ro and La	0	1 (5.5)	2 (8)	0	4 (21)	3 (12)	0	0
Anti-52 kDa and 60 kDa Ro	6 (23)	5 (28)	6 (24)	2 (13)	0	12 (46)	0	2 (33)
Anti-52 kDa and 60 kDa Ro and La	18 (69)	7 (39)	16 (64)	13 (87)	13 (69)	4 (15)*	5 (83)	4 (67)
Total	26	18	25	15	19	26	6	6

* $p < 0.001$, comparisons in other cells were not significantly different. UAS: undifferentiated autoimmune syndrome, CHB: congenital heart block.

tal lupus²⁻⁹. The largest study⁹ examined the profiles of the 3 antibodies in 57 CHB mothers, 12 mothers with children with other manifestations of NL, and 152 women (71 of whom were anti-Ro antibody-positive) with connective tissue disease and unaffected children. In addition to immunoblot, patients were tested for anti-Ro and anti-La by an ELISA that used bovine antigen. The study showed the anti-52 kDa Ro antibody to be more sensitive and specific for CHB mothers than anti-60 kDa Ro antibody by immunoblot. However, as discussed, interpretation of the data and generalizability may have been limited by the methodology.

Our study was designed to address the shortcomings of the previous studies. The LIA assay has the advantage of using natural 60 kDa Ro protein, which retains conformational epitopes more readily than other assays, and thus is more likely to detect positivity for anti-60 kDa Ro antibodies. This is in contrast to many commercial ELISA preparations, which use recombinant protein only.

The differences in methodology did appear to affect the results. The majority of NL mothers were positive for all 3 antibodies. Moreover, the prevalence of the anti-52 kDa Ro antibody was less than that of the anti-60 kDa Ro antibody for CHB mothers and NLM in general. This is in agreement with the finding of Lee, *et al*⁶, who observed that all 20 NL mothers in their study were positive for antibodies to the native 60 kDa Ro/SSA. A comparison for 65 NLM between immunoblot and the LIA confirms that this change in result is due to the increased sensitivity of the LIA in the detection of anti-60 kDa Ro antibodies (Figure 3). Our study showed a corrected odds ratio for having a child with CHB to be 1.54 in mothers who were anti-52 kDa Ro-positive, compared to a corrected odds ratio of 1.02 for mothers who were anti-60 kDa Ro-positive. This might suggest that anti-52 kDa Ro antibodies are more specific than anti-60 kDa Ro antibodies for the identification of CHB mothers, in agreement with other studies^{2-5,8,9}. However, the odds ratio for anti-52 kDa Ro antibodies was not significant (95% CI 0.61–3.93).

Our study has limitations; NL may occur in any birth order and it is therefore possible that any of the Ro-positive mothers whose children are healthy could subsequently give birth to a child with CHB or rash. Thus, there is no “perfect” control group, even if matched for maternal diagnosis. Our study is retrospective and thus has the inherent limitations of a retrospective study. Not all sera were collected at the time of the index pregnancies, and it is possible that the profiles changed subsequent to the index pregnancy. However, data from both CHB mothers^{18,19} and from patients with SLE or SS²⁰⁻²³ show that the antibody profiles remain stable over many years.

There is much data to support a pathological role of anti-52 kDa Ro antibodies in CHB. Boutjdir, *et al* induced CHB in the human fetal heart with IgG and affinity purified anti-

52 kDa Ro antibodies from mothers with children with CHB²⁴. They also showed that these antibodies inhibited L-type Ca²⁺ currents at the whole-cell and single-channel level. However, Ohlsson and colleagues were unable to observe antibodies that functionally inhibited mouse L-type calcium channels in patients with primary and secondary SS²⁵.

In a study by Miranda-Carus and colleagues, female BALB/c mice were immunized with human recombinant 48 kDa SSB/La, 60 kDa SSA/Ro, and 52 kDa SSA/Ro (52-alpha) and 52-beta (amino acids 169–245 deleted) as well as with murine recombinant 52 kDa SSA/Ro. While a minority of the offspring produced in all groups had first-degree heart block, advanced conduction abnormalities were only identified in the offspring of 52-alpha or 52-beta immunized mice²⁶.

In our study, 87% of the CHB mothers were anti-52 kDa Ro antibody-positive, whereas 97% were anti-60 kDa Ro antibody-positive. In addition, the anti-52 kDa Ro antibody was less specific than anti-La for CHB. It therefore seems unlikely that 52 kDa Ro antibodies are uniquely pathogenic.

Several previous studies assessed the frequency of anti-La antibodies in CHB, and observed varying results. In general, studies comparing the frequency of anti-La antibodies in NLM with a control group of women with SS failed to observe an increased frequency of anti-La antibodies in NLM^{2,3}. In series where SLE patients were used as controls an increased frequency of anti-La antibodies was found in the NLM^{2,4,9}. One study by Meilof and colleagues found a significantly greater frequency of anti-La antibodies in their SLE control group than in their NLM group when tested by counterimmunoelectrophoresis, but not when tested by ELISA, immunoblot, or RNA precipitation¹¹. However, this study was small, with 12 controls and 14 NLM. Finally, in a study by Yukiko of 8 NLM and 19 controls, all patients had SS, although some additionally had SLE, mixed connective tissue disease, or discoid lupus. In this study, anti-La antibodies were significantly associated with NLM⁸.

Thus to a certain extent the results of previous studies may be a reflection of the disease profile of the control groups. Our study is the first to adjust statistically for maternal disease. In our study, anti-La antibody was significantly associated with CHB in anti-Ro antibody-positive mothers, with an odds ratio of 3.59 (95% CI 1.68–7.67). This result was highly significant ($p = 0.001$), and has clinical relevance in counseling women for risk of CHB in their offspring. However, why this association between anti-La antibodies and CHB exists is not immediately obvious. The antibody is not essential for the development of CHB, as it was absent in approximately one-fifth of cases. However, there are several possible explanations.

Horsfall, *et al* have described antibody binding to the surface of myocardial fibers in a child who died from CHB²⁷. These surface immunoglobulins on the myocardial fibers

bound to anti-La idiotypes. It is not known whether the myocytes were apoptotic or not. If the myocardial fibers were not apoptotic, the presumed maternal anti-La were not likely to be binding to the myocyte surface, since this antigen is normally sequestered in the interior of the cell. One possibility is that the antibodies were binding to another protein due to a shared epitope. Epitope mapping studies of 60 kDa Ro^{28,29} and La³⁰ have not conclusively demonstrated any epitopes that are specific or sensitive for CHB. Two studies have performed epitope mapping of 52 kDa Ro in the context of NL^{28,31}. Salomonsson, *et al* did find an association of CHB with 2 sequences of amino acids 200–239 and amino acids 176–196³¹. However, this study only assessed sera from 9 mothers of children with CHB and even in this small group the epitopes were neither specific nor sensitive enough to explain the pathogenesis. It was proposed by Eftekhari, *et al* that 52 kDa Ro antibodies cross-reacting with the cardiac 5HT4 serotonergic receptor could explain CHB³². However, a study by Buyon, *et al* was unable to demonstrate antibodies to the 5HT4 peptide in any of the sera from 116 CHB mothers, even though 85% had anti-52 kDa Ro antibodies³³. Additionally, rabbit antisera that recognized the 5HT4 peptide did not react with anti-52 kDa Ro³³. Similarly, Cavill and colleagues were unable to demonstrate cross-reactivity of anti-52 kDa Ro antibodies with the cardiac 5HT4 serotonergic receptor in patients with SLE and primary SS³⁴.

These studies were not exhaustive, nor were all of them able to assess conformational epitopes. These considerations notwithstanding, the results do not support the hypothesis of shared epitopes.

Another possibility is that of a separate but associated antibody that binds to a surface protein on the cardiomyocyte. If such an antibody exists it is likely to be a product of epitope spreading, which is well documented in the Ro-RNP macromolecule and thought to be antigen-driven³⁵. The presence of the La antibody is a sign of advanced epitope spreading within this system.

A further possible explanation relates to apoptosis. It has been found *in vitro* that apoptotic cardiomyocytes express 52 kDa Ro, 60 kDa Ro, and La protein on the cell surface³⁶. Apoptotic cardiomyocytes preincubated with any of the 3 antibodies induce the production of tumor necrosis factor- α from cocultured macrophages, whereas apoptotic cardiomyocytes not preincubated do not³⁶. In the same study anti-La but neither anti-52 kDa nor anti-60 kDa Ro antibodies immunoprecipitated surface biotinylated substrate in apoptotic cultured cardiomyocytes³⁶. Binding of the anti-La antibody to apoptotic fetal cardiomyocytes has been observed *in vivo* in the mouse³⁷. Interestingly, in this model the investigators were unable to show binding of anti-60 kDa Ro antibodies to apoptotic fetal cardiomyocytes (human anti-52 kDa Ro antibodies do not react with mouse 52 kDa Ro). Since apoptosis is more frequent in the developing fetal

heart, this theory would also explain why the disease does not occur in adults. Since both anti-La antibodies and anti-Ro antibodies bind apoptotic cells and induce production of inflammatory cytokines, it would be logical that expression of both anti-Ro and anti-La antibodies would be more pathogenic than one of them alone. The La gene is transcribed about 50 times more frequently than the 2 Ro proteins, making it likely to be a more available target.

Anti-60 kDa Ro is the most sensitive of the 3 antibodies for neonatal lupus and congenital heart block. For optimal sensitivity in prenatal risk assessment for these conditions an assay utilizing intact natural 60 kDa Ro antigen should be used. Anti-La antibody is significantly more specific for CHB, with its additional presence representing a clinically significant increased risk of CHB. These findings have important implications in the risk assessment of Ro-positive pregnant women and the pathogenesis of CHB.

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