Pregnancy Outcomes in Patients with Autoimmune Diseases and Anti-Ro/SSA Antibodies

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Abstract Anti-Ro/SSA antibodies are associated with neonatal lupus (congenital heart block (CHB), neonatal transient skin rash, hematological and hepatic abnormalities), but do not negatively affects other gestational outcomes, and the general outcome of these pregnancies is now good, when followed by experienced multidisciplinary teams. The prevalence of CHB, defined as an atrioventricular block diagnosed in utero, at birth, or within the neonatal period (0-27 days after birth), in the offspring of an anti-Ro/SSA-positive women is 1-2%, of neonatal lupus rash around 10-20%, while laboratory abnormalities in asymptomatic babies can be detected in up to 27% of cases. The risk of recurrence of CHB is ten times higher. Most of the mothers are asymptomatic at delivery and are identified only by the birth of an affected child. Half of these asymptomatic women develop symptoms of a rheumatic disease, most commonly arthralgias and xerophtalmia, but few develop lupus nephritis. A standard therapy for CHB is still matter of investigation, although fluorinated corticosteroids have been reported to be effective for associated cardiomyopathy. Serial echocardiograms and obstetric sonograms, performed at least every 1–2 weeks starting from the 16th week of gestational age, are recommended in anti-Ro/SSA-positive pregnant women to detect early fetal abnormalities that might be a target of preventive therapy.

Keywords Heart block/congenital · Neonatal lupus · Anti-Ro/SSA antibodies

Abbreviations

ANA Anti-nuclear antibodies

AV Atrioventricular

CHB Congenital heart block

ECG Electrocardiogram

NLS Neonatal Lupus Syndromes SLE Systemic lupus erythematosus SS Primary Sjögren's syndrome

UCTD Undifferentiated connective tissue disease

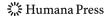
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J. Buyon New York University School of Medicine, New York, NY 10016, USA Anti-Ro/SSA antibodies are linked to the development of congenital heart block (CHB) in utero and to other clinical manifestations in newborns such as skin rash, liver abnormalities, thrombocytopenia, etc. In contrast, it is not clear if these antibodies are linked to other adverse pregnancy outcomes, both in systemic lupus erythematosus (SLE) and in non-SLE women. Neonatal lupus was so termed because the cutaneous lesions of the neonate resemble those seen in SLE. Notably, this might be a misleading name, because the baby does not have SLE and usually neither does the mother.

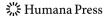


Anti-Ro/SSA and anti-La/SSB antibodies

Ribonucleoproteins (RNPs) are common targets of the humoral autoimmune response. These proteins interact with nucleic acids and RNA in particular. They are evolutionarily conserved molecules and RNPs have putatively been involved in the regulation of transcription and transduction, in RNA trafficking from nucleoli to cytoplasm, ribosome biogenesis, and in RNA rearrangement and complexing during the cell response to stress and apoptosis. Ro/SSA and La/SSB are two of the major immunogenic RNPs and antibodies against them may arise in a number of connective tissue diseases, especially Sjögren's syndrome (SS) and SLE [1]. They can be complexed with a family of small cytoplasmic RNAs called human cytoplasmic (hY) RNA [2]. These are synthesized by the enzyme RNApolymerase III, target of autoimmune responses itself [3]. Ro60 binds YRNAs, while La can interact with other RNApolymerase III products, being indeed considered a crucial factor of the regulation of this enzyme's activity [4]. It is still controversial whether Ro52 actually binds directly to RNA since it unlike Ro60 does not have an RNA recognition motif. Localization of Ro and La can be nuclear when not incorporated in the RNP complex, while is mainly cytoplasmic once bound to their RNA ligands [5]. Ro60 is a 60-kD protein composed of two domains: one resembles the von Willebrand Factor A, also present in extracellular-matrix proteins and peptides that mediate celladhesion. The other domain shows alpha-elical structures arranged together to leave an inner hole and is also responsible for nucleic acid binding [6]. Ro52 has a 52-kD molecular weight. It belongs to a protein family involved in regulation of transcription and ubiquitination characterized by a common conformation made of an Nterminal RING finger, a B-box domain and a coiled-coil domain with a leucine zipper motif [7]. La is a 48 kD molecular weight phosphoprotein with various domains showing N-terminal RNA recognition motifs and Cterminal nuclear localization signals that are necessary to carry out the La housekeeping functions of RNAs' chaperone with a significant influence on their downstream processing [4]. The development of autoantibodies to the Ro/La complex recapitulates most of the common pathways of autoimmunity whose understanding has significantly contributed to clarify at least in part the role of the humoral branch of the immune system in determining/favoring autoimmune diseases and their manifestations. Their frequency varies in different diseases, being higher in SS than in SLE and much lower in other connective tissue diseases such as systemic sclerosis, undifferentiated connective tissue disease (UCTD) or in rheumatoid arthritis (see Table 1) [3, 8]. Anti-Ro/SSA (both to the 52 kD and the 60 kD forms) and anti-La/SSB are strongly associated to given HLA genotypes [9], but genetic susceptibility is not sufficient per se to determine their appearance. Environmental stresses (UV-radiations, viral infection, oxidation, etc.) are crucial contributors to autoantibody production in this setting. During apoptotic rearrangement, intracellular components including both Ro and La proteins may become available to the extracellular compartment. It has been demonstrated that Ro60 is translocated in the early phases followed by a later translocation of the La protein to the surface blebs of apoptotic cells. This different dynamic may suggest an independent regulation of these two epitopes which could be critical in mediating the additive effect of their autoantibody counterparts in increasing the risk of tissue injury, e.g., in apoptotic fetal cardiomyocytes in CHB. It is thus in their involvement in nucleic acid regulation and apoptotic processes that immunogenicity of the Ro-La/RNP complex mainly reside. Autoantibodies recognizing multiple peptide sequences covering almost the entire length of Ro60 have been described and all have been shown to be highly conformation-dependent [4]. Epitope mapping of anti-Ro52 response has identified the middle part of the coiled-coil region as the immunodominant sequence, spanning from aa169 to aa291 containing the leucine zipper domain [7]. A finer specificity of the anti-Ro 52 response has been further clarified to reside on aa200-239 (p200) and specifically defined as anti-p200. Anti-p200 has been the focus of recent attention regarding the risk of CHB [10]. Yet, this higher risk for CHB has not invariably been demonstrated and deserves further investigation [11, 12]. An interesting link between

Table 1 Prevalence of anti-Ro/SSA and anti-La/SSB antibodies in different autoimmune rheumatic diseases

	Anti-Ro/SSA	Anti-La/SSB	Ref.
Sjögren's syndrome	60–90%	30-60%	Routsias JG, et al. Clinic Rev Allerg Immunol 2007
Systemic lupus erythematosus	30-50%	10-40%	Franceschini F, Cavazzana I. Autoimmunity 2005
Systemic sclerosis	12%	4%	Codullo V, et al. Future Rheumatol 2006
Rheumatoid Arthritis	11%	_	Franceschini F, Cavazzana I. Autoimmunity 2005
Undifferentiated connective tissue disease	8–30%	_	Belfiore N, et al. J Bone Spine 2000



the Ro-La/RNP-RNA complex and the immune system has been suggested and can represent a crucial bridge between innate and adaptive immunity in promoting autoimmune mechanisms; Y RNAs are capable of autologous activation of dendritic cells, mainly through the engagement of Tolllike receptors (TLRs). Furthermore, different Y RNAs have different TLR stimulatory capacity and this variable spectrum of interactions is also mirrored by distinct clinicopathologic expressions [2]. This mechanism could possibly account for the perpetuation of damage mediated by anti-Ro/ SSA antibodies, and we could postulate that, in a genetically susceptible environment, the concurrence of stress-induced increased apoptosis, blockade of apoptotic bodies removal and defective YRNAs clearance due to anti-Ro/SSA autoantibodies, can cause exposure to immune cells generating aberrant endogenous signals.

It is not clarified whether antibodies to Ro60, Ro52, and La are the result of an altered immune response which develops independently or are themselves initiators of the immune perturbation characterizing autoimmune diseases where they can be detected. Nevertheless, they represent an invaluable tool for diagnostic purposes, for their clinical associations and for their prognostic meaning (e.g., mandating careful monitoring of patients during pregnancy).

General pregnancy outcome in women with autoimmune diseases and anti-Ro/SSA antibodies

Most of the available data derive from retrospective studies; some of which reported anti-Ro/SSA antibodies as a possible causative factor for reproductive failure and unexplained pregnancy loss in women with autoimmune diseases [13-15]. Small or retrospective studies have addressed this problem, but their retrospective nature is a strong limitation: in fact even data such as the total number of pregnancies might be incorrect in a retrospective analysis. Hull et al. reported on three anti-Ro/SSA-positive SLE women with a history of spontaneous abortion [13]; Barclay et al. reported on one patient with 2 spontaneous abortions and a stillbirth [14], and Watson et al. described a direct correlation of anti-Ro/SSA antibodies with pregnancy loss only in black SLE patients [15]. Julkunen did a retrospective analysis of the fetal outcome in 55 pregnancies in 21 patients with primary SS compared with that in 100 pregnancies in 42 patients with SLE and 94 pregnancies in 42 healthy women. The relative risk for fetal loss in patients with primary SS was 2.7; in SLE was 2.2; most pregnancies in women with primary SS occurred before the onset of the disease. Notably Julkunen observed that the risk of pregnancy loss in primary SS was not associated with the presence of antibodies to Ro/SSA or La/SSB [16]. Another large retrospective study compared the obstetric histories of

154 anti-Ro/SSA-positive women with autoimmune diseases (78 with SLE) to that of both 142 anti-Ro/SSA-negative women matched for disease diagnosis and a control group of 180 healthy women [17]. The authors found that the overall rate of pregnancy loss and adverse pregnancy outcome did not significantly differ among the three groups. Anti-Ro/ SSA-positive SLE women reported a significantly higher rate (18.0%) of the rapeutic abortions compared with anti-Ro/ SSA-negative women (5.6%, p=0.0244) and healthy controls (4.6%, p=0.0013). Anti-Ro/SSA non-SLE-positive women reported a significantly higher rate of recurrent pregnancy loss in comparison to anti-Ro/SSA-negative women (23.7% vs 7.04%, p=0.0063) and healthy controls (6.4%, p=0.0004). The authors concluded that although anti-Ro/SSA antibodies do not adversely affect pregnancy outcome in SLE patients, they appear to be associated with recurrent pregnancy loss in non-SLE patients [17]. In contrast, a small prospective study analyzing pregnancy outcome in SLE showed that fetal loss and IUGR correlated with the absence of anti-Ro/SSA antibodies [18].

Based on these findings, the EULAR recommendations for the management of SLE quoted the retrospective paper by Watson [15] suggesting that anti-Ro/SSA positivity is associated with fetal wastage syndrome in black women with SLE and conclude that SLE may affect the fetus in several ways, especially if the mother has a history of lupus nephritis, anti-phospholipid, anti-Ro/SSA and/or anti-La/ SSB antibodies, and that these conditions are associated with an increase of the risk of miscarriage, stillbirth, premature delivery, intrauterine growth restriction and fetal heart block [19]. We published the largest, prospective study on the impact of anti-Ro/SSA antibodies on pregnancy outcome [20]. In a large multicentric cohort study we have prospectively followed 100 anti-Ro/SSA-positive women (53 SLE) during their 122 pregnancies and 107 anti-Ro/SSA-negative women (58 SLE; 140 pregnancies). Anti-Ro/SSA antibodies were tested by immunoblot and counterimmunoelectrophoresis. Mean gestational age at delivery (38 vs 37.9 weeks), prevalence of pregnancy loss (9.9% vs 18.6%), preterm birth (21.3% vs 13.9%), cesarean sections (49.2% vs 53.4%), PROM (4.9% vs 8.1%), preeclampsia (6.6% vs 8.1%), IUGR (0 vs 2.3%), and newborns SGA (11.5% vs 5.8%) were similar in anti-Ro/ SSA-positive and anti-Ro/SSA-negative mothers (Table 2); findings were similar in SLE (Table 3) and non-SLE (data not shown) women. Our results show that anti-Ro/SSA antibodies do not affect the pregnancy outcomes except for the risk of CHB. In fact, we did not observe any clinically or statistically significant difference between anti-Ro/SSApositive and anti-Ro/SSA-negative women, even if the frequency of pregnancy loss in anti-Ro/SSA-positive compared with anti-Ro/SSA-negative women was slightly lower in SLE (9.9% vs 18.6%; relative risk, 0.53), in

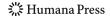


Table 2 Pregnancy outcome of all anti-Ro/SSA-positive vs all anti-Ro/SSA-negative women

	Ro/SSA-positive women		Ro/SSA-negative women	
	Number	Percent ^a	Number	Percent ^a
No. of women	100		107	
No. of pregnancies	122		140	
Twin pregnancies	4		2	
Living newborns	116	95.1%	125	89.3%
Total spontaneous pregnancy losses	10	8.2%	17	12.1%
Pregnancy losses <10 weeks	7	5.7%	10	7.1%
Pregnancy losses >10 weeks	3	2.4%	7	5.0%
therapeutical abortions	0	0	1	0.7%
Prematurity <37 weeks	19	15.6%	16	11.4%
Prematurity <34 weeks	3	2.5%	2	1.4%
Mean gestational age at delivery; weeks (range)	38 (30–42)		38.4(33-42)	
Maternal mean age at delivery; years (range)	31.2 (25–40)		29.8(20-41)	
Previous pregnancies (retrospective analysis)				
Total pregnancies	44		44	
Pregnancy losses <10 weeks	16	36.3%	9	20.4%
Pregnancy losses >10 weeks	5	11.4%	8	18.1%
Total spontenous pregnancy losses ^b	21	47.7%	17	38.6%
Therapeutical abortions	3	6.8%	5	11.4%

^a Percentages are expressed on the number of pregnancies: prospective, 122 for Ro/SSA-positive and 140 for Ro/SSA-negative women; 44 retrospective pregnancies for both

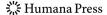
agreement with the data by Le Thi Huong [18], and slightly higher in non-SLE (6.6% vs 1.8%; relative risk, 3.7), in agreement with the data by Mavragani [17]. Apparently, anti-Ro/SSA antibodies seem to have an inverse effect on pregnancy loss in SLE and non-SLE women: looking at pregnancy outcome, anti-Ro/SSA seem to have a favorable impact in patients with SLE which is a severe systemic disease, conversely, they seem to have an adverse effect in patients with milder autoimmune conditions such as UCTD or Sjögren's syndrome. However, no significant differences have been found in our study, therefore this observation should be interpreted with extreme caution. It was also found that the fine specificity of anti-Ro/SSA antibodies did not affect pregnancy outcome.

Considering obstetrical history, approximately half of the previous "historical" pregnancies reported by our women ended with spontaneous or voluntary abortions. The retrospective nature of this analysis does not allow an accurate explanation of these findings. Reasons are probably multifactorial: the management of the current pregnancies by high-risk pregnancy obstetrical team; the preconception planning of these prospective pregnancies; a selection bias, for which women with previous negative obstetrical outcomes might be more readily referred to centers with experience in the field. Moreover, the striking difference emerging suggests how an analysis of data retrospectively reported by the women concerning their previous pregnancies might be of limited value. On the other hand, currently the general outcomes of the pregnancies in these autoimmune "high-risk" women can be excellent when carefully followed by dedicated high-risk obstetrical and multidisciplinary teams.

In conclusion, we observed in a large prospective-controlled multicenter cohort study of anti-Ro/SSA-positive women that the presence of these antibodies is associated with CHB but does not negatively affect other pregnancy outcomes, both in SLE and in non-SLE women. The general outcome of these pregnancies is now very good (especially important to be in remission or relative quiescent at outset), when prospectively followed by multidisciplinary teams with ample experience in this field.

Definition and epidemiology

Neonatal lupus is a rare disease, which affects equally males and females (actually there may be a slight skewing



^b Total spontenous pregnancy losses: in anti-Ro/SSA-positive women, prospective (10/122) vs retrospective (21/44), *p*<0.001; in anti-Ro/SSA-negative women, prospective (17/140) vs retrospective (17/140), *p*<0.001.

Table 3 Pregnancy outcome of SLE anti-Ro/SSA-positive vs SLE anti-Ro/SSA-negative women

	Ro/SSA-positive women		Ro/SSA-negative women	
	Number	Percent ^a	Number	Percent ^a
No. of women	53		58	
No. of pregnancies	61		86	
Twin pregnancies	1		0	
Living newborns	56	91.9%	70	81.4%
Total spontaneous pregnancy losses	6	9.9%	16	18.6%
Pregnancy losses <10 weeks	4	6.6%	10	11.6%
Pregnancy losses >10 weeks	2	3.3%	6	7.0%
Therapeutical abortions	0	0	1	1.1%
prematurity <37 weeks	13	21.3%	12	13.9%
Prematurity <34 weeks	2	3.3%	1	1.1%
Mean gestational age at delivery; weeks (range)	38 (30–41)		37.9(33-42)	
Maternal mean age at delivery; years (range)	30 (23–38)		30 (20–39)	
positivity for aPL	8	14.3%	12	13.9%
SLE plus secondary APS	4	7.1%	9	10.4%
Major flares	10	17.9%	16	18.6%
Previous pregnancies (retrospective analysis)				
Total pregnancies	21		22	
Pregnancy losses <10 weeks	7	33.3%	4	18.1%
Pregnancy losses >10 weeks	3	14.2%	5	22.7%
Total spontenous pregnancy losses ^b	10	47.6%	9	40.9%
Therapeutical abortions	1	4.8%	5	22.7%

^a Percentages are expressed on the number of pregnancies: prospective, 61 for Ro/SSA-positive and 86 for Ro/SSA-negative women; retrospective, 21 for Ro/SSA-positive and 22 for Ro/SSA-negative women

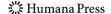
for both rash and CHB toward female in the research registry for neonatal lupus). According to Michaelsson [21], complete CHB has an incidence of 1:20,000 live births; however these numbers include also CHB associated with congenital anatomical malformations and exclude in utero deaths (approximately 20% of fetuses with CHB), so they are not included in statistics which generally focus on live births. More recently Siren [22] found an incidence of CHB in Finland of 1:17,000 live births (95% confidence interval 1:14,000-1:21,000). Confusion exists regarding congenital versus acquired AV block; for these reasons we propose a new definition of congenital AV block which might be acceptable to cardiologists, rheumatologists, pediatricians and obstetricians: "an AV block is defined as congenital if it is diagnosed in utero, at birth or within the neonatal period (0-27 days after birth)"[23, 24]. These blocks are often, but not always, associated with the presence of anti-Ro/SSA antibodies in the mother; 10-20% may be negative for anti-Ro/SSA and other autoantibodies as demonstrated by Brucato [25].

Pathogenesis of neonatal lupus syndromes

Clinical manifestations of neonatal lupus are associated with the presence of anti-Ro/SSA and La/SSB antibodies. These antibodies, in fact, cross the placenta beginning at approximately 12 weeks of gestation and reach the fetal tissues [26]. The antibodies may display at least three effects: (a) theoretically they may induce a *myocarditis*; (b) they can bind apoptotic cells, block presumed physiologic clearance and divert clearance to macrophages, and (c) they might be *arrhythmogenic*. The immune-mediated damage of the cardiac conduction system ultimately ends with fibrotic replacement.

Apoptosis and the road to scar

One of the most intriguing aspects of CHB is that it is an injury nearly unique to some phase(s) of fetal development, because it has only been reported once in the maternal heart [27] despite the presence of identical antibodies in the



^b Total spontenous pregnancy losses: in anti-Ro/SSA-positive women: prospective (6/61) vs retrospective (10/21): *p*<0.001; in anti-Ro/SSA-negative women, prospective (16/86) vs retrospective (9/22), 0.05<*p*<0.10=NS

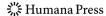
maternal circulation. One of the biological stumbling blocks has been the intracellular localization of the putative antigen targets. It is logical to hypothesize that the target is a cardiac surface protein containing a cross-reactive epitope recognized by anti-Ro/La antibodies. Alternatively, the candidate antigen might be located intracellularly and translocated to the surface during development of the fetal heart. The genetics of a particular fetus might be a second reasonable component. The fact that identical twins are more often discordant than concordant for disease suggests that an environmental factor present in utero might be a third component. In aggregate, the environmental factor would be expected to amplify the extent of injury in susceptible fetuses exposed to antibodies generated in susceptible mothers. In consideration of surface binding, one hypothesis is that apoptosis might result in translocation of intracellular antigens to the external leaflet of the membrane. Applicability of apoptosis to the pathogenesis of CHB is supported by several observations. It is a selective process of physiologic cell deletion in embryogenesis and normal tissue turnover plays an important role in shaping morphologic and functional maturity. In the normal adult myocardium, apoptosis has been observed only rarely [28]. In contrast, apoptosis does occur during the development of the heart [29]. Buyon's group has found that human fetal cardiocytes are readily induced to undergo apoptosis, which results in surface expression of components of the Ro/La complex [30]. Furthermore, cocultured macrophages that phagocytose apoptotic cardiocytes bound by antibodies (opsonized) exhibit an activated phenotype compared with macrophages that phagocytose unbound apoptotic cardiocytes (nonopsonized) [31]. Events subsequent to this inflammatory cascade result in a major alteration of the fibroblast phenotype [32], which ultimately leads to fibrosis of the conducting system, the signature histopathologic finding in CHB observed in all reported autopsies. For example, the spectrum of CHB in the clinically affected fetus includes AV nodal replacement by fibrosis or fatty tissue [33], fibrous structures containing microscopic crystalline structures in the conduction system [34] and altered contractility of the working myocardium secondary to endocardial fibroelastosis [35]. In addition, we evaluated the role of cardiocytes in the physiologic removal of apoptotic cells and the subsequent effect of surface binding by anti-Ro/La antibodies [36]. Regardless of the method used to induce apoptosis, translocation of Ro/La antigens to the fetal cardiocyte plasma membrane was seen. When healthy cardiocytes were cocultured with healthy cardiocytes rendered apoptotic, a previously undescribed clearance mechanism was revealed: proliferating cardiocytes phagocytosed neighboring apoptotic cardiocytes [36]. Phagocytic uptake was inhibited significantly after preincubation of apoptotic cardiocytes with chicken and murine anti-Ro/La,

with IgG from an anti-Ro/La-positive mother of a CHB-child, but not with anti-HLA class I antibody. These results suggest that resident cardiocytes participate in physiologic clearance of apoptotic cardiocytes, but that clearance is inhibited by opsonization via maternal autoantibodies, resulting in accumulation of apoptotic cells promoting inflammation and subsequent scarring.

Genetic considerations

Candidate genes studied have been chosen based on the proposed pathologic cascade of inflammation leading to fibrosis. The human gene encoding trasforming growth factor beta (TGF-β) is on chromosome 19q13 and is highly polymorphic. Awad [37] identified five polymorphisms: two in the promoter region at positions 800 and 509, one at position \$72 in a nontranslated region and two in the signal sequence at positions b869 and b915. The polymorphisms at positions b869 and b915, which change codon 10 (T/C, leucine/proline) and codon 25 (G/C, arginine/ proline), are associated with interindividual variation in the levels of TGF-\$\beta\$ production. Several animal and human studies have found that high TGF-\beta producers develop significantly more lung fibrosis in response to a number of inflammatory triggers, such as radiation [38], chemotherapy [39] and lung transplantation [40]. In codon 25, the Pro25 allele is associated with lower TGF-β synthesis in vitro and in vivo, whereas the Arg25 allele is associated with allograft fibrosis in transbronchial biopsies when compared with controls and with nonallograft fibrosis [41]. With regard to codon 10, it has been reported that lung allograft recipients with the Leu10 allele produced the highest amounts of TGFβ [37], and chronic rejection after lung transplant is linked with high levels of TGF- β [41]. High levels of TGF- β were thus hypothesized to permit the development of CHB caused by enhancement of extracellular matrix and increased fibrosis. To evaluate this hypothesis, codons 10 and 25 of the TGF-β gene were evaluated in 88 children (40 CHB, 17 rash, 31 unaffected siblings) and 74 mothers from the research registry for neonatal lupus [42]. The TGF-β polymorphism Leu10 (associated with increased fibrosis) was significantly higher in CHB-children (genotypic frequency 60%, allelic frequency 78%) than unaffected offspring (genotypic frequency 29%, P=.016; allelic frequency 56%, P=.011) and controls, whereas there were no significant differences between controls and other NLS groups. For the TGF-β polymorphism Arg25 there were no significant differences between NLS groups and controls [42].

The TGF-β polymorphism was evaluated in two families, one with dizygotic twins and one with trizygotic triplets discordant for disease expression [43]. Each family only had one child affected with third-degree CHB,



although another of the triplets had alternating first- and second-degree block. In the twin family, the CHB twin was heterozygous for the TGF- β polymorphism, whereas the unaffected twin did not carry the profibrotic allele. Each of the triplets was also heterozygous for TGF- β . In the two children with third-degree CHB, TGF- β secretion was increased in both spontaneous and mitogen-stimulated peripheral blood mononucleocytes compared with their siblings. These observations support a fetal contribution to the development of neonatal lupus as well as the likelihood that events in utero strongly contribute to the susceptibility of a particular fetus. To help minimize these contributions, several families in the research registry for neonatal lupus have conceived twins via in vitro fertilization in pregnancies carried by anti-Ro/La-negative surrogate mothers.

The neonatal lupus rash resembles that of subacute cutaneous SLE (SCLE) and often is photosensitive. In individuals with SCLE lesions having the haplotype -308A tumor necrosis factor alpha (TNF- α)/DRB1*03, there is evidence that the release of TNF- α by UV light-exposed keratinocytes contributes to these lesions [44]. TNF- α is encoded by a highly polymorphic gene and a substitution of G/A at position -308 (TNF2) in the promoter region has been associated with increased production of this cytokine [45]. The -308G (TNF1) common (wild-type) allele has a frequency of approximately 80% in whites and 92% in African Americans [45]. The link between photosensitivity, anti-Ro/SSA antibodies and TNF-α promoter polymorphisms extends to HLA class II molecules, because the presence of DRB1*03 is common in individuals who synthesize anti-Ro/La antibodies. Additionally, in whites, there is a strong linkage disequilibrium between the -308A allele and HLA-DRB1*03 [46]. DNA from the cohort described above [42] was genotyped for the TNF- α -308 promoter region and HLA-DRB1. In children with NLS rash, there was a significantly higher -308A carrier frequency compared with healthy controls (64% versus 23%; P=.002) [47]. In addition, the DRB1 distribution for DRB1*03 was significantly higher in children with rash compared with controls (64% versus 17%; P=.014) and, interestingly, the carrier frequency of DRB1*03 was also significantly higher than controls for children with CHB (54%) and for mothers (73%) but not unaffected children (39%). The carrier frequency was higher in children with rash compared with children without rash, although the difference did not reach statistical significance. The prevalence of the -308A allele paralleled the prevalence of DRB1*03 in children with rash. For children with rash, the association between the -308A allele and DRB1*03 occurred in every case but one (7%; P=.012 versus control). However, in children who were unaffected and in children with CHB, the presence of -308A in the absence of DRB1*03 was 25% and 38%, respectively, which did not differ significantly from controls [47].

Arrhythmogenesis and electrophysiological effects

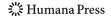
Several publications have shown arrhythmogenic effects of anti-Ro/SSA antibodies in experimental models that used animal or human myocardial tissues [48–51]. In particular, an interference of anti-Ro/SSA IgG with L-type calcium channel has been suggested by some experimental models [50, 52]. Furthermore, animal models have shown that active immunization with the Ro antigen or passive infusion of anti-Ro/SSA IgG into pregnant animals generated varying degrees of AV conduction abnormalities, including complete AV block, in the pups [50, 51], however the penetrance was extremely low paralleling human disease frequencies. Another study showed that 20% of pups born to rats immunized with the p200 peptide developed first-degree AV block [53]. The same authors showed that anti-Ro/SSA antibodies disturb calcium homeostasis of cultured human fetal cardiocytes; in particular they observed that p200-specific autoantibodies cloned from patients bound cultured cardiomyocytes and severely affected Ca2+ oscillations, leading to accumulating levels and overload of intracellular Ca2+ levels with subsequent loss of contractility and ultimately apoptosis [53].

Clinical features

Cardiac manifestations

Third-degree AV block is the most severe manifestation of neonatal lupus, since it is irreversible and carries a high morbidity and mortality rate. The presence of signs or symptoms is mainly related to the ventricular rate, which usually ranges between 30 and 100 beats/minute [54, 55], but may be also related to the cardiac contractility and to the ratio between atrial and ventricular contractions; heart rate usually declines during the pregnancy [56]; the lower the rate, the higher the possibility of fetal hydrops and neonatal cardiac failure and fetal or neonatal death correlates with a ventricular rate in utero less than or equal to 55 bpm [54–57].

CHB (either first, second, or third) is most frequently detected in utero by prenatal ultrasound, between 18 and 24 weeks of gestational age. This "window" coincides with the timing of placental transfer (that becomes effective at about 12 weeks) and the timing of the development of the human fetal conduction system which is formed by 12 weeks (thus explaining the absence of associated structural abnormalities) but presumably undergoes further



remodeling (perhaps explaining the later development of block at 20 weeks or so). In the majority of cases complete block requires a pacemaker implantation, frequently but not necessarily in the neonatal period. In utero death is usually related to intractable heart failure.

A subset of patients with CHB may develop dilated cardiomyopathy, even if the risk seems low, approximately 6% of CHB infants [58–60]. Myocardial biopsy revealed hypertrophy, interstitial fibrosis in most patients and myocyte degeneration in few. The majority of affected children dies from congestive heart failure or requires cardiac transplantation, while a recovery was reported in few cases [59].

CHB may be associated with endocardial fibroelastosis and it has been reported that isolated endocardial fibroelastosis may be independently related to maternal anti-Ro/SSA and anti-La/SSB antibodies [61].

Spectrum and progression of conduction abnormalities in infants born to mothers with anti-Ro/La antibodies

To ascertain the spectrum of arrhythmias associated with maternal anti-Ro/La antibodies, records of all children enrolled in the Research Registry for Neonatal Lupus were reviewed [62]. Of 187 children with CHB whose mothers have anti-Ro/La antibodies, nine had a prolonged PR interval on electrocardiogram (ECG) at birth, four of whom progressed to more advanced AV block. A child whose younger sibling had third block was diagnosed with first block at age 10 year at the time of surgery for a broken wrist. Two children diagnosed in utero with second block were treated with dexamethasone and reverted to normal sinus rhythm by birth, but ultimately progressed to third block. Four children had second block at birth: of these, two progressed to third block. These data have important research and clinical implications.

Perhaps many fetuses sustain mild inflammation, but resolution is variable, as suggested by the presence of incomplete AV block in some cases and progression to complete block in others. Importantly, since subsequent progression of less-advanced degrees of block can occur, an ECG (to identify clinically silent first-degree block) should be performed on all infants born to mothers with anti-Ro/La antibodies.

Other electrocardiographic manifestations

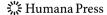
Sinus bradycardia may be a rare and seemingly reversible part of the spectrum of anti-Ro/SSA associated cardiac disease [63]. QT prolongation has been reported both in infants from mothers with anti-Ro/SSA antibodies [64] and in adults positive for anti-Ro/SSA antibodies [65], but these data have not been confirmed by many others [66, 67].

Thus, QT prolongation as a consequence of autoantibody injury remains unproven.

Two useful prospective studies have been recently published by Italian groups. In the first study [68], electrocardiograms prospectively obtained from 51 infants born of anti-Ro/SSA-positive mothers were compared with those obtained from 50 control infants born of mothers with antiextractable nuclear antigen-negative connective tissue disease. One anti-Ro/SSA-exposed infant developed complete CHB. No infant showed sinus bradycardia. First-degree AV block at birth was observed in five study group and no control group infants, P=0.023. First-degree AV blocks spontaneously reverted during the first year of life. Mean corrected OT value of infants born from anti-Ro/SSApositive mothers was slightly prolonged but did not reach statistical significance, as compared with the control group (0.404 vs 0.395 s; P=0.060). In the second study, 60 anti-Ro/SSA-positive and 36 anti-Ro/SSA-negative autoimmune mothers were prospectively followed; ECG and/or ECG-Holter were performed on the offspring at first, third, sixth, and 12th month. One of 61 fetuses of anti-Ro/SSA-positive mothers developed third-degree block, one developed second-degree AV block (30th week). Corrected QT interval ≥440 ms was observed in 13% of cases and in 20% of controls at the first ECG. ECG-Holter showed OTc prolongation ≥440 ms in 59% infants of anti-Ro/SSApositive mothers and in 60% of controls, and QTc ≥470 ms in four infants of anti-Ro/SSA-positive group and in two controls. One baby in the anti-Ro/SSA-positive and two in the anti-Ro/SSA-negative group required beta-blockers. Genetic causes of QTc prolongation were excluded. This prospective study showed that ECG abnormalities (firstdegree block and QTc prolongation) are frequent in infants of mothers with autoimmune diseases particularly if ECG-Holter is done, independently of maternal autoantibody profile [69].

Skin rash

A skin rash can be present at birth, but more frequently, it appears between the second and third months of life [70, 71]. Unlike CHB, it is transient since it disappears with the clearance of maternal autoantibodies from the baby's circulation, usually without any residua. The rash is erythematous and scaly, similar to SCLE. It is frequently annular in shape and most commonly located in sunexposed area with a characteristic predilection for the periorbital area. Ultraviolet exposure may be an initiating factor and can exacerbate an existing rash. Histologically, the lesions are similar to subacute cutaneous lupus, with hyperkeratosis, epidermal atrophy, basal degeneration, interstitial edema and a perivascular mononuclear infiltrate. Immunoglobulin and complement deposition have been



demonstrated by direct immunofluorescence at the dermoepidermal junction. Since these lesions are self-limiting, usually no treatment is required.

Laboratory abnormalities

Hematologic abnormalities are not common but have been described, usually consisting of thrombocytopenia [72], and neutropenia [73]. Hepatic involvement has also been described [74, 75] which can vary from an asymptomatic increase in serum transaminases to severe cholestasis. This has been noted to be present at birth in some cases and in others has not been clinically evident until several weeks after birth. Cimaz observed hematologic abnormalities in 27% of the babies from anti-Ro/SSA-positive mothers and elevation of liver enzymes in 26% [76]. Similar to the cutaneous manifestations but in contrast to CHB, these manifestations are transient and usually do not require medical intervention. On the other hand, Motta in a prospective study observed a lower frequency of hematologic abnormalities and no cases of hepatobiliary disease [68].

Central nervous system involvement

Learning disabilities [77] and hydrocephalus [78] have been associated with in utero exposure to maternal anti-Ro/SSA antibodies, but these findings await confirmation.

Prognosis

Children

Complete CHB is a severe disease. Mortality, usually in utero or in the first three months of life, can reach 30% even after intensive and supportive care. Prophylactic pacemaker treatment might be considered even in asymptomatic patients because of high incidence of unpredictable Stokes-Adams attacks and significant morbidity and mortality. After birth and pacemaker implantation, children can live an almost normal life. The possibility for these children to develop SLE or another connective tissue disease in later life is very uncommon [79, 80]. The risk is apparently not higher than in asymptomatic children of mothers with autoimmune diseases [80, 81]. Data from the Research Registry for Neonatal Lupus [80] supported no increased risk of SLE, but concern was raised regarding the development of autoimmune disease (systemic or organspecific) in early childhood. Specifically, six children (all with neonatal lupus) with definite rheumatic/autoimmune diseases were identified: two with juvenile rheumatoid arthritis, one with Hashimoto thyroiditis, one with psoriasis and iritis, one with diabetes mellitus and psoriasis, and one with congenital hypothyroidism and nephrotic syndrome.

Mothers

Follow-up studies have shown that the long-term prognosis of mothers of babies with NLS is reasonably good, since only approximately half eventually develop a connective tissue disease, which in most cases is mild and non lifethreatening [82-85]. Most recently, mothers enrolled in the Research Registry for Neonatal Lupus were analysed. Of the 321 mothers enrolled, 229 had at least 6 months of follow-up. Twenty-six of the 51 mothers who were asymptomatic at the NL-child's birth progressed: 12 developed pauci-undifferentiated autoimmune syndrome (UAS), two poly-UAS, seven SS, four SLE, and one SLE/ SS. The median time to develop any symptom was 3.15 years. Sixteen of the 37 mothers classified as pauci-UAS at the NL-child's birth progressed: five developed poly-UAS, six SS, four SLE, and one SLE/SS. Of the pauci-UAS mothers enrolled within 1 year, the median time to progression was 6.7 years. Four mothers developed lupus nephritis (two asymptomatic, two pauci-UAS). The probability of an asymptomatic mother developing SLE by 10 years was 18.6%, and developing probable/definite SS was 27.9%. NL-manifestations did not predict disease progression in an asymptomatic mother. Mothers with anti-Ro/SSA and anti-La/SSB were nearly twice as likely to develop an autoimmune disease as mothers with anti-Ro/SSA only [86].

Risk of delivering a child with complete CHB

Since women with anti-Ro/SSA antibodies are relatively frequent but CHB is very rare, we are faced with the following situation: frequent counseling of a rare disease. In a prospective study, we found that the prevalence of complete CHB in newborns of 100 women already known to be anti-Ro/SSA positive and with known connective tissue disease was 2% (95% confidence interval 0.2-7%) [27]. However, we only studied mothers who had been found to be anti-Ro/SSA positive by counterimmunoelectrophoresis, a method with high specificity and rather low sensitivity, to exclude women with low or dubious titers of anti-Ro/SSA. Our results therefore cannot be extrapolated to women with low-positive titers of anti-Ro/SSA antibodies by ELISA, for which the risk, if any, should be even lower. Indeed most women with CHB-children do have very high titers of anti-Ro/SSA antibodies which still do not predict the development of CHB but rather is a characteristic of this autoimmune response. Healthy babies are also born of mothers with very high titers [67]. The low risk of complete CHB has now been confirmed by other groups: Gladman reported no cases of complete CHB in

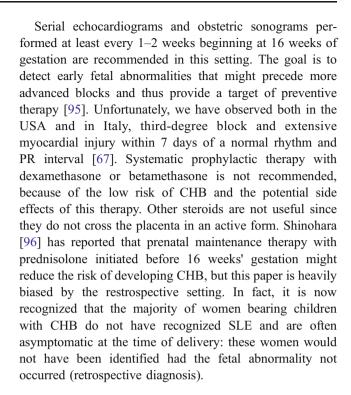
100 live births in 96 anti-Ro/SSA-positive women [87]; Cimaz observed two cases in 128 anti-Ro/SSA-exposed infants (1.6%) [76], Costedoat-Chalumeau of one case out of 99 infants (1%) [66], Gerosa reported a risk of one out of 60 (1.7%) [69], Motta of 1/51 (2%) [68], Friedman of two out of 74 (2.7%) [67] previous pregnancies without affected children, Rein of 0 out of 70 [88]. This risk might be further refined taking into account other factors: anti-La/SSB antibodies and hypothyroidism. The presence of anti-La/ SSB antibodies increases the risk of CHB to 3.1% from a theoretical risk of 2%, while the risk decreases to 0.9% if the mother is anti-La/SSB-negative [89]. One study reported that mothers with hypothyroidism seem to have a 9-fold increased risk over women with normal thyroid function of having a child with CHB (odds ratio 8.63) [90]. Askenase evaluated 54 mothers of children with CHB and identified 14 (25.9%) whose sera contained antibodies to thyroperoxidase and 20 (37%) to thyroglobulin (TG) [91]. Both antibody frequencies were higher than in the general population. Anti-TG antibodies were significantly greater than reported for a cohort of SS patients. Five of these CHB-mothers were hypothyroid which was not increased compared to that reported in SS. In the search for other factors responsible for NL, anti-TG antibodies, while not present in 100% of these sera, might be another contributing factor, the pathologic mechanism of which is not evident.

Risk of recurrence

The mothers have higher probabilities of delivering a second affected child. Few prospective studies exist on this issue; however, the percentage value seems to be comprised between 15% and 20% [92, 93].

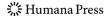
Obstetric management of pregnancy at risk of developing CCHB

A woman is at risk of delivering a baby affected by CHB if she is definitely anti-Ro/SSA positive. There are some suggestions that anti-52-kd Ro/SSA, anti-La/SSB and anti-p200 antibodies are more strongly associated with CHB than anti-60-kd Ro/SSA alone, at least as tested by immunoblot [12, 82, 94], but the data are inconclusive. Furthermore there are issues of reproducibility, sensitivity and specificity of the different tests (commercial kits, research specific to different laboratories). For practical purposes at present the key is a reliable positivity for anti-Ro/SSA antibodies, regardless of the methods employed. If the positivity is uncertain or the titer is very low, we advise to confirm the positivity with standard methods or in reference laboratories.



Assessment of first-degree AV Block in utero

Several methods have been developed with the aim to detect in utero early fetal abnormalities, particularly firstdegree AV block, which might herald CHB. The American group have evaluated the mechanical PR interval. By using the gated-pulsed Doppler technique, time intervals from the onset of the mitral A wave (atrial systole) to the onset of the aortic pulsed Doppler tracing (ventricular systole) within the same left ventricular cardiac cycle may be measured. Recently, 95 pregnant women with anti-Ro/SSA antibodies completed an evaluable course in 98 pregnancies [67]. The protocol included fetal echocardiograms performed weekly from 16 to 26 weeks' gestation. PR intervals >150 ms were considered prolonged, consistent with first-degree block. Ninety-two fetuses had normal PR intervals. Neonatal lupus developed in ten cases; four were neonatal lupus rash only. Three fetuses had third-degree block; none had a preceding abnormal PR interval, although in two fetuses >1 week elapsed between echocardiographic evaluations. Tricuspid regurgitation preceded third-degree block in one fetus and an atrial echodensity preceded block in a second. Two fetuses had PR intervals >150 ms. Both were detected at or before 22 weeks, and each reversed within 1 week with 4 mg dexamethasone. No first-degree block developed after a normal ECG at birth. Heart block occurred in three of 16 pregnancies (19%) in mothers with a previous child with CHB and in 3 of 74 pregnancies (4%) in mothers without a previous child with CHB or rash (P=0.067). The authors concluded that prolongation of the PR interval was



uncommon and did not precede more advanced block and that advanced block and cardiomyopathy can occur within 1 week of a normal echocardiogram without initial first-degree block. Echodensities and moderate/severe tricuspid regurgitation merit attention as early signs of injury.

The Swedish group reported on weekly echocardiograms performed on 24 women with anti-Ro/SSA 52-kd antibodies, between 18 and 24 weeks of gestation, with two Doppler echocardiographic methods designed to estimate the time delay between hemodynamic events caused by atrial and ventricular depolarizations. Fetal AV time intervals were longer and heart rates were slightly lower compared to fetuses not exposed to anti-Ro/SSA antibodies. Eight fetuses had signs of first-degree block. It is of note that the cutoffs for first-degree block were lower than those reported by Friedman [67]. One of these fetuses (whose PR interval was abnormal in the Swedish study but would not have been considered abnormal in the US study) had progression to complete block and another showed recovery from second-degree block to first-degree block with betamethasone treatment. In the remaining six fetuses, spontaneous normalization occurred before or shortly after birth [97].

Recently an Israelian group proposed the tissue velocitybased fetal kinetocardiogram (FKCG) to detect first-degree AVB in fetuses at risk [88]. FKCG was performed in 70 fetuses of 56 anti-Ro/SSA-positive mothers. Fetuses were monitored with weekly FKCG from 13 to 24 weeks gestation and compared to 109 normal fetuses. FKCG was obtained readily in all fetuses; six showed first-degree block (AV conduction time ≥ 2 z scores above normal mean) at 21 to 34 gestational weeks. Immediate maternal treatment with dexamethasone resulted in normalization of AV conduction in all affected fetuses within 3 to 14 days. The ECG PR interval immediately after birth was normal in all affected newborns. No child developed complete CHB or cardiomyopathy in the subsequent 1- to 6-year (median, 4 years) follow-up. What is unknown from these three studies is the natural history of so called first-degree block. There are no data on spontaneous reversibility and progression to advanced block has not been reproducibly documented to date. This is critical and investigators are encouraged to share echocardiographic tapes to validate the reliability of this candidate biomarker.

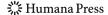
Treatment

In utero therapy

There is no known effective therapy for CHB. Prenatal interventions consist of drugs given to the mothers, the purposes of which are to diminish the maternal autoimmune response and/or the fetal cardiac inflammatory injury and in

some cases to increase the fetal heart rate. Corticosteroids have been used, particularly dexamethasone and bethametasone, since they are not metabolized by the placenta and are thus available to the fetus in an active form. Steroid therapy has been reported as effective in the resolution of inflammatory signs (pleural effusions, ascites, and hydrops fetalis) in a few case reports [98, 99]. However, treatment is probably unable to revert third-degree heart block once established (presumably, fibrosis of conducting system). Different case series gave conflicting results. Fluorinated steroids seemed at least partially effective in improving overall prognosis in American [100, 101], Swedish [97] and Israelian [88] experiences; in these studies they have been associated with improvement in AV conduction in first- and second-degree AV blocks [88, 97, 98], or with disappearance of fetal effusions and improvement in survival [98, 100, 101]. In other series, these drugs have not been reported to have favorable effects [57, 102]. Maternal risks of fluorinated steroids are similar to any glucocorticoid and include infection, osteoporosis, osteonecrosis and diabetes. Specific fetal risks include intrauterine growth restriction, oligohydramnios and possibly adrenal suppression. Besides possible maternal side effects similar to any glucocorticoid therapy, specific fetal risks exist (oligohydramnios). Only a randomized controlled trial will define the role of fluorinated steroids in the treatment of CHB. However, this may not be feasible given the rarity of disease, the difficulty in matching the severity of cases and resistance on the part of the mothers to agree to randomization [100]. We presently suggest the following schema. If the block is incomplete (e.g., second degree), bethametasone or dexamethasone 4-8 mg daily to the mother is started, with a note of caution: to differentiate incomplete from complete AV block may be difficult in utero and usually requires expertise by a pediatric cardiologist. If the block is recent (the more common clinical situation), bethamethasone or dexamethasone is recommended as well; the dose is tapered and the drug is discontinued if no change occurs after several weeks. If the block is associated with signs of myocarditis, heart failure or hydropic changes, betamethasone/dexamethasone is recommended. If the block is complete and present for more than 2-4 weeks, with no effusions and no signs of hydrops, only serial echograms should be performed, with no therapy. In Italy, we have recently treated with IVIG two mothers whose fetus had complete CHB and clear evidence of myocarditis: the myocarditis quickly resolved, but the complete AV block persisted. A possible therapeutic window for IVIG might be in the early treatment of complete or incomplete AV block, or even in its prevention for high-risk cases which is currently under study [103, 104].

On the cardiological side, salbutamol, a selective beta 2 adrenergic agonist, also named albuterol, may be useful to



increase the fetal heart rate, to improve ventricular function and fetal hydrops. Maternal continuous ritodrine infusion or terbutaline may be attempted [105]. Beta 2 adrenergic agonists may be useful particularly as a bridge to reach a more advanced gestational age [106]. Salbutamol is the preferred sympathomimetic agent since it can be given orally to the mother, at a dosage of 2 mg six to ten times daily, according to maternal compliance.

The in utero environment is preferred as long as possible because of the low resistance circulatory pathways, thereby affording minimal work to maintain cardiac output, as well as the immaturity of the lungs and other organs. However, this approach must be balanced against the heightened transfer of maternal antibodies as the pregnancy progresses, although the window of cardiac vulnerability may have passed.

The problem of neuropsychological development

Evidence has accumulated on the potential harm of repeated courses of steroids for the mother and the fetus. Animal models suggest that repeated antenatal steroid doses can interfere with the growth and development of the immature brain and human observations suggest that antenatal and postnatal dexamethasone may negatively affect the child's neuropsychological development [107– 109]. The possible negative effects seem more linked to dexamethasone than betamethasone and it has been suggested that betamethasone should be preferred when available [110, 111]. Moreover, separate meta-analysis of the data in the Cochrane review show that only betamethasone and not dexamethasone significantly reduces neonatal mortality [112]. For these reasons, we are now moving in Europe to use bethametasone instead of dexamethasone in the context of CHB.

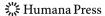
The presence of maternal anti-Ro/SSA antibodies per se may be associated with learning disabilities in the offspring [77]. In light of these findings, CHB babies who are both treated in utero with high-dose dexamethasone and exposed to maternal anti-Ro/SSA antibodies could be at risk for neuro-developmental defects. We have therefore tested our Italian CHB patients for neuropsychological development, IQ and learning disabilities: 16 children were enrolled in this study and all had normal IQ [113]. Although it is quite possible that repeated course of dexamethasone may be detrimental to the newborn's neurodevelopment, a child's final intellectual maturation remains an extremely complex process, involving the interplay of many biological, social, and cultural factors. We observed no negative effects on the neurodevelopment of our patients, many of them exposed to very high dosages of dexamethasone (much higher than those used to enhance fetal lung maturity) and to maternal anti-Ro/SSA antibodies. CHB is a rare disease, but these reassuring findings might be clinically relevant also for the large number of newborns treated with repeated courses of antenatal fluorinated steroids to induce fetal lung maturity.

Postnatal treatment

Postnatal treatment of CHB is based on pacemaker implantation. Frequently a pacemaker is implanted in the neonatal period. Due to the very low weight of these subjects (often <2.5 kg) the pacemaker is implanted by thoracotomic or sternotomic route, with an electrode on the epicardial surface which is connected to an impulse generator placed in an abdominal subfascial pouch. In the presence of extreme bradycardia, isoproterenol (0.1–0.3 µg/kg per minute) needs to be administered as well. Pacing should be considered based on the clinical condition and not considered mandatory in any infant. Non-cardiac manifestations such as skin rash or hematology abnormalities do not require any treatment, since they are reversible and spontaneously disappear, usually during the second semester of life.

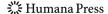
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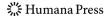
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