Congenital Heart Block Not Associated with Anti-Ro/La Antibodies: Comparison with Anti-Ro/La-positive Cases

ANTONIO BRUCATO, CHIARA GRAVA, MARIA BORTOLATI, KEIGO IKEDA, ORNELLA MILANESI, ROLANDO CIMAZ, VERONIQUE RAMONI, GABRIELE VIGNATI, STEFANO MARTINELLI, YOUCEF SADOU, ADELE BORGHI, ANGELA TINCANI, EDWARD K.L. CHAN, and AMELIA RUFFATTI

ABSTRACT. ***Objective.*** To study anti-Ro/La-negative congenital heart block (CHB).

Methods. Forty-five fetuses with CHB were evaluated by analysis of anti-Ro/La antibodies using sensitive laboratory methods.

Results. There were 9 cases of anti-Ro/La-negative CHB; 3 died (33.3%). Only 3 (33.3%) were complete *in utero* and 5 (55.5%) were unstable. No specific etiology was diagnosed. Six infants (66.6%) were given pacemakers. There were 36 cases of anti-Ro/La-positive CHB. All except 2 infants (94.4%) had complete atrioventricular block *in utero*. Ten died (27.8%), one (2.7%) developed severe dilated cardiomyopathy, and 26 (72.2%) were given pacemakers.

Conclusion. Nine of the 45 consecutive CHB cases (20%) were anti-Ro/La-negative with no known cause. They were less stable and complete than the anti-Ro/La positive cases. (First Release July 1 2009; J Rheumatol 2009;36:1744–8; doi:10.3899/jrheum.080737)

Key Indexing Terms:
HEART BLOCK ANTINUCLEAR ANTIBODIES AUTOIMMUNE DISEASES

Most cases of congenital heart block (CHB) detected *in utero* and unrelated to structural cardiac abnormalities are associated with anti-Ro/La antibodies, although the percentages of positive cases vary[1-5]. Descriptions of the prevalence and outcome of anti-Ro/La-negative atrioventricular (AV) block also tend to differ[2-10]. According to some investigators, mortality and morbidity are similar to those in anti-Ro/La-positive AV block[3,4,7,8], while others report spontaneously reversible CHB and a more favorable course[6,9,10].

From the Departments of Internal Medicine and Pediatric Cardiology and Neonatal Intensive Care Unit, Ospedali Riuniti, Bergamo, Italy; Departments of Clinical and Experimental Medicine, Rheumatology, and Pediatric Cardiology, University of Padova, Padova, Italy; Department of Oral Biology, University of Florida, Gainesville, Florida, USA; Department of Pediatric Rheumatology, Meyer Hospital, Florence, Italy; Departments of Obstetrics and Gynecology and Pediatric Cardiology, and Neonatal Intensive Care Unit, Niguarda Hospital, Milano, Italy; and Departments of Rheumatology and Clinical Immunology, Spedali Civili and University of Brescia, Brescia, Italy.

Dr. Chan was supported in part by US National Institutes of Health grant AI47859.

A. Brucato, MD, Department of Internal Medicine, Ospedali Riuniti; C. Grava, MD, PhD; M. Bortolati, MD, Department of Rheumatology, University of Padova; K. Ikeda, MD, PhD, Department of Oral Biology, University of Florida; O. Milanesi, MD, Department of Pediatric Cardiology, University of Padova; R. Cimaz, MD, Department of Pediatric Rheumatology, Meyer Hospital; V. Ramoni, MD, Department of Internal Medicine, Ospedali Riuniti; G. Vignati, MD; S. Martinelli, MD, Departments of Obstetrics and Gynecology and Pediatric Cardiology, and Neonatal Intensive Care Unit, Niguarda Hospital; Y. Sadou, MD, Neonatal Intensive Care Unit, Ospedali Riuniti; A. Borghi, MD, Department of Pediatric Cardiology, Ospedali Riuniti; A. Tincani, MD, Departments of Rheumatology and Clinical Immunology, Spedali Civili and University of Brescia; E.K.L. Chan, PhD, Department of Oral Biology, University of Florida; A. Ruffatti, MD, PhD, Department of Rheumatology, University of Padova.

Address correspondence to Dr. A. Brucato, Medicina Interna, Ospedali Riuniti, Largo Barozzi 1, 24128 Bergamo, Italy. E-mail: albrucato@ospedaliriuniti.bergamo.it

Accepted for publication March 2, 2009.

MATERIALS AND METHODS

Forty-five consecutive fetuses with AV block were observed from 1990 to 2007 in 5 tertiary referral centers in Northern Italy (2 rheumatological, 2 cardiological, and one obstetric clinic). The inclusion criteria were congenital AV block detected *in utero* or at birth by fetal echocardiography and electrocardiogram. The exclusion criteria were structural cardiac abnormalities, congenital long QT syndrome[11], mothers who had taken drugs during pregnancy that could induce fetal bradycardia, mothers who had had infectious diseases during pregnancy, or who had tested positive to hepatitis B/C viruses or human immunodeficiency virus, or to IgM anticytomegalovirus, Herpes or rubella virus and toxoplasma at the beginning of pregnancy.

Maternal sera were collected when CHB was detected and at delivery and tested for autoantibodies to Ro/SSA and La/SSB ribonucleoproteins using ELISA. Sera were tested a second time at the Padua University Hospital rheumatology laboratory employing a custom-designed counterimmunoelectrophoresis (CIE) method[12]; the fine specificities for 52-kDa and 60-kDa anti-Ro/SSA and 48-kDa anti-La/SSB were determined using a commercial ELISA (Diamedix, Delta Biologicals, Rome, Italy) and a line-blot assay (Inno-Lia, Innogenetics, Ghent, Belgium). Maternal sera negative to anti-Ro/SSA and anti-La/SSB antibodies were tested for confirmation by immunoblotting analysis using human salivary gland cell lysates, by ELISA using recombinant Ro52 protein, and by immunoprecipitation analysis with Ro60 and La *in vitro* translated proteins[13], all at the laboratory of the University of Florida Department of Oral Biology.

Maternal sera were also tested at the Padua laboratory for a battery of autoantibodies, including antinuclear (ANA), anti-dsDNA, anti-extractable nuclear antigens (ENA), anticardiolipin, and anti-ß$_2$-glycoprotein I antibodies.

Statistical analysis was done using SPSS software, version 14.0.

RESULTS

Forty-five fetuses with CHB were examined. Thirty-six were born to anti-Ro/La-positive mothers (80%) and 9 to anti-Ro/La-negative mothers (20%). Negative maternal sera lacked reactivity to both Ro/SSA and La/SSB according to ELISA, CIE, and line-blot assays. These results were confirmed by the University of Florida Department of Oral Biology.

Anti-Ro/La-negative CHB infants (Table 1). Five (55.5%) of the 9 infants were female. Three developed complete AV block *in utero* (Patients 1, 2, 3) and one also presented congenital sensorineural deafness. Two others had a stable second-degree AV block (Patients 4 and 5). Two fetuses had a second-degree AV block, one progressing to complete block soon after birth and the other at 3 months (Patients 6 and 7). The block alternated with normal sinus rhythm in the other 2 infants (Patients 8 and 9) and reverted to a stable normal sinus rhythm in Patient 9. Five blocks were unstable, changing their degree (nos 3,6,7,8,9).

Six blocks (66.6%) were detected *in utero*. Three (33.3%) were diagnosed at birth (Table 1 and 2) when cesarean delivery was needed because of fetal bradycardia. Six were given pacemakers. Two presented signs of heart

Table 1. Features of fetuses/infants born to anti Ro/La-negative mothers.

Patient	Sex	Year of Birth	GA CHB	Lower Fetal HR	GA at Delivery	HR at Birth	Apgar Score	Fetal Heart Failure	Age at Permanent Pacing	Features of Block	Outcome (2007)
1	M	2005	22	35	30	27	7–8	Ascites, LV dilatation	1 day	Stable 3rd degree	Died (at 5 mo from respiratory distress complicated by sepsis)
2	F	1996	32	30	36	58	8–9	No	1 day	Stable 3rd degree Dexamethasone 84 mg in utero pending autoantibody finding	Alive (congenital sensorineural deafness)
3	F	2005	19	45	37	40	8–8	Hydrops, LV dilatation	20 days	2nd degree, progressed to 3rd degree in utero and then stable. Betamethasone 102 mg in utero pending autoantibody finding	Died (suddenly at 2 mo despite pacing)
4	F	1999	At birth	80	41	100	8–10	No	1 yr	Stable 2nd degree	Alive
5	M	2001	29	80	34	70	7–9	No	No pacing	Stable 2nd degree	Alive
6	F	2005	At birth	80	34	75	7–8	No	20 days	2nd degree at birth; 3rd degree at 3 days	Alive
7	M	2001	At birth	96	41	96	9–10	No	4 mo	Extrasystoles at 29 and 30 wks; 2nd degree AV block at birth, 3rd degree at 3 mo	Alive
8	F	1996	29	40	35	60	5–7	No	No pacing	2nd degree alternating with 3rd degree in utero. Betamethasone 24 mg to enhance fetal lung maturity. 3rd degree at birth, reverted to NSR 30 min after birth but worsened to 2nd degree block the next day	Died (at 15 days from respiratory distress, pulmonary hypertension, and cardiac failure)
9	M	2005	29	80	34	110	9–9	No	No pacing	28 wks fetus in NSR; at 29 wks complete AV block was repeatedly found in 2 referral centers, over 3 days; betamethasone 12 mg was given to induce lung maturity; NSR the day after; 2 days later 3rd degree AV block recurred, and betamethasone was restarted, pending autoantibody finding; after 2 days NSR again, stable till delivery, at 34 wks; mother continued high-dose betamethasone (4 mg daily), from 29 to 34 wks	Alive (NSR at 2 yrs of age)

GA CHB: gestational age at detection of CHB weeks; HR: heart rate; AV: atrioventricular; LV: left ventricle; NSR: normal sinus rhythm.

Table 2. Comparison of anti-Ro/La-positive (n = 36) and anti-Ro/La-negative (n = 9) CHB.

	Ro/La-positive	Ro/La-negative	Method	p*
Total deaths (%)	10 (27.78)	3 (33.33)	Fisher	0.384**
Male/female	11/25	4/5	Fisher	0.454
Mean gestational age at detection, wks	23.03 ± 3.90	30.56 ± 7.30	U test	0.005
Mean lowest fetal HR (bpm)	57.17 ± 11.92	62.89 ± 24.93	U test	0.628
Mean gestational age at delivery, wks	34.67 ± 3.41	35.67 ± 3.53	t test	0.400
Mean weight at birth, g	2168.67 ± 606.61	2567.78 ± 659.05	t test	0.098
Mean length at birth, cm	38.16 ± 8.15	44.44 ± 5.98	U test	0.073
Mean Apgar 1 min	7.63 ± 2.24	7.44 + 1.24	U test	0.241
Mean Apgar 5 min	8.71 ± 1.52	8.78 ± 1.00	U test	0.433
Mean HR at birth (bpm)	59.20 ± 21.13	70.67 ± 27.82	U test	0.165
Incomplete AV block at end of followup (%)	1 (2.78)	4 (44.44)	Fisher	0.004
AV block complete in utero (%)	34 (94.44)	3 (33.33)	Fisher	0.000
AV block detected at birth (%)	0	3 (33.33)	Fisher	0.006
Pacemaker (%)	26 (72.22)	6 (66.67)	Fisher	0.704
Mean age at pacemaker insertion, days	454.12 ± 1056.89	87.83 ± 142.93	U test	0.497
Asymptomatic mothers (%)	11 (30.55)	8 (88.89)	Fisher	0.002

* Significant at $p < 0.05$. ** To calculate this p value the 4 terminations are considered as missing values. AV: atrioventricular; Fisher: Fisher's exact test.

failure *in utero* and 3 died (33.3%) shortly after birth. The 6 survivors had a mean age of 5.5 ± 3.5 years at the end of followup. Echocardiography showed no signs of cardiomyopathy or myocarditis.

Anti-Ro/La-negative mothers. Eight mothers were asymptomatic and one had photosensitivity and Raynaud's phenomenon. All were negative for ANA, anti-dsDNA, anti-ENA, and antiphospholipid antibody. Eight had further pregnancies, with no recurrences. One had a family history of AV block (11.1%).

Anti-Ro/La-positive CHB infants (Table 3). Twenty-five (69.4%) cases were female. None of the 34 presenting with complete AV block *in utero* reverted to a lesser degree (Table 2 and 3), but the 2 infants with incomplete block did. One of the fetuses with a second-degree AV block reverted to a normal sinus rhythm after therapy with high-dose dexamethasone and was born with a first-degree block that remained stable throughout the followup. The other fetus, with a second-degree AV block, reverted to an alternating pattern between second-degree block and normal sinus rhythm during treatment with dexamethasone 4 mg daily and weekly plasmapheresis; at age 27 months she developed a complete AV block.

AV block was always diagnosed *in utero* on the basis of fetal bradycardia (mean gestational age 23.03 weeks). There were 2 cases of sudden death *in utero* and 4 were aborted, 3 with severe heart failure. Three died immediately after birth due to heart failure and a fourth, who was given a pacemaker shortly after birth, died suddenly at age 21 months. Twenty-six neonates (72.2%) were given a pacemaker (Table 2 and 3); 4 of them died. Twenty-six infants were alive at the end of the followup, with mean age 6.6 ± 4.5 years. Echocardiography showed myocarditis *in utero* in 2 cases (5.5%). One male received a heart transplant at age 17 months because of severe dilated cardiomyopathy.

Anti-Ro/La-positive mothers. Eleven mothers were asymptomatic; 12 had Sjögren's syndrome, 11 undifferentiated connective tissue disease, and 2 systemic lupus erythematosus. Thirteen (36.1%) were anti-La/SSB-positive. Only 4 had been diagnosed with connective tissue disease before the index pregnancy. Twelve had further pregnancies and there were no recurrences. There was a family history in one case (2.7%)[14].

DISCUSSION

Cardiologists have been skeptical in the past about the existence of immune-mediated CHB, and some rheumatologists may still be doubtful about the existence of CHB not associated with anti-Ro/La antibodies. The exact percentages of anti-Ro/La antibody positivity in mothers of fetuses with CHB detected *in utero* and not associated with structural cardiac abnormalities are not known. Immunological studies using the most sensitive laboratory methods give positive results close to 100%, but these mothers were selected mainly from rheumatology centers[2]. Cardiological studies report lower percentages of anti-Ro/La-positive cases but do not always provide details about the laboratory methods[3,6-10]. Schmidt, *et al*[3] and Maeno, *et al*[7] reported 35% of anti-Ro/La negativity but did not indicate the laboratory tests. Using Ouchterlony double-diffusion and quantitative radioligand assays, Villain, *et al*[5] reported that 55/111 cases (49.5%) of complete AV block detected before age 15 years were anti-Ro/La-negative. They also reported that 6/56 blocks (10.7%) detected *in utero* were anti-Ro/La-negative, and another 25 had to be excluded because their antibody status was not known[5]. Lopes, *et al*[15] reported that 41/57

Table 3. Fetal-newborn features of infants born to anti-Ro/La-positive mothers.

Patient	Sex	Year of Birth	GA CHB	Lower Fetal HR	GA at Delivery	HR at Birth	Apgar Score	Fetal Heart Failure	Age at Permanent Pacing	Features of Block	Outcome (2007)
1	F	2000	19	69	33	147	9–9	No	No	2nd degree block in utero. 1st degree at birth	Alive (1st degree stable)
2	F	2002	22	64	39	60	ND	No	4 yrs	2nd degree block alternating with NSR at birth	Alive (3rd degree block at 27 mo)
3	M	1998	37	100	43	76	9–10	No	6 days	3rd	Alive
4	M	1996	21	64	38	60	8–9	No	3 yrs	3rd	Alive
5	F	2004	20	80	36	80	8–9	No	17 mo	3rd	Alive
6	F	2005	23	45	35	45	9–9	No	12 days	3rd	Alive
7	F	1995	26	60	38	52	8–9	No	8 mo	3rd	Alive
8	F	1998	20	47	33	48	9–9	No	1 mo	3rd	Alive
9	M	1997	23	62	33	50	8–9	No	3 mo	3rd	Alive
10	F	1997	20	42	29	50	ND	Yes (in utero)	1 day	3rd	Died
11	F	2001	18	70	35	80	9–9	No	1 yr	3rd	Alive
12	F	2000	24	62	38	70	8–9	No	3 mo	3rd	Alive
13	F	1992	29	50	37	50	9–9	No	10 yrs	3rd	Alive
14	M	2001	23	50	37	65	ND	No	2 days	3rd	Alive (heart transplant)
15	F	2004	23	60	27	55	7–8	No	1 day	3rd	Alive
16	M	2000	20	50	38	60	8–9	No	2 days	3rd	Alive
17	M	2000	20	55	32	50	9–9	No	2 mo	3rd	Alive
18	M	2002	28	48	34	40	5–8	Yes (in utero)	7 days	3rd	Alive
19	F	1990	28	32	29	32	0–2	Yes (in utero)	2 days	3rd	Died
20	M	1998	22	55	29	40	ND	Yes (in utero)	1 day	3rd	Died
21	M	2006	22	55	35	50	8–9	No	1 day	3rd	Alive
22	F	2001	22	62	36	62	9–10	No	2 mo	3rd	Alive
23	M	2002	24	45	34	45	5–8	No	1 mo	3rd	Alive
24	F	2001	24	52	33	50	3–9	Yes (in utero)	1 mo	3rd	Alive
25	F	2001	23	66	35	47	ND	No	23 days	3rd	Died
26	F	1992	32	55	32	92	ND	No	11 yrs	3rd	Alive
27	M	2007	20	57	36	60	9–9	No	No	3rd	Alive
28	F	2007	23	55	34	55	9–9	No	22 days	3rd	Alive
29	F	2007	26	55	35	55	ND	No	No	3rd	Alive
30	F	2007	24	45	37	50	8–9	No	No	3rd	Alive
31	F	2006	22	60				No	No	3rd	Died in utero
32	F	1993	22	50				No	No	3rd	Died in utero
33	F	2001	19	65				Yes (in utero)	No	3rd	Aborted
34	F	1996	20	45				Yes (in utero)	No	3rd	Aborted
35	F	1999	20	60				Yes (in utero)	No	3rd	Aborted
36	F	2007	20	66				No	No	3rd	Aborted

GA CHB: gestational age at detection of CHB, weeks; HR: heart rate; ND: not determined; NSR: normal sinus rhythm.

(71.9%) mothers of fetuses with isolated AV block "were seropositive for antinuclear antibodies, most often anti-Ro antibodies," but did not indicate the test method. In agreement with our findings that report showed that spontaneous regression of AV block was possible in cases of anti-Ro/SSA-negative block and that mortality was similar in anti-Ro/SSA-positive and negative blocks, so these blocks too call for close followup.

Referral and ascertainment bias might have skewed our results. However, we used state of the art laboratory methods and took the extra step of investigating further the initially negative reactivity for anti-Ro/SSA and La/SSB. In fact there were concerns that the negative reactivity was the result of low sensitivity of some assays or of failure to detect conformational epitopes (e.g., Ro60), which are readily detectable with immunoprecipitation of radiolabeled recombinant Ro60. By including these extra steps, we are confident about the negative reactivity reported here.

It has been suggested that cases of complete *in utero* AV block are often anti-Ro/La-positive, while incomplete blocks are generally negative[5,6,8-10]. Our study is the first to specifically compare anti-Ro/La-negative CHB with positive cases (Table 2) using sensitive laboratory methods. Twenty percent of our consecutive CHB cases were negative, but only 8.1% were negative when the analysis was limited to AV blocks that became complete *in utero*.

As no definite etiology (such as tumors, myocarditis, drugs) was found in the cases of anti-Ro/La-negative CHB, these must be considered "idiopathic." It is possible that there was some viral involvement, but it goes beyond the aims of our study to assess the role of viruses in the pathogenesis of CHB, whether anti-Ro/La-positive or negative. That one anti-Ro/La-negative female infant presented congenital sensorineural deafness is an interesting finding that warrants further study, since it has been correlated with congenital long QT syndrome[11].

The early diagnosis of Ro/La-positive CHB cannot be explained by the fact that the mothers were attending rheumatology centers, as 32 of the 36 mothers were unknown to us before the blocks were detected.

The mortality rate was similar in the anti-Ro/La-negative and positive groups (33.3% and 27.8%, respectively), in agreement with Lopes, *et al*[15], but the causes were different. No anti-Ro/La-negative fetuses developed cardiomyopathy. The cardiological courses of the cases of negative blocks were partially different for later gestational age and because they presented less often as complete, but often had a changing or progressive course.

While these findings have all the limitations of an observational study, there can be no doubt about the antibody negativity of the 9 cases we found. Although no single variable clearly distinguished Ro/La-positive from negative cases of CHB, the latter were often incomplete, were detected later during pregnancy, and were less stable.

REFERENCES

1. Friedman DM, Rupel A, Buyon JP. Epidemiology, etiology, detection, and treatment of autoantibody-associated congenital heart block in neonatal lupus. Curr Rheumatol Rep 2007;9:101-8.
2. Brucato A, Jonzon A, Friedman D, et al. Proposal for a new definition of congenital complete atrioventricular block. Lupus 2003;12:427-35.
3. Schmidt KG, Ulmer HE, Silverman NH, et al. Perinatal outcome of fetal complete atrioventricular block: a multicenter experience. J Am Coll Cardiol 1991;91:1360-6.
4. Groves AMM, Allan LD, Rosenthal E, et al. Outcome of isolated congenital complete heart block diagnosed in utero. Heart 1996;75:190-4.
5. Villain E, Coastedoat-Chalumeau N, Marijon E, Boudjemline Y, Piette J-C, Bonnet D. Presentation and prognosis of complete atrioventricular block in childhood, according to maternal antibody status. J Am Coll Cardiol 2006:48:1682-7.
6. Chang YL, Hsieh PCC, Chang SD, et al. Perinatal outcome of fetus with isolated congenital second degree atrioventricular block without maternal anti-SSA/Ro-SSB/La antibodies. Eur J Obstet Gynecol Reprod Biol 2005;122:167-71.
7. Maeno Y, Himeno W, Saito A, et al. Clinical course of fetal congenital atrioventricular block in the Japanese population: a multicentre experience. Heart 2005;91:1075–9.
8. Berg C, Geipel A, Kohl T, et al. Atrioventricular block detected in fetal life: associated anomalies and potential prognostic markers. Ultrasound Obstet Gynecol 2005;26:4-15.
9. Lin MT, Hsieh FJ, Shyu MK, et al. Postnatal outcome of fetal bradycardia without significant cardiac abnormalities. Am Heart J 2004;147:540-4.
10. Breur JM, Oudijk MA, Stoutenbeek P, et al. Transient non-autoimmune fetal heart block. Fetal Diagn Ther 2005;20:81-5.
11. Chiang CE, Roden DM. The long QT syndromes: genetic basis and clinical implications. J Am Coll Cardiol 2000;36:1-12.
12. Bernstein RM, Bunn CC, Hughes GR. Identification of antibodies to acidic antigens by counterimmunoelectrophoresis. Ann Rheum Dis 1982;41:554-5.
13. 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.
14. Ruffatti A, Favaro M, Cozzi F, et al. Anti-SSA/Ro-related congenital heart block in two family members of different generations: comment on the article by Clancy et al. Arthritis Rheum 2005;52:1623-5.
15. Lopes LM, Penha Tavares GM, Damiano AP, et al. Perinatal outcome of fetal atrioventricular block. One hundred sixteen cases from a single institution. Circulation 2008;118:1268-75.