Anti-SSA/Ro positivity and congenital heart block: obstetric and foetal outcome in a cohort of anti-SSA/Ro positive pregnant patients with and without autoimmune diseases

M. Fredi[1], L.M. Argolini[2], F. Angeli[1], L. Trespidi[3], V. Ramoni[4], S. Zatti[5], T. Vojinovic[1], D. Donzelli[6], F.G. Gazzola[3], B. Xoxi[4], L. Andreoli[1], A. Lojacono[7], E. Ferrazzi[3], C. Montecucco[4], C.B. Chighizola[8], P.L. Meroni[9], F. Franceschini[1], R. Cimaz[8], R. Caporali[6], A. Tincani[1,10], M. Gerosa[6]

[1]Rheumatology and Clinical Immunology Unit, ASST Spedali Civili and Clinical and Experimental Science Department, University of Brescia, Italy; [2]Division of Clinical Rheumatology, ASST Gaetano Pini-CTO, Milan, Italy; [3]Department of Obstetrics and Gynaecology, Fondazione Cà Granda, Ospedale Maggiore Policlinico, Milan, Italy; [4]Division of Rheumatology, University of Pavia and Fondazione IRCCS Policlinico San Matteo; Pavia, Italy; [5]Obstetrics and Gynaecology Unit, ASST Spedali Civili of Brescia, Italy; [6]Division of Clinical Rheumatology, ASST Gaetano Pini-CTO, Research Center for Adult and Paediatric Rheumatic Diseases, Department of Clinical Sciences and Community Health, University of Milan, Italy; [7]Obstetrics and Gynaecology Unit, ASST Garda, Desenzano Hospital, Italy; [8]Paediatric Rheumatology Unit, ASST G. Pini-CTO, Research Center for Adult and Paediatric Rheumatic Diseases, Department of Clinical Sciences and Community Health, University of Milan, Italy; [9]Experimental Laboratory of ImmunoRheumatological Researches, IRCCS Istituto Auxologico Italiano, Cusano Milanino, Italy; [10]I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation Moscow, Russia.

Abstract

Objective

Neonatal lupus (NL) is an acquired disease caused by the transplacental passage of anti-SSA/Ro antibodies. The rate of congenital heart block (CHB), its most serious manifestation, ranges from 1 to 5%. The aim of this study was to retrospectively assess the prevalence of CHB in anti-SSA/Ro positive pregnant women with or without systemic autoimmune diseases from 2010 to 2020.

Methods

Patients underwent monthly visit and a shared follow-up programme of weekly (16^{th}-24^{th} week) foetal heart rate assessment by obstetric ultrasound.

Results

322 pregnancies in 258 anti-SSA/Ro patients were included; 314 were followed from the beginning of pregnancy because of the known presence of anti-SSA/Ro autoantibodies and 1 case of CHB occurred in an anti-SSA/Ro+ asymptomatic subject (0.3%). In the same period, 8 additional patients were referred to our clinics after in utero CHB diagnosis and subsequent discovery of anti-SSA/Ro without a disease diagnosis. Globally, 9 cases of congenital CHB (2.8%) occurred: 7 complete, 1 II-III degree and 1 first degree CHB. Anti-SSB/La positivity was associated with a higher risk of CHB (7.8% vs. 1.2%; p=0.0071). No differences in maternal or foetal outcomes were found in comparison with a large cohort of unselected pregnancies except for caesarian section. Hydroxychloroquine (HCQ) was used in 58.3% pregnancies, with a different prevalence according with maternal diagnosis.

Conclusion

Our data suggest that anti-SSA/Ro positive patents with a defined systemic autoimmune disease undergoing a strict follow-up since positive pregnancy test display a low risk of pregnancy complications, including but not limited to NL.

Key words

anti-SSA, pregnancy, congenital heart block, neonatal lupus, outcome, therapy

Micaela Fredi, MD
Lorenza Maria Argolini, MD
Fabrizio Angeli, MD
Laura Trespidi, MD
Veronique Ramoni, MD
Sonia Zatti, MD
Tamara Vojinovic, MD
Davide Donzelli, MD
Federica Giulia Gazzola, MD
Blerina Xoxi, MD
Laura Andreoli, MD, PhD
Andrea Lojacono, MD
Enrico Ferrazzi, MD
Carlomaurizio Montecucco, MD
Cecilia Beatrice Chighizola, MD, PhD
Pier Luigi Meroni, MD
Franco Franceschini, MD
Rolando Cimaz, MD
Roberto Caporali, MD, PhD
Angela Tincani, MD
Maria Gerosa, MD, PhD

Please address correspondence to:
Maria Gerosa
Divisione di Reumatologia Clinica,
ASST Istituto Gaetano Pini-CTO,
Via Pini 9,
20122 Milano, Italy.
E-mail: maria.gerosa@unimi.it

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Introduction

Neonatal lupus (NL) is a rare acquired disorder caused by the transplacental passage of maternal anti-SSA/Ro and/or anti-SSB/La autoantibodies (1, 2), usually during the second trimester of gestation (3, 4). The most common clinical features affect the skin and the heart, while liver damage or cytopenia are less frequent (5, 6). Cardiac involvement represents the most serious manifestation and is characterised by congenital heart block (CHB) in an otherwise structurally normal heart.

CHB can occur in the offspring of mothers with a diagnosis of specific autoimmune disease, but most cases are reported in asymptomatic carriers of anti-SSA/Ro antibodies (7-10). Not conclusive data are available regarding the relationship between maternal diagnosis and CHB outcome. In the American Research Registry for Neonatal Lupus more than 50% of 297 patients were asymptomatic or had insufficient symptoms for a formal diagnosis of connective tissue disease (CTD) when CHB was detected; however, a trend towards an increased mortality was found in the offspring of mother with systemic lupus erythematosus (SLE) compared to mothers affected by other autoimmune diseases or asymptomatic carriers (8). This association was not found in the French Registry (195 mothers with 214 pregnancies): only 26% of patients had a diagnosis of CTD and the maternal diagnosis was not associated with an increased risk of foetal or neonatal mortality (10).

The precise molecular mechanism through which maternal autoantibodies affect the foetal heart is not completely understood but is linked to inflammation (macrophage infiltration and giant cell formation), calcification and fibrosis, which lead to aberrant signal conduction at the atrio-ventricular node (11, 12).

Anti-SSA/Ro autoantibodies are directed against Ro52 and Ro60 autoantigens, represented by distinct cellular proteins of 52 and 60 kDa and are found in approximately 85–90% of mothers of children with CHB (13).

Congenital heart block is a rare event, and a Swedish study reported an incidence of second and third-degree block of 1/23,300 (9).

Prospective studies of pregnancies in anti-SSA/Ro positive patients with or without systemic autoimmune disease were revised in a systematic review published in 2015 that included 705 SSA/Ro positive mothers with 823 pregnancies with a prevalence of 1.2% (1). In another study, 7 second-third degree CHB were reported in 199 prospectively followed patients, with a prevalence of 3.5% (7). Recurrence rate of CHB in subsequent pregnancies is about 12–19% (1, 14, 15).

Inconclusive data are available about the association of these antibodies and other obstetric complications. Previous studies have suggested a relationship between the presence of anti-SSA/Ro antibodies and several pregnancy complications such as pregnancy loss (16, 17) but these results were not confirmed in subsequent studies (18, 19, 20).

The aim of our study was to assess the prevalence of CHB and other obstetrical and neonatal complications in a cohort of anti-SSA/Ro positive pregnant women prospectively followed up in three Italian tertiary referral centres.

Materials and methods

Inclusion criteria for the study were the confirmed positivity for SSA/Ro antibodies in pregnant women attending 3 referral centres (Rheumatology Departments with consolidated experience on management of patients with autoimmune diseases during pregnancy) from January 2010 to December 2020. In each centre, autoantibodies were determined with routinely methods (*i.e.* counterimmunoelectrophoresis, immunoblotting, enzyme-linked immunosorbent assay) and were performed in a referral laboratory certified for diagnosis.

The clinical data of these women and their diagnosis were retrospectively evaluated and collected by review of clinical charts. The women were grouped according to their rheumatologic diagnosis; patients with a confirmed positivity for anti-SSA/Ro, but asymptomatic at time of conception and without any sign or symptoms suggestive for connective tissue disease were defined as "anti-SSA/Ro carriers".

The 3 referral centres applied a shared follow-up programme of anti-SSA/Ro

positive pregnant women that requires a weekly assessment of foetal heart rate (FHR) by obstetric Doppler ultrasound from 16th to 24th gestational week. If a FHR below 100 bpm is detected, Doppler echocardiography is performed. Congenital heart block (CHB) I° was defined as mechanical PR interval ≥150 ms, inferred from the delay between left atrial systole and left ventricular systole, measured by using pulsed Doppler echocardiography in the left ventricular outflow tract to assess simultaneously mitral valve inflow and aortic outflow. CHB II° was defined as intermittent mechanical dissociation between atrial and ventricular contraction diagnosed with M-mode echocardiography and CHB-III° as a complete mechanical dissociation of atrial and ventricular activation diagnosed by M-mode (21).

Cutaneous NL was defined as annular or elliptic erythematous lesions in typical sites and with a neonatal onset (22). Hepatic involvement was considered in case of elevation of aminotransferase or hepatomegaly (5, 23) and haematological involvement was defined as one or more cytopenia (anaemia, neutropenia and thrombocytopenia) (23).

The following data were obtained from clinical charts: demographic features, age at the beginning of pregnancy and gestational week at first rheumatological examination, obstetrical history, autoantibody profile, maternal diagnosis at conception, organ autoimmune disease, other obstetrical risk factors, pregnancy complications and outcome, previous and ongoing therapies during pregnancy. Preeclampsia is defined as the new onset of hypertension and proteinuria or the new onset of hypertension and significant end-organ dysfunction with or without proteinuria after 20 weeks of gestation or postpartum in a previously normotensive woman; small for gestational age was defined as birth weight in the <5th percentile for gestational age.

The following data were collected for each foetus/child: foetal/neonatal outcomes, time at diagnosis of CHB and its treatment, other cardiac abnormalities related or not with neonatal lupus; cutaneous, hepatic, or haematologic involvement of neonatal lupus.

Table I. Number of patients and pregnancies according to maternal diagnosis.

Diagnosis	Patients (n=258, %)	Pregnancies (n=322, %)
SLE	65 (25.2)	82 (25.5)
UCTD	65 (25.2)	82 (25.5)
Anti-SSA/Ro carrier	53 (20.5)	57 (17.7)
SSj	53 (20.5)	73 (22.7)
Other CTD	22 (8.5)	28 (8.7)

The general obstetrical population (GOP) of Spedali Civili of Brescia of 2018 (3065 deliveries of 3178 newborns) was used as comparison population for the following outcomes: preterm delivery (before 37 weeks of gestation), severe preterm delivery (before the 34 weeks of gestation) and Caesarean sections. Outcomes that were not retrievable from the GOP were compared with data from a multicentric study cohort of patients with undifferentiated connective tissue disease (UCTD) (live birth rate, preeclampsia, intrauterine growth restriction, gestational diabetes and hypertension) (24).

This study was performed according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Brescia Promoting Centre (approval no. 2417) and by the other participating centres.

Statistical analysis

Categorical variables were reported as proportion and/or percentage, while continuous variables were expressed as mean (±standard deviation) values. Quantitative variables were compared using the t-test or non-parametric Mann Whitney test. Categorical variables were compared using contingency tables and *p*-value was calculated with Chi-Square or Fisher's exact test, as appropriate. *p*-values <0.05 were considered statistically significant.

Results

Description of the cohort

Two hundred and fifty-eight women with 322 pregnancies met the inclusion criteria. Maternal diagnosis was distributed as follows: systemic lupus erythematosus (SLE) (n=65, 25.2%), UCTD (n=65, 25.2%), Sjögren's syndrome (SSj) (n=53, 20.5%), other CTD (n=22, 8.5%) (rheumatoid arthritis n=7, primary antiphospholipid syndrome n=5, systemic sclerosis n=4, poly/dermatomyositis n=3, mixed connective tissue disease n=2, seronegative spondyloarthritis n=1) and anti-SSA/Ro carrier (n=53, 20.5%) (Table I). The majority of patients Caucasian (n=218, 84.5%), followed by Asian (n=13, 5%), African (n=8, 3.1%), Afro-Caribbean (n=5, 1.9%) and others (n=14, 5.4%).

An organ-specific autoimmune disease was diagnosed in 79 women (30.6%): the majority had an autoimmune thyroiditis (n=56, 21.7%), followed by coeliac disease (n=12, 4.7%), primary biliary cholangitis (n=2, 0.8%), myasthenia gravis, psoriasis, autoimmune hepatitis, multiple sclerosis with one case for each condition (0.4%); the remaining five patients had two organ diseases with 3 cases autoimmune thyroiditis and coeliac disease and two autoimmune thyroiditis and vitiligo.

One hundred and thirty-seven women (53%) previously had at least one pregnancy and five patients previously experienced a pregnancy complicated by CHB (3.6%): three were labelled as anti-SSA/Ro carriers and two had Sjögren's syndrome.

The mean age at the beginning of pregnancy was 33.9 years (SD= 4.9, range 19–48), maternal body mass index at the onset of pregnancy was 22.3 (SD= 3.9, range 17–40). Current smoke was present in 28 (9.3%) patients, 10 were obese at time of conception (3.2%) and 11 had a previous diagnosis of hypertension (3.4%).

Serological characteristics

In each hospital, the evaluation of the presence of anti-SSA/Ro autoantibody were performed according to the routine diagnostic tests, therefore further characterisation of SSA/Ro was possible only for few patients (16, 4.1%): 5 were anti-Ro52 positive, 7 anti-Ro60 positive and 4 (1.5%) anti-Ro52+Ro60

Table II. Description of the CHB cases occurring in our cohort.

	Number of cases (pregnancies n. 322, %)
CHB type III	7 (2.2)
CHB intermittent type II-III	1 (0.3)
CHB type I	2 (1 at 28.5w gestation, 1 by first year of life) (0.6)
Week at diagnosis of foetal CHB	26 ± 6.4

Description of the 9 foetal CHB cases

Patients	CHB detection week	Max CHB degree	Maternal diagnosis	Hydrops	Therapy	Gestational outcome	Foetal outcome
N1	25	III	Anti-SSA/Ro carrier	No	No	CS, preterm delivery (34+4w)	LB, PM at birth
N2	21+2	III	Anti-SSA/Ro carrier	Yes	Fluorinated steroid proposed, never taken	CS, severe preterm delivery (31w)	Perinatal death
N3	23	III	Anti-SSA/Ro carrier	No	Fluorinated steroid 4mg for 1 week, then stopped	CS, preterm delivery (36+5w)	LB
N4	35	III	Anti-SSA/Ro carrier	No	No	CS, preterm delivery (36w)	LB
N5	22	III	Anti-SSA/Ro carrier	No	Fluorinated steroid 4mg/die from CHB detection to delivery	CS, preterm delivery (34+6w)	LB, Temporary PM; chylothorax
N6	20	III	Anti-SSA/Ro carrier	No	Fluorinated steroid 4mg/die and HCQ a fter CHB detection	IUGR, LPL	IUD
N7	28+5	I	Rheumatoid arthritis	No	Fluorinated steroid 5mg/die	CS, preterm delivery (34+4w)	LB; respiratory distress
N8	38	III	Anti-SSA/Ro carrier	No	HCQ (before pregnancy)	VD	LB; trisomy 21
N9	22	III	Anti-SSA/Ro carrier	No	Fluorinated steroids (8 mg for 1 week, 4 mg for 4 weeks)	CS	LB

CS: Caesarean section; HCQ: hydroxychloroquine; IUD: intrauterine death; IUGR: intra-uterine growth restriction; LB: live birth; LPL: late pregnancy lost; PM: pacemaker; VD: vaginal delivery.

positive. Sixty-one (23.6%) patients tested positive also for anti-SSB/La.

Results of antiphospholipid profile were the following: anti-cardiolipin (aCL) was positive in 35 (13.6%) women, anti-β2GPI in 39 (15.1%) and LAC in 29 (11.2%).

Neonatal lupus in offspring

Nine cases of congenital CHB among 322 pregnancies (2.8%) were collected: 7 presented a complete CHB (2.2%), 1 alternating II-III degree (0.3%) and 1 first degree (0.3%) (Table II). All cases occurred in singleton pregnancies of different women and none of them had a previous pregnancy affected by NL.

Three hundred and fourteen pregnancies were surveyed throughout the pregnancy because of the known presence of anti-SSA/Ro autoantibodies and underwent the FHR monitoring protocol described in the methods. Among these pregnancies, 1 case of congenital heart block occurred (0.3%).

In addition, in the same period, 8 patients were referred to our clinics after the detection of a FHR below 110 bpm during routinely obstetric US that lead to CHB diagnosis by foetal echocardiography. All these 8 patients were subsequently found to be anti-SSA/Ro antibody positive.

Maternal diagnosis at the time of the CHB detection was as follows: 8 mothers were anti-SSA/Ro carriers and 1 had a diagnosis of rheumatoid arthritis. Notably, 1 asymptomatic patient developed SLE 7 years after the pregnancy complicated by CHB.

Among the 9 patients that presented a foetal CHB 6 were positive for both SSA/Ro and SSB/LA and 3 for only SSA/Ro. Globally, the 61 patients SSA/Ro + SSB/LA double positive patients had 77 pregnancies; therefore, the presence of SSB/La was associated with a higher risk of developing CHB ((6/77, 7.8%) *vs*. (3/245, 1.2%) p=0.0071, OR=6.81; 95%CI 1.76–25.19)).

All cases of CHB were diagnosed in utero and gestational outcome of these 9 pregnancies is reported in Table II: 7 live births, 1 perinatal death after a preterm delivery induced for the rapid worsening of foetal condition and 1 intra-uterine foetal death (CHB III degree). In two cases a pacemaker was implanted immediately after birth. One foetus presented trisomy 21, one newborn had chylotorax.

Eight of these women had not taken any medication in previous pregnan-

Table III. Comparison of demographic features and obstetrical outcome according to maternal diagnosis.

Pregnancy assessment	All pregnancies n=311 (%)	SLE n=79 (%)	UCTD n. 81 (%)	SSA/Ro carrier n=55 (%)	SSj n=68 (%)	Other CTD n=28 (%)	*p*-value
Mother age (beginning of pregnancy) (years)	34 ± 5	34 ± 5	33 ± 5	34 ± 4	35 ± 5	35 ± 4	NS
Gestational week at first examination	11.1 ± 5.9	9.3 ± 4.2	11.2 ± 6.3	14.9 ± 7.6	10.7 ± 5.1	9.6 ± 3.9	<0.0001*
MAP	27 (8.7)	6 (7.6)	7 (8.6)	2 (3.6)	7 (10.3)	5 (17.9)	NA
Bigemine pregnancies (pregnancy otucome)	16 (5.1) (27LB)	4 (5) (7LB, 1VTP)	8 (9.9) (13LB, 2EPL, 2LPL)	1 (1.8) (2LB)	2 (2.9) (4LB)	1 (3.6) (2LB)	NS
Pregnancy outcome							
Live births/all foetuses	304/327 (93)	82/83 (98.8)	79/89 (88.7)	50/56 (88.7)	65/70 (92.9)	28/29 (96.6%)	NS
Foetal losses	23/327 (7)	1/83 (1 VTP) (1.2)	10/89 (11.2) (7EPL, 3LPL)	6/56 (10.7) (1 VTP, 2EPL, 3LPL)	5/70 (7.1) (5EPL)	1/29 (3.4) (VTP)	NS
Perinatal deaths	2/304 (0.7)	1/82 (1.2)	0	1/50 (2)	0	0	NA

LB: live birth, EPL: early pregnancy lost; LPL: late pregnancy lost; MAP: Medically Assisted Procreation; VTP: voluntary termination of pregnancy. NA: not applicable; NS: not significant.
*A significant difference was found among the subgroups; the difference remains significant (p=0.0076) even excluding the 9 CHB. Comparing individual diagnostic group with each other there is a significant difference between "Ro carrier" women compared to all the other groups: anti-SSA/Ro carrier *vs*. SLE (p<0.0001), anti-SSA/Ro carrier *vs*. UCTD (p=0.0028), anti-SSA/Ro carrier *vs*. SSj (p=0.0005), anti-SSA/Ro carrier *vs*. other CTD (p<0.0001). There is also a difference between SLE and UCTD (p=0.028).

cies or before CHB detection. Only one woman (11%) was on pre-conceptional hydroxychloroquine (HCQ) at the time of CHB diagnosis. Proposed treatment after CHB discovery is reported in Table II: the most frequently proposed therapy was fluorinated corticosteroids (6/9, 66.6%), but one patient refused the treatment, and one was treated only for one week.

In addition, a case of first degree CHB within the first year of life was diagnosed in a child of a patient with seronegative spondyloarthritis that did not present any sign of foetal cardiac involvement during pregnancy, who was on acetylsalicylic acid, low molecular weight heparin and 5 mg of corticosteroids daily. No cases of recurrency of CHB occurred, in fact all the five pregnancies in patients with a previous CHB were uneventful.

The data concerning non-cardiac neonatal lupus were not recorded for all pregnancies. Cutaneous neonatal lupus was detected in 3/164 (1.8%) newborns: 1 of a mother affected by SLE, anti-SSB/LA negative and negative history for others obstetric complications and two siblings born from a mother with SSj with also a positivity for SSB/LA. All the three cases were exposed to hydroxychloroquine before and during pregnancies. Finally, 9 cases of possible haematologic-hepatic manifestations were recorded (9/138, 6.5%), mostly with a mild increase of hepatic enzymes: 4 in newborns of SLE patients, 3 in mothers affected by UCTD and 2 by SSj.

Obstetric and neonatal outcome

Out of the 322 pregnancies included, complete information concerning all the obstetric and neonatal outcome were available for 311 pregnancies, with 16 multiple pregnancies. Twenty-seven pregnancies (8.7%) were obtained with assisted reproductive technologies (ART). Obstetrical and neonatal outcomes between study and control populations were evaluated. Studied population displayed a significantly higher rate of live birth (92.9% *vs*. 79%, p<0.0001, OR=3.51, 95%CI: 2.06–5.97) when compared with a recent Italian multi-centre cohort of UCTD patients (24). Moreover, our cohort had significantly lower risk for severe preterm delivery than GOP (2.9% *vs*. 6.3%, p=0.025, OR=0.41, 95% CI: 0.22–0.88), both including or excluding CHB cases in the analysis (2.7% *vs*. 6.3%, p=0.017, OR=0.041, 95% CI: 0.20–0.84). Otherwise, caesarean section more frequently occurred in our cohort, both with (45.5% *vs*. 29.3%, p<0.0001; OR=1.98, 95% CI: 1.54–2.53) and without CHB cases (44.3% *vs*. 29.3%, p<0.0001; OR=1.92, 95% CI: 1.49–2.47). No differences were found concerning the occurrence of preeclampsia, intrauterine growth restriction, gestational diabetes or hypertension (Supplementary Table S1).

Then, we compared outcomes according to maternal diagnosis (Table III). No differences were found in the live births rate and in the mean gestational week at delivery. The average gestational week at first examination was 11.1 ± 5.9 weeks with significant differences among the subgroups: anti-SSA/Ro carrier women had a delay at first examination compared to women with SLE (p<0.0001), SSj (p=0.0005), other CTD (p<0.0001), UCTD (p=0.0028). The difference remained significant after the exclusion of CHB cases, apart from the comparison between anti-SSA/Ro carriers and UCTD.

Concerning neonatal outcomes, mean Apgar scores at fifth minute displayed a significant difference among groups, with lower scores for anti-SSA/Ro carriers compared with the other groups,

Table IV. Therapies in studied population, based on maternal diagnosis.

Therapy	All pregnancies n=317 (%)	SLE n=81 (%)	UCTD n=82 (%)	SSA/Ro carrier n=57 (%)	SSj n=70 (%)	Other CTD n=27 (%)	*p*-value
HCQ	188 (59.3)	70 (86.4)	49 (59.8)	20 (35.1)	37 (52.8)	12 (44.4)	<0.0001*
CS	111 (35)	56 (69.1)	16 (19.5)	6 (10.5)	19 (27.1)	14 (51.8)	<0.0001**
LDA	210 (66.2)	66 (81.4)	52 (63.4)	33 (57.9)	39 (55.7)	20 (74.1)	0.0054#

CS: corticosteroids; HCQ: hydroxychloroquine; LDA: low-dose acetylsalicylic acid.
* SLE *vs*. UCTD (p<0.0001; OR=4.28; 95% CI: 1.97–9.29), SLE *vs*. anti-SSA/Ro carrier (p<0.0001; OR=11.77; 95% CI: 5.09–27.17); SLE *vs*. SSj (p<0.0001; OR=5.67; 95% CI: 2.57–12.50); SLE *vs*. other CTD (*p*<0.0001; OR=7.95; 95% CI: 2.95–21.41). UCTD compared to anti-SSA/Ro carrier (*p*=0.0057; OR=2.74; 95% CI: 1.36–5.53). SSj compared to anti-SSA/Ro carrier (*p*=0.051; OR=2.07; 95% CI: 1.01–4.25).
** SLE *vs*. UCTD (*p*<0.0001; OR=9.24; 95 % CI: 4.49–19.01), SLE *vs*. anti-SSA/Ro carrier (*p*<0.0001; OR=19.04; 95% CI: 7.22–50.14), SLE *vs*. SSj (p<0.0001 OR=6.01; 95% CI: 2.96–12.19). UCTD *vs* other CTD (*p*=0.003; OR=0.22; 95% CI: 0.08–0.57), anti-SSA/Ro carrier *vs*. SSj (*p*<0.0001; OR=0.31; 95% CI: 0.11–0.85), anti-SSA/Ro carrier *vs*. other CTD (*p*<0.0001; OR= 0.10; 95% CI: 0.03–0.33). SSj *vs*. other CTD (*p*=0.039; OR=0.34, 95% CI: 0.13–0.86).
# SLE *vs*. UCTD (*p*=0.016; OR=2.53; 95% CI: 1.23–5.20), SLE *vs*. anti-SSA/Ro carrier (*p*=0.005; OR=3.20; 95% CI: 1.48–6.90), SLE *vs*. SSj (*p*=0.001; OR=3.49; 95% IC: 1.68–7.27).

and, again, these results remained significant after the exclusion of the CHB cases (Table III).

Therapy

Corticosteroids (CS) and low-dose acetylsalicylic acid (LDA) use was recorded in 111 (34.4%) and 210 (65.2%) pregnancies, respectively (Table IV). Statistical analysis showed a different prevalence in the prescription of both CS (*p*<0.0001) and LDA (p=0.0054), with the highest frequency in SLE. Prescription of CS was more frequent in women with SLE compared to: UCTD (*p*<0.0001) and anti-SSA/Ro carrier (*p*<0.0001). Corticosteroids were less frequently used in anti-SSA/Ro carrier women than SSj (*p*<0.0001), in UCTD compared to other CTD (*p*=0.003) and in SSj *vs*. Other CTD (*p*=0.039). Women with SLE were more frequently treated with LDA than patients with UCTD (*p*=0.0016), anti-SSA/Ro carrier (*p*=0.005) and SSj (*p*<0.0001).

Hydroxychloroquine was globally used in 188 (58.3%) pregnancies, and significant discrepancies were found among the groups, with the highest prevalence in SLE (86.4%), significantly higher than UCTD (*p*=0.0002), anti-SSA/Ro carrier (*p*<0.0001), SSj (*p*<0.0001) and other CTD (*p*<0.0001); moreover, the prevalence was higher in UCTD patients if compared with anti-SSA/Ro carrier (*p*=0.0057) and in SSj compared to anti-SSA/Ro carrier (*p*=0.051).

Analysing the occurrence of CHB in pregnancies exposed to HCQ before conception or from positive gravindex (152 pregnancies) one case of CHB occurred, whereas 0 case among the 162 pregnancies regularly followed but not treated with HCQ (1/151 *vs*. 0/162, *p*=0.46) without any significant association.

If we consider the whole population, including also the 8 non-prospectively followed up pregnancies (referred to our clinics after CHB diagnosis and subsequent anti-SSA/Ro positivity detection), the CHB prevalence was lower in the group treated with HCQ but without a statistically significant difference: 0.54% (95% CI -0.51–1.59) *vs*. 5.26% (95% CI -5.05–15.58).

Hydroxychloroquine was proposed in 4 out the five pregnancies in mother that had a previous CHB, in one case before conception and in the other three since gravindex.

Out of the 188 women that were treated with HCQ, 143 (76.1%) started the treatment before conception with a significant difference among groups (*p*=0.0003) (Table IV).

SLE patients started therapy earlier than anti-SSA/Ro carrier (*p*<0.0001) and SSj (*p*=0.021); UCTD patients started earlier than anti-SSA/Ro (*p*=0.0035;) and other CTD started therapy earlier than anti-SSA/Ro (*p*=0.028).

The reason for the introduction of HCQ resulted different among the groups: 18 out of 20 (90%) anti-SSA/Ro carrier pregnancies were treated with HCQ for primary or secondary prevention of CHB, whereas in the other groups this indication less frequently occurred.

Finally, 143/188 (76.1%) women started HCQ at preconception with the highest prevalence in SLE patients (61 out of 70, 87.1%) while the lower prevalence was in anti-SSA/Ro patients (8 out of 20, 40%).

Discussion

Neonatal lupus represents one of the most relevant matter of concern in anti-SSA/Ro positive female patients with rheumatologic diseases willing to conceive, since CHB, its most serious manifestation, has a mortality rate of about 20% (1). In our study, only one (0.3%) among 314 pregnancies followed from positive pregnancy test throughout the pregnancy because of the known anti-SSA/Ro positivity was complicated by CHB, in an asymptomatic anti-SSA/Ro positive woman taking HCQ, while none of the patients with a confirmed diagnosis of systemic autoimmune disease developed CHB during gestation. Moreover, no cardiac complications of NL were observed in the few patients with previous CHB. Thus, in this cohort, the prevalence of cardiac manifestations of NL was lower than expected based on what reported in the literature (1, 5, 16). By contrast, most part of pregnancies complicated by foetal heart conduction abnormalities were referred to our pregnancy clinics in the second trimester, exactly because of the diagnosis of CHB that led to subsequent anti-SSA/Ro autoantibody detection, in the absence of any rheumatologic manifestation. Overall, our results testify that patients with an overt rheumatologic disease display a very

low risk of CHB. This finding can have several explanations: first, anti-SSA/Ro positive rheumatic patients usually receive a chronic immune-modulating and/or immunosuppressant therapy including HCQ, that has been shown effective in CHB recurrence prevention and therefore might be also exerting primary prevention (8, 25); secondly, according to the previously reported rate of recurrence (from 12 to 25%), in our cohort at least one case of recurrence was expected, confirming what previously reported (25, 26); lastly, on the other hand, the only prospectively followed up patient who developed CHB was taking HCQ 400 mg from 6 months before positive pregnancy test and during the whole pregnancy, leaving the discussion open. However, the large majority of women with affected children were not taking any medication, since anti-SSA/Ro were investigated because of CHB occurrence, impairing any possible statistical analysis about a possible protective role of HCQ. It is worth noting that no relevant unwanted effects were observed in patients treated with HCQ, confirming the safety profile of this drug during pregnancy (27). In this view, the balance between potential beneficial effects and low hazard of adverse events is in favour of the use of HCQ in all pregnant patients with anti-SSA/Ro and without specific contraindications.
In the last few years, cardiac complications possibly related to HCQ use have been extensively investigated in patients with connective tissue disease and more recently after the use of HCQ as a possible therapeutic option in the context of Sars-Cov2 infection. A possible foetal/neonatal cardiotoxicity was recently investigated in a substudy of the aforementioned PATCH study, and the authors reported a normal corrected QT interval (QTc) in almost 90% of the 45 neonates included (28). These data confirm a previous French study that did not found differences in mean QTc in 47 new-borns exposed to HCQ and 45 non-exposed during pregnancy (29). Moreover, the autopsy of a foetus with complete CHB exposed to HCQ did not reveal any histological sign of HCQ cardiotoxicity (30).
Previous studies have raised the hypothesis that the underlying systemic autoimmune disease could have an impact on CHB development in agreement with the Italian registry that reports a higher prevalence of CHB in patients with an established autoimmune disease (15). On the other hand, some authors described a higher risk of NL in asymptomatic anti-SSA/Ro positive subjects as it was seen in the present study. This suggests a selection bias of previous cases only recruited among patients attending specialistic institutions while now with the spread on knowledge the target population is significantly enlarged.
In our retrospective cohort, there was a huge heterogeneity in antibody assessment making difficult to know anti-SSA/Ro titres possibly linked to an increased risk of CHB (31, 32). It can be speculated that in patients with an established autoimmune disease, long term immuno-modulatory or immuno-suppressive therapy could have impaired autoantibody production and contributed to reduce the inflammatory milieu that apparently interfere with the proper intra-uterine heart conduction development (33).
Moreover, the majority of our women received a pre-conception counselling, suggesting that an accurate pregnancy planning, during a phase of tight control of the underlying disease associated with a strict follow-up during gestation, could help in reducing the risk of adverse pregnancy outcomes including CHB occurrence. In line with this hypothesis, all but one woman developing CHB were asymptomatic anti-SSA/Ro carriers, that did not receive specific pre-conception advise.
The concomitant presence of anti-SSB/La autoantibodies has been addressed as a risk factor for CHB (1,18). Actually, in our study, anti-SSB/La positivity provided an additional risk of CHB (OR of 6.8).
Morbidity and mortality associated with CHB in our cohort are in line with previous studies reporting an *in utero* mortality rate ranging between 6 and 30% (8, 10, 15, 34-36). Actually, we observed only one intrauterine death and one perinatal death due to severe prematurity. As previously reported, the use of fluorinated steroids does not seem to improve foetal outcome in established III degree AV block (37). Additional therapies, including IVIG has been proposed for CHB treatment. However, none of our cases received therapies other than fluorinated steroid.
The few studies evaluating the impact of anti-SSA/Ro positivity on other pregnancy outcomes report conflicting data, such as an inconstant association with early pregnancy loss, pre-term delivery and utero-placental insufficiency that has been attributed by some authors to the underlying disease being significantly more frequent in SLE patients and/or subjects with both anti-SSA/Ro and anti-phospholipid antibodies (9, 18, 31, 38). Taken as whole cohort, our anti-SSA/Ro positive patients did not display a significantly higher risk of pregnancy complications in comparison with both general population and a group of women suffering from connective tissue diseases. To note, in our cohort we observed a significantly lower rate of severe preterm delivery. This finding is only apparently surprising as our patients received a very tight follow-up in tertiary centres dedicated to high-risk pregnancy by a multidisciplinary team. In addition, more than 60% of pregnancies were treated with low-dose aspirin, one of the few drugs that have been demonstrated to reduce the risk of adverse pregnancy outcome including preeclampsia (39). Moreover, our patients showed a significantly higher rate of live births in comparison to UCTD. No differences in the rate of adverse outcome were observed among the different disease subgroups.
The current study has some limitations. The lack of centralisation of immunological test prevented a detailed analysis of anti-SSA/Ro antibodies, therefore the exact prevalence or the titre of anti-Ro52 and 60 kDa in our cohort is not known. Moreover, although the cohort was prospectively followed, the data were retrospectively analysed and some information was missing.
In conclusion, our data suggest that anti-SSA/Ro positive patents with a defined systemic autoimmune disease undergoing a periodical follow-up dis-

play a very low risk of pregnancy complications, including but not limited to NL. This finding could be helpful for pre-conception counselling to reassure rheumatologic patients with known anti-SSA/Ro positivity willing to conceive and it highlights the importance of a regular and tight follow-up by an experienced multidisciplinary team during the whole pregnancy to minimise the risk of adverse events. Moreover, even if the retrospective nature of the study precludes from drawing a definite conclusion, the addition of hydroxychloroquine also in asymptomatic subjects could be considered in the presence of anti-SSA/Ro positivity due to its very safe profile and its possible protective role for CHB.

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