

Autoimmune Response in Mothers of Children with Congenital and Postnatally Diagnosed Isolated Heart Block: A Population Based Study

HEIKKI JULKUNEN, AARO MIETTINEN, TIMO K. WALLE, EDWARD K.L. CHAN, and MARIANNE ERONEN

ABSTRACT. *Objective.* To study the autoimmune response in mothers of children with isolated congenital heart block (CHB) and heart block (HB) diagnosed postnatally.

Methods. We reviewed the Finnish hospital registries for patients born between 1950 and 2000 and diagnosed with isolated HB before the age of 16 years. Clinical data and sera for the determination of autoantibodies were available from 67 mothers of children with CHB and from 37 mothers of children with postnatally diagnosed HB 9.9 years and 22.6 years (mean) after the index delivery, respectively. Maternal antibodies to 52 kDa and 60 kDa SSA and 48 kDa SSB were determined by time-resolved fluoroimmunoassay (TR-FIA) and by immunoblotting. Other marker antibodies for connective tissue diseases (CTD) were determined by immunoblot and/or by immunofluorescence. The control group comprised 136 mothers with primary Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), or other CTD with healthy children.

Results. Sixty of our 67 mothers (90%) of children with CHB had antibodies to SSA or SSB by the methods initially used in this study. When retests and tests performed previously were taken into account, only 3 (4%) of the 67 mothers did not have any autoantibodies. Two (3%) of the 67 mothers had antibodies to dsDNA and one (1%) each to Jo-1/HRS, RNP-70 kDa, and histone proteins. Of 37 mothers of children with postnatally diagnosed HB, only 3 (8%) had any autoantibodies. Increased risk of having a child with CHB was indicated by maternal primary SS and high levels of anti-SSA and anti-SSB by all assays, whereas low risk was indicated by maternal SLE or other CTD and undetectable or low levels of the antibodies. No single anti-SSA or anti-SSB test was clearly superior to others, but in general, immunoblots were more specific than TR-FIA.

Conclusion. Maternal autoimmune disorder is almost always associated with CHB but only rarely with postnatally diagnosed HB. Anti-SSA and anti-SSB are marker antibodies for mothers of children with CHB, and an increased risk of having an affected child is indicated by maternal primary SS and high titer antibodies to SSA and SSB. (J Rheumatol 2004;31:183–9)

Key Indexing Terms:

NEONATAL LUPUS ANTIBODIES SSA SSB CONGENITAL HEART BLOCK

From the Department of Internal Medicine, Peijas Hospital, Helsinki University Hospital, Vantaa; the Department of Immunology, HUCH Laboratory Diagnostics, Helsinki University Hospital; the Hospital for Children and Adolescents, Helsinki University Hospital, Helsinki, Finland; and the Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California, USA.

Supported in part by Helsinki University Hospital Research Foundation, the Sigrid Juselius Foundation, Finska Läkaresällskapet, and Helsinki University Hospital.

H. Julkunen, MD, Rheumatologist, Department of Internal Medicine, Peijas Hospital; A. Miettinen, MD, Clinical Immunologist; T.K. Walle, MD, Clinical Immunologist, Department of Immunology, HUCH Laboratory Diagnostics; E.K.L. Chan, PhD, Department of Molecular and Experimental Medicine, The Scripps Research Institute; M. Eronen, MD, Pediatric Cardiologist, Hospital for Children and Adolescents, Helsinki University Hospital.

Address reprint requests to Dr. H. Julkunen, Department of Internal Medicine, Peijas Hospital, Helsinki University Hospital, 01400 Vantaa, Finland. E-mail: heikki.julkunen@hus.fi

Submitted October 11, 2002; revision accepted July 24, 2003.

According to the most plausible hypothesis, congenital heart block (CHB) without intracardiac anatomic malformations is caused by maternal antibodies to SSA and/or SSB that cross the placenta during the second trimester of pregnancy and damage the fetal conduction system by immunological mechanisms¹. There are, however, reports of cases of isolated CHB not associated with antibodies to SSA and/or SSB^{1,2}.

The cause of isolated HB in children detected after the newborn period (postnatally diagnosed HB) is largely unknown. It has been suspected that many cases previously detected in early infancy are congenital and this is explained by failure to diagnose overt third-degree HB during pregnancy³. Another explanation for late detection of isolated CHB is the possible progression of first or second-degree HB to third-degree after birth^{1,4,5}. Therefore, mothers of children

with postnatally diagnosed HB may have abnormal immunological findings including antibodies to SSA and/or SSB.

According to previous studies, one-third to two-thirds of mothers of children with CHB have or will later develop a connective tissue disease (CTD)⁶⁻⁸, most commonly primary Sjögren's syndrome (SS) or systemic lupus erythematosus (SLE). Other more rarely reported diseases include rheumatoid arthritis, mixed connective tissue disease (MCTD), ankylosing spondylitis, or linear scleroderma^{7,9}. Although most mothers of children with CHB appear to develop at least some symptoms and/or signs suggesting a subclinical autoimmune disease, there are some affected mothers who, even decades after the birth of their child with CHB, are totally asymptomatic⁸. Whether mothers of children with CHB have marker antibodies that predict future development of a specific CTD is not well known.

About 2% of women with antibodies to SSA are at risk of having a child with CHB¹⁰, suggesting that only some antigenic subspecificities of the heterogeneous antibodies may have harmful effects on some fetal heart tissues. According to previous studies, risk factors for having a child with CHB have been strong reactions by practically all anti-SSA and anti-SSB assays and especially the presence of anti-52 kDa SSA by immunoblot^{11,12}. One of the highest risk factors, however, has been the birth of a previous child with CHB (the risk of recurrence is 15–17%)^{13,14}.

In this population-based study from Finland, we sought answers to the following questions: Are maternal antibodies to SSA and/or SSB a prerequisite for CHB? How often is postnatally diagnosed HB associated with maternal antibodies to SSA and/or SSB, suggesting that HB dates from birth? Do mothers of children with CHB have marker antibodies predicting future development of a specific CTD? Who has a high or low risk of having a child with CHB?

MATERIALS AND METHODS

Mothers of children with isolated HB. The recruitment of these mothers has been described^{8,15}. A pediatric cardiologist (ME) sought all children who had been diagnosed with isolated HB and who were born in Finland between 1950 and 2000. Children were identified from hospital discharge registries and pacemaker insertion registries of all 5 university central hospitals in Finland. The obstetric case records of the mothers and the hospital records of the neonates, children, and adults were carefully reviewed. Children were included in the study only if the HB was second or third-degree and was diagnosed before the age of 16 years, and there were no intracardiac anatomic malformations considered to be causally related to HB. Children whose HB had been diagnosed during pregnancy by ultrasound or by electrocardiogram (ECG) immediately after birth were defined as having CHB; all others had postnatally diagnosed HB.

Mothers of these children were identified and contacted personally, by mail and/or by telephone by the responsible rheumatologist (HJ), and demographic and clinical data were collected according to a protocol that consisted of 39 items. In addition, all medical records of these mothers from different hospitals and/or health care centers in Finland were examined to collect signs, symptoms, and laboratory values suggesting an underlying autoimmune disorder and to classify definitive autoimmune diseases. We identified 78 living mothers of children with CHB and 47 living mothers of children with postnatally diagnosed HB. Sera from the determi-

nation of immunological tests could be collected from 67 (86%) mothers of children with CHB and from 37 (78%) mothers of children with postnatally diagnosed HB a mean of 9.9 years (SD 8.8, range 0–49 yrs) and 22.6 years (SD 13.1, range 4–47 years) after the index delivery, respectively. Of the 67 mothers of children with CHB, 23 had been described by us and tested for antibodies to SSA and SSB by recombinant ELISA and immunoblot¹⁶. Of the 37 cases of postnatally diagnosed HB, 15 had been detected after birth but before the age of 1 year, 13 between ages 1 to 5 years, and 9 between ages 6 to 16 years. We feel that we identified the majority of all children diagnosed with isolated HB in Finland, and that we could clinically characterize and collect sera from most mothers of these children.

Controls. Controls with CTD were consecutive parous women attending 2 outpatient rheumatology departments of the Helsinki University Hospital, and consisted of 90 patients with SLE¹⁷, 32 with primary SS¹⁸, and 14 with other CTD (7 MCTD¹⁹, 4 scleroderma²⁰, one polymyositis²¹, and 2 with fibrosing alveolitis associated with SS and primary biliary cirrhosis, respectively). Healthy controls included 89 parous women without a previously diagnosed chronic autoimmune disease from the department staff of the Helsinki University Hospital. All controls had borne at least one child, none of whom was known to have cardiac conduction abnormalities. Sera from the patients and controls were stored at –20°C for further testing. In general, there was only one sample per mother.

Antibody detection. Antibodies to 52 kDa SSA, 60 kDa SSA, and 48 kDa SSB antigens were assayed by a time-resolved fluoroimmunoassay (TR-FIA). The wells of the microtiter plates (Greiner Bio-One, Frickenhausen, Germany) were coated with the respective recombinant human proteins that are produced as 6xHis tag fusion proteins in BL21(DE3) *E. coli* and were purified twice using nickel column chromatography^{22,23} [0.5 µg/ml in PBS (100 µl/well) at 4°C overnight]. After washing with phosphate buffered saline, 150 µl of TBS-T buffer (50 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.6) with 0.1% bovine serum albumin (BSA) and 0.05% Tween 20 was added in the wells and incubated at room temperature for 1 h to block nonspecific protein binding. After discarding the blocking solution, test sera diluted 1:40 in TBS-T with 0.1% BSA and positive and negative control sera were added (125 µl/well), and the wells were incubated on a shaker in humidified chambers at room temperature for 2 h. The wells were washed with TBS-T, and affinity purified goat anti-human IgG (Fc-specific; Jackson ImmunoResearch, West Grove, PA, USA) coupled with Europium-chelate (Delfia, Wallac, Turku, Finland) according to the instructions of the manufacturer, diluted 1:10,000 in assay buffer (Wallac), was added in the wells (100 µl/well). After further incubation at room temperature for 1 h, the wells were washed with TBS-T, enhancer solution (Wallac) was added in the wells, and fluorescence was read on a Victor multi-label analyzer (Wallac). Known positive and negative samples were used to construct standard curves for the assay. The upper reference values for anti-52 kDa SSA, anti-60 kDa SSA, and anti-48 kDa SSB were set at the 97th percentile for 74, 70, and 73 healthy controls, respectively.

For the immunoblotting assay we used the Inno-Lia™ ANA test kits (Innogenetics NV, Ghent, Belgium) as suggested by the manufacturer. For this assay the sera were diluted 1:200 in the sample diluent, and goat anti-human IgG labeled with alkaline phosphatase was used as the secondary antibody. Affinity purified (SSA/Ro 60), synthetic (SmD), or recombinant (SmB, RNP-70 kDa, RNP-A, RNP-C, SSA/Ro 60, SSB, CenpB, TopoI/Scl-70, Jo-1/HRS, Ribosomal RNP, and histone proteins) human antigens are bound to known sites of the test strips. The strips were screened on a Hewlett Packard Scanjet 6300 C scanner, and the results were analyzed using Lia-LiPA Interpretation software and Lia-Scan ANA Update Version 1.0-990X. The relative intensities of the staining reactions given by the test sera as compared to the cutoff control of the kits were expressed as arbitrary units (U).

Antinuclear (ANA) and anti-dsDNA antibodies were detected by the indirect immunofluorescence technique using HEP-2 cells (Nova Lite™ ANA, Inova Diagnostics, San Diego, CA, USA) and *Criethidia luciliae* cells (Nova Lite™ dsDNA, Inova) as substrates, respectively.

Seronegative sera of mothers of CHB children (as determined by the above assays) were retested in another laboratory (by EC) by ELISA using affinity purified recombinant antigens, by immunoblotting using MOLT-4 extracts²³, and by indirect immunofluorescence on commercially prepared HEp-2 cell substrates (HEp-2000; Immuno Concepts Inc., Sacramento, CA, USA) using sera diluted at 1/100 and an Alexa 488 conjugated goat anti-human Ig was used as described^{24,25}.

Statistical analyses. Nonparametric data were analyzed using the Mann-Whitney U-test. Spearman's rank correlation was applied in studying correlations between the assays for anti-SSA and anti-SSB. Odds ratios (OR) were calculated by confidence interval (CI) analysis. The sensitivities, specificities, and positive and negative predictive values (PPV and NPV, respectively) were calculated as indicated in Table 1. All p values were 2-tailed, and differences at 0.05 were considered significant.

RESULTS

Autoantibodies in mothers of children with CHB. Of 67 mothers of children with CHB, 7 (10%) were negative by all anti-SSA and anti-SSB assays initially performed (Table 1).

The 7 seronegative sera were retested by ELISA, immunoblot, and immunofluorescence as described above. One of the 7 sera was found to have antibodies to 60 kDa SSA protein by ELISA and was also found to be weakly positive by immunoblot (60 kDa SSA band). In addition, one serum sample was found to be positive by the HEp-2000 test (the patient also had ANA using HEp-2 cells).

In a previous study, we had tested sera from 3 of these 7 initially seronegative mothers and all were found to have antibodies to SSA and/or anti SSB¹⁶.

Altogether, only 3 (4%) of the 67 mothers of children with CHB were seronegative by all assays performed in this study or previously. One of the 3 mothers had had grand mal epilepsy 9 years before the birth of a child with CHB and had been photosensitive since the birth of her child with CHB. The remaining 2 seronegative mothers (3%) were completely asymptomatic 2 and 21 years after the index delivery, respectively.

Two (3%) of the 67 mothers of children with CHB had

anti-dsDNA. One of them satisfied criteria for SLE 20 years after the index delivery and had 3 children with CHB (including concordant identical female twins). The other of these 2 mothers satisfied criteria for primary SS 2 years after the index delivery and fulfilled 3 criteria for SLE. Fifty-one (77%) out of 66 mothers tested for ANA were positive (titers ≥ 320). By immunoblot, one (2%) mother out of 67 had antibodies to RNP 70 kDa, one to Jo-1/HRS, and one to histone proteins. Excluding anti-SSA and anti-SSB, all other antibodies by immunoblot were negative in these mothers.

Autoantibodies in mothers of children with post-natally diagnosed HB. Of 37 mothers of children with post-natally diagnosed HB, 2 (5%) were positive by at least one anti-SSA or anti-SSB assay. One of these 2 mothers developed mild symptoms of dry eyes 7 years after the index delivery and had only a very marginally elevated titer of anti-SSB by TR-FIA. Her child's HB was detected in 1991 at the age of 5 years.

The other of these 2 seropositive mothers had 7 children, one of whom was diagnosed as having isolated HB in 1961 at the age of 10 years. The mother had high-titer antibodies to 52 kDa SSA by TR-FIA and immunoblot and to 48 kDa SSB by TR-FIA. She had an episode of arthritis 4 years before the index delivery and later developed dry eyes and was found to have leukopenia.

One (3%) of the 37 mothers of children with post-natally diagnosed HB had high-titer ANA (≥ 5000) but no antibodies to SSA or SSB. This mother was completely asymptomatic, and her child's HB was diagnosed at the age of 11 months in 1966.

Thirty-four (92%) mothers of 37 children with postnatally diagnosed HB were negative by all immunological assays performed in the study.

Antibodies to SSA and SSB in mothers of children with CHB and in controls. The sensitivities and specificities, PPV, and NPV of the tests for having a child with CHB are shown in

Table 1. Prevalence of antibodies to SSA and SSB in 67 mothers of children with CHB and in 136 mothers with SLE (90), primary SS (32), and other CTD (14) with healthy children. Sensitivity is the rate of true positives (number of patients with thrombosis and a positive test result divided by the number of patients with thrombosis); specificity is the rate of true negatives (number of patients without thrombosis and a negative test result divided by the number of patients without thrombosis); PPV is the number of true positives from all positives; NPV is the number of true negatives from all negatives.

	% Positive (n)	CHB	Sensitivity, %	Specificity, %	PPV	NPV
Any test	69 (130/188)	60/67	90	42	46	88
Any TR-FIA	65 (128/198)	60/67	90	48	47	90
52 kDa SSA	57 (112/198)	58/67	87	59	52	90
60 kDa SSA	28 (55/198)	28/67	42	79	51	73
48 kDa SSB	43 (84/197)	42/66	64	68	50	79
Any immunoblot	59 (112/189)	59/67	88	57	53	90
52 kDa SSA	56 (106/190)	59/67	88	62	56	90
60 kDa SSA	54 (103/189)	58/67	87	63	56	90
48 kDa SSB	42 (79/189)	46/67	69	73	58	81

CHB: congenital heart block; SLE: systemic lupus erythematosus; TR-FIA: time-resolved fluoroimmunoassay.

Table 1. In general, TR-FIA and immunoblot were both sensitive, but immunoblot was more specific. PPV were somewhat higher by immunoblot than by TR-FIA, but NPV were similar. Spearman correlation coefficients of the tests were good ($r = 0.506\text{--}0.889$, $p < 0.001$).

Table 2 shows that OR for having a child with CHB were highest among anti-52 kDa and anti-60 kDa SSA by immunoblot and anti-52 kDa SSA by TR-FIA and lowest by anti-60 kDa SSA by TR-FIA. In general, OR by immunoblot were somewhat higher than by TR-FIA.

When the antibody levels of different assays were compared among each group of patients, no statistically significant differences were found between mothers of children with CHB and mothers with primary SS ($p = 0.62\text{--}1.03$ for TR-FIA and $p = 0.70\text{--}0.93$ for immunoblot). In comparison with SLE and CTD (together, $n = 104$), mothers of children with CHB had significantly higher antibody titers ($p < 0.001$ for all assays). The immunoblot findings in the different groups are shown in Figure 1. The figures were similar by TR-FIA.

DISCUSSION

Are maternal antibodies to SSA and/or SSB a prerequisite for the development of CHB? Only 3 (4%) out of the 67 mothers in our study had no autoantibodies. One of these 3 mothers had epilepsy and photosensitivity, suggesting an underlying autoimmune disorder. There were only 2 (3%) mothers who were totally asymptomatic and who were negative by all immunological tests performed. It is possible that these 2 mothers are true seronegative and CHB could be caused by other mechanisms than those associated with anti-SSA and/or anti-SSB².

One explanation for false seronegative CHB could be that antibodies present in the circulation during the index pregnancy are nondetectable at another time point many years after the pregnancy. This, however, is quite rare, since the antibodies to SSA and SSB are under genetic regulation²⁶, and they have been found to maintain a very stable profile for many years^{27,28}. Antibody levels may change, but

only very little. Weakly positive sera may become negative, and just negative may become weakly positive when retested, which we also found in our study.

Another explanation for seronegativity is that antibodies to SSA and SSB are determined only once and by insensitive methods. It is also possible that very low concentrations (repeatedly below detection) of maternal antibodies can mediate CHB. Thus, according to this study and others^{1,11,29}, isolated CHB is practically always associated with maternal antibodies to SSA and/or SSB and, therefore, has a tendency to recur. True seronegative CHB is rare.

How often is HB diagnosed postnatally dating from birth? To answer this question more definitively would require ECG taken at birth on every child who will later develop HB, which is not possible. We can, however, speculate on this issue. Since almost all mothers of children with CHB have autoantibodies and/or at least some symptoms or signs suggesting an underlying autoimmune disorder, the complete absence of all these manifestations in a mother whose child is diagnosed with HB postnatally would suggest that HB did not date from birth. Conversely, if a mother whose child is diagnosed with HB postnatally (e.g., at the age of 10 years as in a case in our study) has very high levels of antibodies to SSA and SSB and clinical manifestations of autoimmune disease, it would strongly suggest that the HB dated from birth. However, this speculation is self-fulfilling and does not prove that postnatally diagnosed HB cannot be congenital if the mother is seronegative. It can be congenital, and is likely to be congenital, at least in very young infants, but it is very rarely associated with antibodies to SSA and/or SSB according to our study.

We found that only 3 (8%) out of the 37 mothers of children with postnatally diagnosed HB had elevated levels of autoantibodies with or without symptoms suggesting a subclinical maternal autoimmune disease. In these 3 mothers, late detection or possible progression of CHB as a cause of postnatally diagnosed HB was most obvious. In the remaining 34 mothers of 37 children with postnatally diagnosed HB, all immunological tests performed were negative.

Table 2. Relationship of individual anti-SSA and anti-SSB tests and a history of having a child with CHB (67 mothers of children with CHB, 90 mothers with SLE, 32 with primary SS, and 14 with other CTD).

Test	Negative (%)	Positive (%)	OR (95% CI)
TR-FIA			
52 kDa SSA	9/86 (11)	58/112 (52)	9.2 (4.2–20.1)
60 kDa SSA	39/143 (27)	28/55 (51)	2.8 (1.5–5.3)
48 kDa SSB	24/113 (21)	42/84 (50)	3.7 (2.0–6.9)
Any TR-FIA	7/70 (10)	60/128 (47)	7.9 (3.4–18.7)
Immunoblot			
52 kDa SSA	8/84 (10)	59/106 (56)	11.9 (5.3–27.2)
60 kDa SSA	9/77 (12)	58/103 (56)	11.0 (5.0–24.4)
48 kDa SSB	21/110 (19)	46/79 (58)	5.9 (3.1–11.3)
Any immunoblot	8/77 (10)	59/112 (53)	9.6 (4.2–21.8)
Any test	7/58 (12)	60/130 (46)	6.2 (2.6–14.8)

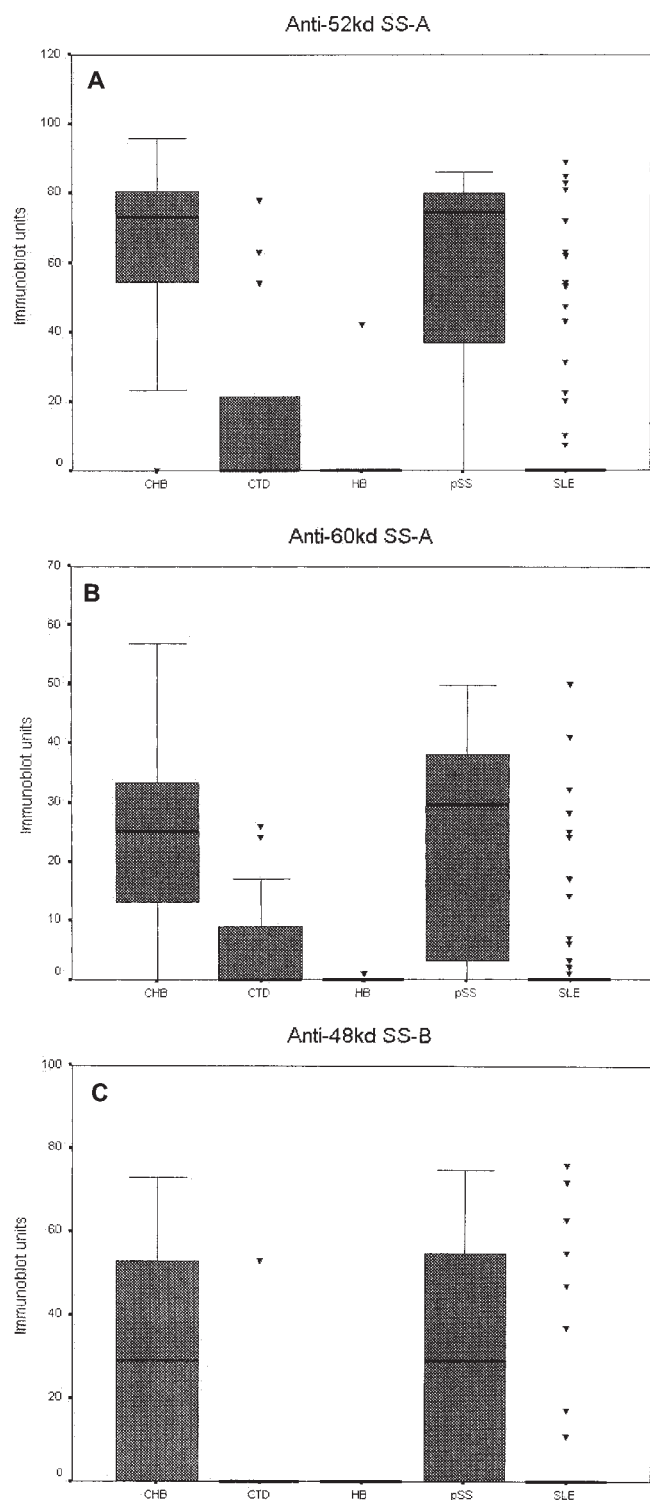


Figure 1. Antibody profiles of sera [quantitative immunoblot for (A) anti-52 kDa, (B) anti-60 kDa SSA, and (C) anti-48 kDa SSB] from 67 and 37 mothers of children with CHB and postnatally diagnosed HB, respectively, compared with 14 mothers with CTD, 32 with primary Sjögren's syndrome (pSS), and 90 with SLE who had healthy children. No statistical differences were found between mothers of children with CHB and mothers with pSS. In comparison with SLE and CTD, mothers of children with CHB had significantly higher antibody titers by all immunoblot assays ($p < 0.001$).

Our findings are in contrast to those of Hubscher, *et al*³, who reported that 9 (82%) out of their 11 mothers whose children were diagnosed with complete HB after birth but before the age of 3 months had autoantibodies. In our study, 15 cases of postnatally diagnosed HB were diagnosed between 0 and 12 months of age, but none of them was diagnosed between 0 and 3 months. In those 15 mothers whose children were diagnosed with HB between 4 and 12 months of age, one had ANA. We were quite surprised by this finding. We had expected that most mothers whose children had been diagnosed with HB during their first year of life would have had several immunological abnormalities, but it was not so. The most likely explanation for the discrepancy between our results and those of Hubscher, *et al* is that methods to diagnose CHB in the past have been different in Finland and in Argentina. Since the 1950s, regular checkups for pregnant women have included measurement of fetal heart rates in regional maternity welfare centers in Finland. We believe that third-degree CHB has seldom escaped detection during pregnancy or at birth in our country even decades ago. In our series, there are obvious undiagnosed cases, but they are rare.

Hubscher, *et al* also reported that out of their 6 mothers whose children were diagnosed with HB between ages 17 months and 10 years, only one had autoantibodies. This finding is in good agreement with our results: we found that only 2 out of 22 mothers whose children were diagnosed with postnatal HB between 1 and 16 years of age had autoantibodies. In other studies, the absence of anti-SSA is mostly restricted to mothers of children with HB diagnosed after 1 year of age^{29,30}.

What is the etiology of postnatally diagnosed HB in children if the disease is not associated with maternal antibodies to SSA or SSB? Literature data to answer this question are quite limited. There are occasional reports of atrioventricular block associated with myocarditis, endocarditis, cardiomyopathy, inherited collagen diseases, different medications, and rare environmental exposure³¹. In our study, we could find no overt explanation for postnatally diagnosed HB in our children during the clinical followup and by examining the hospital case records. It therefore appears that the etiology of HB first detected in childhood is still, for the most part, a mystery. Postnatally diagnosed HB in a young infant (< 1–3 years) is more likely to be congenital and may be associated with mass lesions of the conducting system, e.g., fibroma, rhabdomyoma, or hemangioma of the atrial cavity².

Do mothers of children with CHB have marker antibodies for clearly defined CTD? Only 2 of our mothers had anti-dsDNA, and, with the exception of anti-SSA and anti-SSB, practically all other antibodies were negative. These findings clearly support previous clinical findings that the majority of mothers of children with CHB have or will later develop subclinical or overt primary SS⁸.

We found increased risk for having a child with CHB was indicated by maternal primary SS and high levels of antibodies to SSA and SSB, whereas low risk was indicated by maternal SLE and CTD and undetectable or low levels of the antibodies. Some individual anti-SSA and anti-SSB assays gave higher OR than others (especially immunoblots), but we considered the differences to have limited clinical significance. Further, we could find no differences in the antibody levels or specificities between mothers of children with CHB and mothers with primary SS. These findings from our population-based study are similar to those reported by others^{3,7,10,11,29}.

Although antibodies to SSA and/or SSB appear to be necessary for the development of CHB, it is possible that they are only an unspecific marker for the disease. Autoantibodies against M3-type muscarinic receptors have been described in patients with SS affecting parasympathetic neurotransmission and the function of lacrimal or salivary glands³². It is possible that these or other receptor antibodies described in patients with primary SS, but not in SLE, either independent or associated with anti-SSA and/or SSB, might affect fetal cardiac conduction³³.

Antibodies to SSA and/or SSB are not sufficient to cause CHB. This is clearly shown by the rarity of CHB in seropositive mothers¹⁰, and by reports on twins and siblings both discordant and concordant for CHB³⁴. Thus, there must be other unknown intrinsic (fetal) or extrinsic (infectious or chemical) factors that ultimately determine which of the mothers (usually with moderate to high titer antibodies to SSA and/or SSB) will have a child with CHB and which of their children is going to be affected.

In this population-based study, we show that almost all mothers of children with CHB have antibodies to SSA and/or SSB, and that HB diagnosed postnatally is very rarely associated with any maternal autoimmune disorder. Antibodies to SSA and SSB are marker antibodies for mothers of children with CHB, confirming previous clinical studies that found the majority of these mothers have or will later develop either subclinical or overt primary Sjögren's syndrome. There is no unique antibody profile for CHB. Mothers with primary SS and/or high levels of antibodies to SSA and/or SSB in nearly all assays are at increased risk of having a child with CHB, whereas low risk is associated with maternal SLE and other CTD, and with low levels of the antibodies.

REFERENCES

- Buyon JP, Kim MY, Copel JA, Friedman DM. Anti-Ro/SSA antibodies and congenital heart block: necessary but not sufficient. *Arthritis Rheum* 2001;44:1723-7.
- Allan L, Hornberger L, Sharland G. Fetal cardiac tumors. In: Allan L, Hornberger L, Sharland G, editors. *Textbook of fetal cardiology*. London: Greenwich Medical Media Limited; 2000:358-65.
- Hubscher O, Batista N, Rivero S, et al. Clinical and serological identification of 2 forms of complete heart block in children. *J Rheumatol* 1995;22:1352-5.
- McCarron DP, Hellman DB, Traill TA, Watson RM. Neonatal lupus erythematosus syndrome: late detection of isolated heart block. *J Rheumatol* 1993;20:1212-4.
- Gegge RL, Tucker L, Szer I. Postnatal progression from second- to third-degree heart block in neonatal lupus erythematosus syndrome. *J Pediatrics* 1998;113:1049-52.
- Waltuck J, Buyon JP. Auto-antibody-associated congenital heart block: outcome of mothers and children. *Ann Intern Med* 1994;120:544-51.
- Press J, Uziel Y, Laxer RM, Luy L, Hamilton RM, Silverman ED. Long-term outcome of mothers of children with congenital heart block. *Am J Med* 1996;100:328-32.
- Julkunen H, Eronen M. Long-term outcome of mothers of children with isolated heart block in Finland. *Arthritis Rheum* 2001;44:647-52.
- Nolan RJ, Shulman ST, Victorica BE. Congenital complete heart block associated with maternal mixed connective tissue disease. *J Pediatr* 1979;95:420-2.
- Brucato A, Frassi M, Franceschini M, et al. Risk of congenital complete heart block in newborns of mothers with anti-Ro/SSA antibodies detected by counterimmunoelectrophoresis: a prospective study of 100 women. *Arthritis Rheum* 2001;44:1832-5.
- Buyon JP, Winchester RJ, Slade SG, et al. Identification of mothers at risk for congenital HB and other neonatal lupus syndromes in their children. *Arthritis Rheum* 1993;36:1263-73.
- Julkunen H, Kaaja R, Siren MK, et al. Immune-mediated congenital heart block (CHB): Counseling pregnancies and identifying risk factors for CHB. *Semin Arthritis Rheum* 1998;28:97-106.
- Buyon JP, Hiebert R, Copel J, et al. Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. *J Am Coll Cardiol* 1998;31:1658-66.
- Julkunen H, Eronen M. Rate of recurrence of congenital heart block — a population based study. *Arthritis Rheum* 2001;44:487-8.
- Eronen M, Siren MK, Ekblad H, Tikanoja T, Julkunen H, Paavilainen T. Short and long-term outcome of the children with isolated congenital complete heart block diagnosed in utero or as newborn. *Pediatrics* 2000;106:86-91.
- Julkunen H, Kurki P, Kaaja R, et al. Isolated congenital heart block: long-term outcome of mothers and characterization of the immune response to SSA/Ro and SSB/La. *Arthritis Rheum* 1993;36:1488-98.
- 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.
- Vitali C, Bombardieri S, Moutsopoulos HM, et al. Preliminary criteria for the classification of Sjögren's syndrome. Results of an EEC prospective concerted action. *Arthritis Rheum* 1993;36:340-7.
- Alarcon-Segovia D, Cardiel MM. Comparison between three diagnostic criteria for mixed connective tissue disease. Study of 593 patients. *J Rheumatol* 1989;16:328-34.
- Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581-90.
- Bohan A, Peter JB. Polymyositis and dermatomyositis. Part I and part II. *N Engl J Med* 1975;292:344-7,403-7.
- Chan EKL, Sullivan KF, Tan EM. Ribonucleoprotein SS-B/La belongs to a protein family with consensus sequences for DNA-binding. *Nucl Acid Res* 1989;17:2233-44.
- Chan EKL, Hamel JC, Buyon JP, Tan EM. Molecular definition and sequence motifs of the 52-kd component of human SS-A/Ro autoantigen. *J Clin Invest* 1991;87:68-76.
- Fritzler MJ, Hanson C, Miller J, Eystathioy T. Specificity of autoantibodies to SS-A/Ro on a transfected and overexpressed

- human 60 kDa Ro autoantigen substrate. *J Clin Lab Anal* 2002;16:103-8.
25. Keech CL, Howarth S, Coates T, Rischmueller M, McCluskey J, Gordon TP. Rapid and sensitive detection of anti-Ro (SS-A) antibodies by indirect immunofluorescence of 60kDa Ro HEp-2 transfectants. *Pathology* 1996;28:54-7.
 26. Arnett FC, Bias WB, Reveille JD. Genetic studies in Sjögren's syndrome and systemic lupus erythematosus. *J Autoimmun* 1989;2:403-13.
 27. Tseng CE, Di Donato F, Buyon JP. Stability of immunoblot profile of anti-SSA/Ro-SSB/La antibodies over time in mothers whose children have neonatal lupus. *Lupus* 1996;5:212-5.
 28. Yamagata H, Akizuki M, Tojo T, Homma M. Anti-Ro/SSA and -La/SSB antibodies in patients with connective tissue diseases. *Scand J Rheumatol* 1986;15 Suppl:98-101.
 29. Brucato A, Franceschini F, Gasparini M, et al. Isolated congenital HB: Long-term outcome of mothers, maternal antibody specificity and immunogenetic background. *J Rheumatol* 1995;22:533-40.
 30. Scott JS, Maddison PJ, Taylor PV, Essher E, Scott O, Skinner P. Connective tissue disease, antibodies to ribonucleoprotein and congenital heart block. *N Engl J Med* 1983;309:209-12.
 31. Bharati S, Lev M. Pathology of atrioventricular block. *Cardiovasc Clin* 1984;2:741-51.
 32. Waterman SA, Gordon TP, Rischmueller M. Inhibitory effects of muscarinic receptor autoantibodies on parasympathetic neurotransmission in Sjögren's syndrome. *Arthritis Rheum* 2000;43:1647-54.
 33. Eftekhari P, Salle L, Lezoualch F, et al. Anti-SSA/Ro52 autoantibodies blocking the cardiac 5-HT₄ serotonergic receptor could explain neonatal lupus congenital heart block. *Eur J Immunol* 2000;30:2782-90.
 34. Buyon JP. Neonatal lupus syndrome. In: Lahita R, editor. *Systemic lupus erythematosus*. 3rd ed. New York: Academic Press; 1998:337-59.