RHEUMATOLOGY

Original article

doi:10.1093/rheumatology/kex191

Prenatal exposure to antimalarials decreases the risk of cardiac but not non-cardiac neonatal lupus: a single-centre cohort study

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Abstract

Objective. Recent studies have suggested that prenatal exposure to HCQ reduces the risk of cardiac neonatal lupus. The aim of this study is to assess if maternal intake of antimalarials (AMs) throughout pregnancy lowered the risk of cardiac and non-cardiac neonatal lupus.

Methods. Consecutive children seen between 1 January 1984 to 1 October 2013 born to women with a CTD and positive anti-Ro and/or anti-La antibodies were eligible for this single-centre retrospective cohort study. A total of 315 individuals were screened and 268 participants were included. Exposure to AMs was defined as HCQ or chloroquine throughout pregnancy. Outcomes were cardiac and non-cardiac neonatal lupus. Frequentist and Bayesian analyses were performed. We hypothesized that prenatal AM exposure would decrease the risk of cardiac but not non-cardiac neonatal lupus.

Results. A total of 268 pregnancies were included; 73 were exposed to AMs throughout pregnancy. Ninety-nine children developed neonatal lupus, 117 remained unaffected and 52 children did not develop cardiac neonatal lupus but could not be categorized as unaffected since their full non-cardiac neonatal lupus status was unknown. Logistic regression suggested a protective effect of AM on cardiac neonatal lupus, but results were not statistically significant [odds ratio (OR) 0.21; $P = 0.07$]. Bayesian analysis showed that the probability of obtaining a protective effect (OR < 1.0) for cardiac neonatal lupus was significant (98.7%). The effect of AMs on non-cardiac neonatal lupus was not significant (OR 0.78; $P = 0.21$).

Conclusion. In this large single-centre cohort study, exposure to AMs throughout pregnancy was associated with a decreased probability of developing cardiac but not non-cardiac neonatal lupus.

Key words: neonatal lupus erythematosus, antimalarials, congenital heart block

CLINICAL SCIENCE

Rheumatology key messages

- The probability that prenatal antimalarial exposure decreased the risk of cardiac neonatal lupus was 98.7%.
- Prenatal antimalarial exposure did not significantly decrease the risk of non-cardiac neonatal lupus.

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Submitted 24 June 2016; revised version accepted 10 April 2017

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Introduction

Neonatal lupus erythematosus (NLE) is an autoimmune disease associated with the transplacental transfer of maternal anti-Ro, anti-La and/or anti-RNP antibodies. The most serious manifestation is cardiac NLE [1]. The reported prevalence of antibody-exposed pregnancies complicated by congenital heart block (CHB) is 1–2%, but increases to ~17% in women with a previously affected child [2–4]. Most of the non-cardiac manifestations of NLE are benign and/or transient. Although the

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long-term outcome of non-cardiac NLE is very good, these manifestations lead to increased health care service utilization.

There are currently no therapies to prevent NLE. It had recently been suggested that prenatal exposure to HCQ reduced the risk of cardiac NLE, possibly through disruption of toll-like receptor (TLR) signalling [5, 6]. However, the effect of prenatal antimalarial (AM) exposure on the risk of NLE *per se*, specifically non-cardiac NLE, has not been studied. We hypothesized that prenatal AM exposure would lower the risk of cardiac but not non-cardiac NLE. The aims were to determine whether prenatal exposure to AMs decreased the risk of cardiac NLE and determine the effect of AM exposure on the risk of non-cardiac NLE.

Methods

Study population

A single-centre, retrospective cohort study was performed on children exposed to anti-Ro and/or anti-La antibodies seen consecutively at the NLE clinic of the Hospital for Sick Children of Toronto from 1 January 1984 to 1 October 2013. The NLE clinic database comprises a prospectively collected cohort of pregnant women with a CTD who were known to be positive for anti-Ro and/or anti-La antibodies. The state of care in the greater Toronto area, beginning in the early 1980s, was that the majority of pregnant women with a known CTD and anti-Ro and/or anti-La antibodies were referred to one physician and subsequently to our centre for regular foetal echocardiographic screening during pregnancy. After birth, children were seen in the NLE clinic, regardless of the presence or absence of NLE features, and every child had a standardized clinical (clinic visit at ages 2, 4 and 12 months and then every 2 years) and laboratory assessment (complete blood count, aspartate aminotransferase, alanine aminotransferase, bilirubin) and an ECG.

Inclusion criteria

The inclusion criteria were first child born to a woman positive for anti-Ro and/or anti-La antibodies documented during pregnancy or soon after delivery and with a diagnosis prior to conception of either SLE, cutaneous lupus, SS, RA or DM; the mother underwent foetal echocardiographic screening during her pregnancy and/or the child had a post-natal ECG; maternal medication intake during pregnancy was documented and the child was $\geqslant$6 months old as of 1 October 2013. Women in whom anti-Ro and/or anti-La antibodies or a CTD were diagnosed due to the identification of NLE in their offspring were excluded from this study.

Definitions

NLE was defined as either cutaneous involvement; cardiac involvement, including first- (only if documented postnatally), second- or third-degree heart block, cardiomyopathy and/or endocardial fibroelastosis; haematological involvement, including thrombocytopenia ($<100 \times 10^9$/l), neutropenia ($<1.0 \times 10^9$/l) and/or anaemia [haemolytic (haemoglobin $<$100 g/l with positive direct Coomb's test, elevated unconjugated bilirubin, lactate dehydrogenase and decreased haptoglobin) or aplastic (haemoglobin $<$100 g/l with bone marrow aspiration showing decreased red blood cell precursors)]; liver involvement, including elevation of liver enzymes (greater than the upper limit for age) with or without cholestasis (direct bilirubin greater than the upper limit for age) and isolated cholestasis; or neurological involvement, including macrocephaly or hydrocephalus. Non-cardiac NLE manifestations were considered as NLE features only if other underlying causes were absent, that is, infections, medication-induced or haemodynamic disturbances. Unaffected children were those who did not develop any of these NLE features. The primary outcome, cardiac NLE, was defined as a child with one or more cardiac manifestation. The secondary outcome, non-cardiac NLE, was defined as a child with one or more non-cardiac manifestation. Children were considered exposed to AMs (HCQ 200–400 mg/day or chloroquine 250 mg/day) and AZA (any dose) if their mother had documented intake of these medications throughout pregnancy. Prenatal exposure to non-fluorinated steroids was defined as any exposure during pregnancy. Prenatal exposure to fluorinated steroids and IVIG was also defined as any exposure, but the exposure had to occur before the development of NLE. The anti-Ro and anti-La antibody levels were treated as categorical variables ($<$50 or $\geqslant$50 U/ml), as high titres of these antibodies have been associated with an increased risk of cardiac NLE [7].

Data collection

Eligible cases and controls were identified in our NLE and foetal cardiology databases, which contain prospectively collected data since 1984 and 1999, respectively. Information was extracted on mothers (age, CTD diagnosis, maternal level of anti-Ro and anti-La antibodies, medication intake throughout pregnancy and previously affected child with CHB) and their children (gender, year of birth, NLE status, intake of steroid and IVIG). Twin pregnancies were included but each twin set was analysed as one child to avoid confounding by within-family data correlation. Twin sets discordant for their NLE status were categorized as having NLE. The study was approved by the Hospital for Sick Children (REB1000037488) and Mount Sinai Hospital (REB12-0261-C) Ethics Boards. A waiver of consent was granted for this study.

Statistical analysis

Univariable and multivariable logistic regression were used to study the association between prenatal use of AMs and NLE. The multivariable model examining the association between prenatal use of AMs and cardiac NLE included the following variables: exposure to AMs, anti-Ro antibody $\geqslant$50 U/ml, anti-La antibody $\geqslant$50 U/ml, maternal age and maternal diagnosis (SS *vs* other diagnoses). The multivariable model for non-cardiac NLE included the five

covariates mentioned above as well as maternal intake of fluorinated steroids and/or IVIG and infant's intake of steroid and/or IVIG. One-sided *P*-values <0.05 were considered statistically significant. In addition to the standard maximum likelihood–based fitting of the logistic regression models, the multivariable model was refit using Bayesian inference with informative prior distributions. Considerable statistical uncertainty should be expected when making inferences on the moderately large number of effect sizes in the multivariable model with a dataset as small as this dataset. Informative prior distributions provide a compromise between excluding an explanatory variable (and implicitly assuming it has an effect size of zero) and accepting the loss of statistical power that results from adding further variables to a maximum likelihood–based logistic regression analysis. Bayesian inference requires the specification of prior distributions for each variable's effect size, which in this case were derived by an NLE expert (co-author E.D.S.) based on published data on this topic (see Cardiac NLE outcome and Non-cardiac NLE outcome sections of supplementary data, available at *Rheumatology* Online). Data analysis was performed using SPSS Statistics Version 21.0 (IBM, Armonk, NY, USA), R Version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria) and JAGS version 3.14.

Results

Characteristics of the study population

A total of 315 prospectively followed pregnancies were screened (Fig. 1). Forty-seven pregnancies were excluded due to the inability to confirm the maternal diagnosis, antibody status, medication intake, foetal echocardiography screening and/or post-natal ECG or the infant outcome. The study population consisted of children born from 268 pregnancies. The cardiac NLE status was known for all 268 foetuses/infants, with full laboratory data in 216 infants. The 52 children without full laboratory data were classified as having no cardiac NLE involvement but could not be categorized as unaffected and therefore were only included for cardiac NLE analysis. Six of the 268 pregnancies were twin pregnancies. In three twin sets, both twins did not develop NLE; these children were designated as three unaffected children. The remaining three twin sets were classified as NLE: in two sets, one had NLE and one was unaffected while in the other pair, both developed NLE.

Maternal characteristics

Table 1 shows the clinical and laboratory characteristics of the maternal cohort. Among the 268 pregnancies, the most frequent maternal diagnoses were SLE [$n=203$ (75.7%)] and SS [$n=41$ (15.3%)]. Seventy-three women (27.2%) took AMs throughout pregnancy. Women taking AMs were more likely to have a diagnosis of SLE and to be on AZA or non-fluorinated steroids than those not taking AMs. The proportion of mothers with high-titre anti-Ro antibodies was slightly higher in the group of women not taking AMs. The proportion of mothers with high-titre anti-La antibodies and who had a previously affected child with CHB was similar in both groups. The majority of children exposed to AMs were born after the year 2000, a

Fig. 1 Flow diagram of study participants

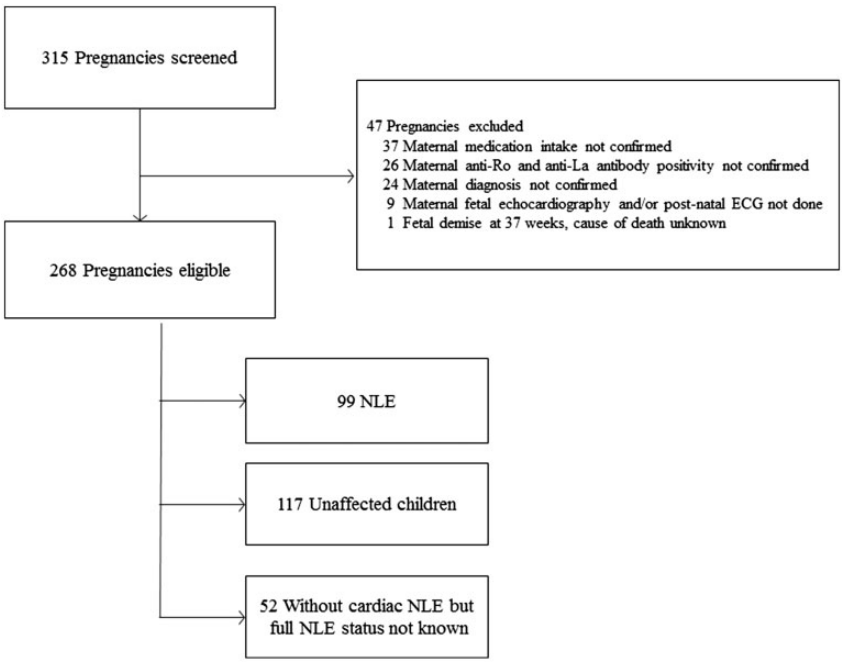

Table 1 Characteristics of 268 mothers and children exposed and unexposed to AMs

Characteristics	Exposed to AMs (n = 73)	Unexposed to AMs (n = 195)	P-value
Mothers			
Age at birth of child, mean (S.D.), years	31.8 (4.4)	32.1 (4.8)	0.669
Diagnosis			0.002
SLE	65 (89.1)	138 (70.8)	
Cutaneous lupus	1 (1.4)	4 (2.0)	
SS	2 (2.7)	39 (20.0)	
RA	5 (6.8)	13 (6.7)	
DM	0	1 (0.5)	
Anti-Ro antibody titre $\geqslant$50 U/ml[a]	33 (56.9)	117 (70.5)	0.058
Anti-La antibody titre $\geqslant$50 U/ml[b]	17 (28.0)	47 (27.5)	0.830
AZA use	14 (19.2)	7 (3.6)	$<$0.001
Non-fluorinated steroid use	36 (49.3)	55 (28.2)	0.001
Fluorinated steroid use prior to cardiac NLE	0	0	NR
IVIG use prior to cardiac NLE	1 (1.4)	1 (0.5)	0.471
Previous child with CHB	1 (1.4)	6 (3.1)	0.677
Children			
Female/male, n[c]	30/36	81/95	0.937
Year of birth $\geqslant$2000	68 (93.2)	106 (54.4)	$<$0.001
Infant's intake of steroid ± IVIG	1 (1.4)	5 (2.6)	0.999

Data presented as n (%) unless otherwise specified. [a]Quantitative titre available for n = 224 women. [b]Quantitative titre available for n = 232 women. [c]Data available for n = 242 children. NLE: neonatal lupus erythematosus; NR: not reported.

date when the safety of AMs in pregnancy had been well recognized.

Infant outcome

Of the 216 pregnancies in which we had full data, NLE developed in 99 (45.8%) infants. Thirteen children developed cardiac NLE and 92 children had at least one non-cardiac NLE manifestation (Table 2). Haematological abnormalities were the most frequent non-cardiac involvement. Six children developed both cardiac and non-cardiac NLE features.

Effect of *in utero* AM exposure on the risk of cardiac NLE

Among the 73 children exposed prenatally to AMs, only 1 developed cardiac NLE as compared with 12/195 unexposed children. Univariable and multivariable logistic regression models suggested that prenatal exposure to AMs was associated with a decreased risk of cardiac NLE, but results were not statistically significant (Table 3). Multivariable Bayesian analysis revealed a probability of 98.7% that prenatal AM exposure would be protective against cardiac NLE (OR $<$ 1.0).

Effect of *in utero* AM exposure on the risk of non-cardiac NLE

Among the 216 infants with full NLE status available, 24/62 AM-exposed patients developed non-cardiac NLE as compared with 68/154 unexposed patients. Prenatal exposure to AMs was associated with a decreased risk of non-cardiac NLE in univariable and multivariable logistic regression models, but results were not statistically significant (Table 4). The probability of obtaining a protective effect (OR $<$ 1.0) for non-cardiac NLE features was 78.6% in the multivariable Bayesian analysis.

Discussion

Cardiac involvement in NLE is a significant cause of morbidity and mortality during pregnancy and in offspring. The results of our prospectively collected cohort of 268 pregnancies of women with an autoimmune disease and anti-Ro and/or anti-La antibodies followed in a single centre with routine pregnancy and post-natal follow-up demonstrated a probability of 98.7% that maternal use of AMs throughout pregnancy was associated with a decreased risk of cardiac NLE in the foetus/infant. Two previous retrospective multicentre studies have suggested that maternal exposure to HCQ is associated with a decreased risk of CHB in the foetus [5, 6]. The first study examined 201 foetuses/infants born to women with SLE and anti-Ro and/or anti-La antibodies [5]. Seven of the 50 children with cardiac NLE were exposed to HCQ, compared with 56 of the 151 controls, leading to an OR of 0.28 (95% CI 0.12, 0.63; P = 0.002). The second study addressed the effect of HCQ exposure on the recurrence rate of cardiac NLE in 257 pregnancies, irrespective of maternal health [6]. The OR for cardiac NLE associated with HCQ use was 0.23 (95% CI 0.06, 0.92; P = 0.04) when accounting for maternal race, the presence of anti-La antibodies and the source of data. Preliminary results of the Preventive Approach to Congenital Heart Block with Hydroxychloroquine study (NCT01379573), a prospective non-randomized trial of maternal use of HCQ throughout the pregnancies of women with anti-Ro and/or anti-La antibodies, suggested that HCQ use was associated with a decrease in the recurrence rate of cardiac NLE [8].

Table 2 Features of the 99 NLE cases

Feature	*n*	Laboratory values in affected infants	Laboratory values in unaffected infants
Cardiac			
Third-degree CHB	7		
Third-degree CHB and endocardial fibroelastosis	3		
Second-degree alternating with third-degree CHB	1		
Second-degree CHB	1		
Cardiomyopathy and endocardial fibroelastosis	1		
Non-cardiac			
Haematological	44		
Neutropenia, $\times 10^9$/l	41	0.64 (0.52–0.83)	1.76 (1.34–2.36)
Thrombocytopenia, $\times 10^9$/l	3	75 (55–94)[a]	444 (355–530)
Elevated liver enzymes	38		
Aspartate aminotransferase, U/l		155 (124–174)	43 (35–55)
Alanine aminotransferase, U/l		74 (66–111)	34 (26–44)
Cutaneous	33		
Extraventricular obstructive hydrocephalus	4		

Data presented as *n* or median (interquartile range) unless otherwise specified. [a]refers to Median (range). CHB: congenital heart block.

Table 3 Effect of prenatal exposure to antimalarials on the risk of cardiac NLE

Analysis	Frequentist OR (95% CI)	Frequentist *P*-value	Bayesian OR (95% CrI)	Probability of OR < 1.0 (%)
Univariable				
Frequentist prior	0.21 (0.03, 1.66)	0.07	NA	NA
Multivariable				
Frequentist prior	0.39 (0.05, 3.39)	0.20	NA	NA
Bayesian informative prior	NA	NA	0.42 (0.12, 0.90)	98.7

CrI: credible interval; NA: not applicable.

Table 4 Effect of prenatal exposure to antimalarials on the risk of non-cardiac NLE

Analysis	Frequentist OR (95% CI)	Frequentist *P*-value	Bayesian OR (95% CrI)	Probability of OR < 1.0 (%)
Univariable				
Frequentist prior	0.78 (0.43, 1.42)	0.21	NA	NA
Multivariable				
Frequentist prior	0.74 (0.36, 1.54)	0.21	NA	NA
Bayesian informative prior	NA	NA	0.83 (0.45, 1.23)	78.6

CrI: credible interval; NA: not applicable.

It has been suggested that a complex formed by anti-Ro antibodies and Ro antigens can activate macrophages through TLR-7 signalling [9]. This signalling then leads to secretion of pro-fibrotic factors, resulting in cardiac fibrosis, a histopathological hallmark of cardiac NLE [10]. The mechanism through which AMs may confer protection for cardiac NLE might be its effect on TLR-7 activation, as both HCQ and chloroquine have been shown to inhibit TLR-7 stimulation and thereby inhibit macrophage secretion of pro-fibrotic factors [11, 12]. Regarding cutaneous NLE, TLR-7 and TLR-9 have been shown to be involved in the initiation and maintenance of interface dermatitis in lupus-prone New Zealand Black/White F1 mice [13]. As the histopathological pattern in these mice is similar to

what is found in cutaneous NLE, we suggest that AMs could exert a protective effect on cutaneous involvement through this mechanism [14]. The rationale underlying a potential protective effect of prenatal AM exposure on the haematological, hepatic and neurological NLE manifestations is not as straightforward. The role played by TLRs in the pathogenesis of these specific disease features has not been characterized and TLRs may not even be part of the pathogenic cascade. Direct antibody-mediated cytotoxicity may be involved in some of these NLE manifestations. Specifically, neutropenia in NLE may be the result of anti-Ro antibodies binding to an antigen on the neutrophil surface membrane that has significant sequence homology to Ro60 [15]. Therefore it is possible that AMs are effective in the prevention of some but not all non-cardiac NLE manifestations and our sample size was too small to study non-cardiac manifestations individually. Another potential cause of non-efficacy in some NLE manifestations may be that sufficient blood levels of AMs are not obtained in neonates and/or the therapeutic blood level declines after birth and therefore the protective effect for non-cardiac NLE is lost during the at-risk period.

Our study is the first study to use Bayesian analysis to explore the effect of AMs on the risk of NLE. One of the main advantages of Bayesian analysis is that it provides an explicit probability statement for the association being studied [16]. The use of prior information allows it to perform better than frequentist statistical methods with small sample sizes, as found in our study and the previous studies on AM use and cardiac NLE [17]. In this study, results of the frequentist analyses for the outcome cardiac NLE were inconclusive due to the small number of events and lack of power. Bayesian inference with informative prior distributions was used to overcome this limitation, specifying a fairly restricted range of probable effect sizes (see Cardiac NLE outcome section of supplementary data, available at *Rheumatology* Online). Although informative priors ensure results will be sensible even when the dataset in question is small, the results should be expected to be highly sensitive to the choice of prior distributions used. This issue is partly addressed by performing frequentist logistic regression analyses alongside the Bayesian analyses in order to provide a contrast between the subjective Bayesian conclusions and the purely data-driven inferences obtained from maximum likelihood estimation. Although *P*-values obtained from the frequentist analyses for the outcome cardiac NLE did not show a statistically significant effect, considering the small number of events in the cardiac NLE dataset and the number of covariates fitted in the multivariable logistic regression model, *P*-values of 0.07 (univariable analysis) and 0.20 (multivariable analysis) may be considered relatively small under these limiting circumstances. In addition, incorporating information obtained from two previous studies with our data, the Bayesian analysis results showed that AM exposure was highly likely to offer protection against cardiac NLE. We believe that these results support the hypothesis that AMs are a plausible candidate for cardiac NLE prevention.

Our study is the first to address the effect of prenatal AM exposure on the risk of non-cardiac NLE features. Results from the logistic regression analyses did not suggest a statistically significant protective effect from AM exposure if the 95% threshold is strictly adhered to, as the 95% credible interval derived from the multivariable Bayesian analysis produced a posterior probability of a protective effect in those exposed to AMs of 78.6%, which seems considerable. A further point to consider is that no past studies have assessed the effect of AM exposure on non-cardiac NLE manifestations such that derivation of the informative prior for the Bayesian model was not based on actual data but was based on an NLE expert opinion (see Non-cardiac NLE outcome section of supplementary data, available at *Rheumatology* Online). Therefore the informative prior could have been more subjective and results obtained may have been biased accordingly. However, the 95% CIs obtained from the frequentist analyses were narrow enough, therefore we believe that the frequentist results are sufficiently accurate to draw conclusions. Based on those results, we cannot conclude that exposure to AMs significantly decreases the risk of non-cardiac NLE.

Potential limitations need to be acknowledged. Medication adherence was not measured specifically during pregnancy, but mothers were queried about medication intake during routine prenatal rheumatology follow-up and at the first NLE clinic visit. It is therefore possible that some children categorized as exposed to AMs were not exposed throughout gestation due to maternal non-adherence to therapy. Women not taking AMs had a higher frequency of high-titre anti-Ro antibodies and a diagnosis of SS was more commonly found, which might have put them at a higher baseline risk of having a child with cardiac NLE. This potential bias was accounted for by including these important variables in the multivariable analysis. Women taking AMs were more likely to be taking AZA or non-fluorinated steroids. A confounding effect by these drugs is not likely, as the placenta acts as a relative barrier to AZAs main metabolite and inactivates non-fluorinated steroids [18]. It is possible that the prevalence of non-cardiac NLE (specifically increased liver function tests and neutropenia) might have been overestimated if certain manifestations were not a consequence of NLE but were due to other factors (i.e. infections, medication induced, hemodynamic instability). However, all children were serially examined at ~2, 4 and 12 months. Examination ruled out infection, none of the infants were on any medication and the effect of maternal medication would not be present at these ages. Furthermore, the incidence of non-cardiac NLE was similar to our previous study that systematically examined a prospective cohort from a second group from Italy [2]. There are no other systemic prospective studies that have examined infants born to mothers with anti-Ro antibodies to compare. Lastly, the effect of AM exposure on non-cardiac NLE features was analysed as a whole without looking at the effect on each individual manifestation. As suggested in the section on mechanisms of

action of AMs and mechanisms leading to NLE, it is possible that AM exposure only alters the risk of certain non-cardiac features and therefore our analysis did not capture the effect on individual features.

The cohort described in this study is unique, as all children born to mothers who had foetal echocardiograms in the greater Toronto area were referred to the NLE clinic and these children were seen regardless of the presence or absence of NLE features or whether there was a family history of NLE. Therefore we suggest that the cohort described in this study is an unbiased representative cohort of all foetuses exposed *in utero* to anti-Ro and/or anti-La antibodies. It is possible that children born to women without a CTD could benefit from prenatal AM exposure, but this specific question would need to be addressed in a prospective study, as children born to these women were excluded from our study as a result of having a very low risk of AM exposure during pregnancy.

In this study of the largest single-centre cohort of children born to anti-Ro and/or anti-La antibody–positive women with a CTD, using Bayesian analysis we have shown that the probability that AM exposure was associated with a decreased risk of cardiac NLE was 98.7%. AM exposure did not significantly decrease the risk of non-cardiac NLE. Due to the small number of events in this study, our findings need to be confirmed in an independent cohort before definite conclusions can be drawn about the efficacy of AMs in preventing NLE.

Acknowledgements

The authors thank Michelle Wang (MD, University of Toronto) and Shazia Ali (BSc, Hospital for Sick Children, Toronto) for assistance with data collection and extraction. No financial compensation was given for their contributions. We wish to thank all the rheumatologists for referring patients to C.A.L. and for foetal echocardiography and all of the obstetricians at Mount Sinai Hospital's high-risk unit, without whose help this study would not have been possible. During the course of the study E.D.S. was the holder of the Ho Family Chair in Autoimmune Diseases at the Hospital for Sick Children and J.B. was supported for her fellowship funding by GlaxoSmithKline, the Canadian Rheumatology Association (CRA), the Canadian Network for Improved Outcomes in SLE (CaNIOS), the Arthritis Society and CHU Sainte-Justine.

Funding: No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article.

Disclosure statement: The authors have declared no conflicts of interest.

Supplementary data

Supplementary data are available at *Rheumatology* Online.

References

1 Izmirly PM, Saxena A, Kim MY *et al*. Maternal and fetal factors associated with mortality and morbidity in a multiracial/ethnic registry of anti-SSA/Ro-associated cardiac neonatal lupus. Circulation 2011;124:1927–35.

2 Cimaz R, Spence DL, Hornberger L, Silverman ED. Incidence and spectrum of neonatal lupus erythematosus: a prospective study of infants born to mothers with anti-Ro autoantibodies. J Pediatr 2003;142:678–83.

3 Buyon JP, Hiebert R, Copel J *et al*. Autoimmune-associated congenital heart block: demographics, mortality, morbidity and recurrence rates obtained from a national neonatal lupus registry. J Am Coll Cardiol 1998;31:1658–66.

4 Llanos C, Izmirly PM, Katholi M *et al*. Recurrence rates of cardiac manifestations associated with neonatal lupus and maternal/fetal risk factors. Arthritis Rheum 2009;60:3091–7.

5 Izmirly PM, Kim MY, Llanos C *et al*. Evaluation of the risk of anti-SSA/Ro-SSB/La antibody-associated cardiac manifestations of neonatal lupus in fetuses of mothers with systemic lupus erythematosus exposed to hydroxychloroquine. Ann Rheum Dis 2010;69:1827–30.

6 Izmirly PM, Costedoat-Chalumeau N, Pisoni CN *et al*. Maternal use of hydroxychloroquine is associated with a reduced risk of recurrent anti-SSA/Ro-antibody–associated cardiac manifestations of neonatal lupus. Circulation 2012;126:76–82.

7 Jaeggi E, Laskin C, Hamilton R, Kingdom J, Silverman E. The importance of the level of maternal anti-Ro/SSA antibodies as a prognostic marker of the development of cardiac neonatal lupus erythematosus a prospective study of 186 antibody-exposed fetuses and infants. J Am Coll Cardiol 2010;55:2778–84.

8 Izmirly PM, Costedoat-Chalumeau N, Saxena A *et al*. First stage of a Simon's two-stage optimal approach supports placental transfer of hydroxychloroquine and a reduced recurrence rate of the cardiac manifestations of neonatal lupus. Arthritis Rheum 2013;65:S1212.

9 Clancy RM, Alvarez D, Komissarova E *et al*. Ro60-associated single-stranded RNA links inflammation with fetal cardiac fibrosis via ligation of TLRs: a novel pathway to autoimmune-associated heart block. J Immunol 2010;184:2148–55.

10 Llanos C, Friedman DM, Saxena A *et al*. Anatomical and pathological findings in hearts from fetuses and infants with cardiac manifestations of neonatal lupus. Rheumatology 2012;51:1086–92.

11 Sacre K, Criswell LA, McCune JM. Hydroxychloroquine is associated with impaired interferon-alpha and tumor necrosis factor-α production by plasmacytoid dendritic cells in systemic lupus erythematosus. Arthritis Res Ther 2012;14:R155.

12 Lee J, Chuang TH, Redecke V *et al*. Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of toll-like receptor 7. Proc Natl Acad Sci USA 2003;100:6646–51.

13 Guiducci C, Tripodo C, Gong M *et al*. Autoimmune skin inflammation is dependent on plasmacytoid dendritic cell activation by nucleic acids via TLR7 and TLR9. J Exp Med 2010;207:2931–42.

14 Penate Y, Guillermo N, Rodriguez J *et al*. Histopathologic characteristics of neonatal cutaneous lupus erythematosus: description of five cases and literature review. J Cutan Pathol 2009;36:660–7.

15 Kurien BT, Newland J, Paczkowski C, Moore KL, Scofield RH. Association of neutropenia in systemic lupus erythematosus (SLE) with anti-Ro and binding of an immunologically cross-reactive neutrophil membrane antigen. Clin Exp Immunol 2000;120:209–17.

16 van de Schoot R, Kaplan D, Denissen J *et al*. A gentle introduction to Bayesian analysis: applications to developmental research. Child Dev 2014;85:842–60.

17 Spiegelhalter D, Adams K, Myles J. Bayesian Approaches to Clinical Trials and Health-Care Evaluation. West Sussex, UK: John Wiley & Sons, 2004.

18 Hutson JR, Lubetsky A, Walfisch A *et al*. The transfer of 6-mercaptopurine in the dually perfused human placenta. Reprod Toxicol 2011;32:349–53.