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BIRTH OUTCOMES

Associations of Rhesus and non-Rhesus maternal red blood cell alloimmunization with stillbirth and preterm birth

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Abstract

Background: Although the risks of adverse pregnancy outcomes associated with anti-D antibodies are well-recognized, much less is known concerning alloimmunization with other red blood cell antibodies detected during routine maternal screening. To date, most reports of adverse pregnancy outcomes associated with non-anti-D antibodies have been from small case studies. The aim of this study was to examine the associations of maternal alloimmunization with specific red blood cell antibodies and the risks of preterm birth and stillbirth in the Swedish population.

Methods: All antibody screening, outcome and covariate data were obtained through linkages of Swedish national health and data registers. Follow-up in these population-based registers was available up to 31 December 2002. The final study sample consisted of 1 022 569 singleton births from 668 952 mothers during 1987–2002.

Results: In total, 1.3% of the 1 022 569 study pregnancies were alloimmunized. In adjusted logistic regression models, compared with having no antibodies, alloimmunization with anti-D, anti-E, anti-C and anti-c was associated with increased risk of both stillbirth and preterm birth. In addition, anti-Kell was associated with increased risk of preterm birth and anti-Lea with increased risk of stillbirth. Compared with firstborn children, risk of preterm birth associated with alloimmunization was greater in subsequent births

Conclusions: In the largest study to date, alloimmunization with Rhesus, K- and -Lea red blood cell antibodies increased the risk of preterm birth and/or stillbirth. The association of anti-Lea with stillbirth was an unexpected finding. Further study of the consequences of non-anti-D alloimmunization is warranted.

Key words: maternal screening, erythrocyte antibodies, alloimmunisation, Lewis system, Kell system

Key Messages

- Whereas maternal alloimmunization with anti-D antibody is recognized as a major contributor to fetal morbidity and mortality, the consequences of alloimmunization with other red blood cell antibodies are not well studied, especially with regard to stillbirth and preterm birth.
- This study found evidence that alloimmunization with anti-D, -E, -C, -c and -Lea antibodies was associated with increased risk of stillbirth.
- With regard to preterm birth, alloimmunization with anti-D, -E-, -C-, -c and -Kell antibodies was associated with increased risk in non-primiparous women. Alloimmunization with anti-D and Kell antibodies was also associated with increased risk in primiparous women.
- The findings, especially concerning anti-Lea, should be cautiously interpreted and warrant further investigation.

Introduction

Preterm birth and stillbirth are adverse pregnancy outcomes that present serious challenges to maternal care all over the world. Preterm birth, defined by the World Health Organization (WHO) as live birth before 37 completed weeks of gestation, is a leading cause of infant mortality and lifelong morbidity.[1] In 2010, 14.9 million preterm births were estimated to have occurred, accounting for 11.1% of all live births in the world.[2] Worldwide, an estimated 2.64 million stillbirths occurred in 2009, corresponding to a rate of 19 stillbirths per 1000 deliveries.[3]

Although preterm birth and stillbirth are distinct birth outcomes, proximate risk factors and mechanisms common to both have been identified.[4,5] In particular, immunological pathways are relevant for at least a portion of cases of preterm birth and stillbirth. For example, a meta-analysis found that increased pro-inflammatory cytokines in cervicovaginal fluid and amniotic fluid are associated with spontaneous preterm birth,[6] whereas placental inflammation is associated with increased risk of term stillbirth.[7] To date the role of immunological pathways in instigating adverse birth outcomes is not well understood, and risk factors other than inflammation may be implicated.

Screening for the presence of red blood cell (RBC) antibodies is a standard antenatal procedure, with the goal of detecting maternal RBC antibodies that can cross the placental barrier and attack fetal RBCs. In particular, maternal alloimmunization to anti-Rhesus-D (anti-D) antibody is recognized as a major contributor to fetal morbidity and mortality.[8] Whereas the focus of this screening to date has been on determining the presence of anti-D antibody, antibodies against more than 50 other RBC antigens have been implicated in haemolytic disease of the fetus/newborn.[9] However, the consequences of maternal RBC alloimmunization are not well known with regard to stillbirth and preterm birth. Case reports and small studies suggest that preterm birth may be caused by various maternal RBC antibodies, including anti-c,[10–12] -E[12] and -Kell.[13] Similarly, case reports suggest that stillbirth is associated with other maternal RBC antibodies in the MNS system[13–15] and Kell system.[16–18] However, because of the low prevalence of maternal RBC alloimmunization (affecting approximately 1% of all births, and considerably lower for most specific antibodies[19]), there is a lack of population-based studies concerning the contribution of specific RBC antibodies to risks of stillbirth and preterm birth.

In the present study, we examine a population-based cohort of more than one million pregnancies in Sweden from 1987 to 2002 to investigate the associations of maternal RBC antibodies with stillbirth and preterm birth.

Methods

Data sources

Various national computerized health and population data registers were used in the analysis, with electronic linkages conducted via the unique national registration number assigned to each person in Sweden.[20] The Medical Birth Register and Hospital Discharge Register were used to obtain detailed information on births and mothers' medical history, respectively. The Medical Birth Register contains records on approximately 99% of all births in Sweden from 1973[21] and includes information on maternal age, parity, multiple or singleton birth, stillbirth and infant diagnoses classified according to ICD (International Classification of Diseases) codes. The Hospital Discharge

Register, starting from 1964–65 and with nearly complete national coverage of the population from 1987[22] includes records on admission and discharge dates and hospital codes, with diagnoses coded using the ICD-9 and -10 systems and procedures coded using the Swedish Classification of Operations and Major Procedures.

Pregnant women in Sweden undergo blood screening at the first antenatal appointment for ABO/RhD type as well as for the presence and identification of RBC antibodies. These laboratory tests are conducted and recorded at the local blood banks. The Scandinavian Donations and Transfusions database contains all transfusion and blood screening data on virtually all persons registered at computerized local blood banks in Sweden and Denmark since 1966[20] and from these data we constructed a database of routine maternal screening records in Sweden.[19] The presence or absence of specific RBC antibodies detected during antenatal screening, as well as whether RhD prophylaxis was administered, was determined through a computerized search of free-text laboratory results. The accuracy and completeness of this automated data extraction approach has been validated.[19] Follow-up in the registers described above was available up to 31 December 2002 and, after linkage, all data were de-identified by Statistics Sweden and remain anonymous to us permanently. Ethical approval for this study was obtained from the Stockholm Regional Ethics committee (Diary numbers 2008/672-32 and 2012/1133-31/1).

Definition of study sample

From 1987 to 2002, there were 1 568 348 births with valid identifiers in the Medical Birth Register. Of these, a total of 1 124 193 births had any maternal antibody screening information available in the aforementioned database. We restricted the analysis to the 1 086 030 singleton births, because multiple births more often present pregnancy complications that result in higher incidence of stillbirth and preterm birth. After removal of births with missing antibody data, the study sample consisted of 1 022 569 singleton births from 668 952 women with complete information on maternal antibody screening and birth outcomes.

Variables

The exposures of interest were the specific maternal RBC antibodies detected during pregnancy. Indicator variables for the presence of specific antibodies were created based on any mention of those antibodies in maternal screening records. For anti-D antibody, we considered a woman to be alloimmunized only if the positive screen was not due to RhD prophylaxis, which latter information was also recorded in the screening records.

The adverse pregnancy outcomes of interest were stillbirth and preterm birth. In the Swedish Medical Birth Register, stillbirth (fetal death after 28 full weeks of gestation according to Swedish law) is recorded as an indicator variable. Preterm birth is recorded as ICD-9 codes 644 (early or threatened labour) or 765 (disorders relating to short gestation and low birthweight) and ICD-10 codes O601(preterm spontaneous labour with preterm delivery) or P073 (at least 28 completed weeks of gestation but less than 37).

We considered a number of maternal and pregnancy characteristics as potential covariates: maternal blood group, age, maternal body mass index (BMI) at first antenatal visit, maternal country of birth, maternal smoking status at first antenatal visit, parity, gestational hypertension during the indicated pregnancy, sex of the fetus and transfusion history prior to pregnancy. Mothers' ABO blood group and RhD status were classified into four (O, A, B and AB) and two (RhD-positive and RhD-negative) categories, respectively. Maternal age at delivery was classified into six categories (<20 years, 20–<25 years, 25–<30 years, 30–<35 years, 35–<40 years, and ≥40 years). Data on maternal height and weight were recorded at registration for maternity care from 1992 onwards. Maternal weights <30 kg or >140 kg or heights <120 cm or >200 cm were considered as errors and replaced by missing values. BMI was calculated and classified into five groups (<20, 20–<25, 25–<30, ≥30 and missing). Maternal country of birth was categorized into three groups (Sweden, other Nordic and non-Nordic countries). Smoking status was defined according to the number of cigarettes smoked per day (no smoking, 1–9 cigarettes/day and ≥10 cigarettes/day). Parity was classified into three levels (1, 2, ≥3). An indicator variable for diagnosis of gestational hypertensive disorders during pregnancy was created for ICD-9 codes 642 or ICD-10 codes O13, O14 or O15.[21] History of receiving blood transfusion prior to pregnancy was classified as either yes or no.

Statistical analysis

We analysed the associations of RBC antibodies with stillbirth and preterm birth using unadjusted and adjusted logistic regression models. For stillbirth, the study sample consisted of all 1 022 569 births. For preterm birth, the study sample was split into firstborn children and later-born children, since risk of preterm birth is parity specific.[22] After removal of the 3349 stillbirths, there were 406 500 firstborn children and 580 367 later-born children in the analyses of preterm birth.

In both stillbirth and preterm birth analyses, the estimates of association between antibodies and outcome were adjusted for covariates defined a priori from known associations with the outcomes (maternal BMI, smoking, gestational hypertensive disorders, sex of fetus), or of the covariate with both maternal RBC alloimmunization and the outcomes (country of origin, age, parity, birth year).

The maternal antibody screening database consists of information on over 60 RBC antibodies, many of which were rarely detected even in 21 years of nationwide screening. Therefore, we limited the present analysis to antibodies to the four most immunogenic RBC antigen systems (Rh, Lewis, MNS and Kell systems), along with specific antibodies in the Rh and Lewis systems which occurred at a sufficient frequency for meaningful analysis. For each antibody or group of antibodies, logistic regression models were fitted and adjusted in the same way, with births without the specific antibody or antibodies as the unexposed group. All data preparation and analysis was conducted using SAS version 9.3 (SAS Institute, Cary, NC, USA) and R version 2.15.2.

In sensitivity analyses, we examined whether transfusion history prior to pregnancy or gestational hypertensive disorders influenced observed results, by removing affected persons from the dataset and repeating analyses. In addition, we tested whether associations of a particular antibody with an outcome might be independent of other antibodies, by examining a logistic regression model that included in the same model an indicator for anti-D antibodies and four indicator variables for any non-anti-D antibody in the Rh system, Lewis system, MNS system and Kell system.

Results

Description of study sample

Selected characteristics of the study sample are described in Table 1. From 1987 to 2002, stillbirth occurred in 3349 of 1 022 569 (0.3%) singleton births, whereas preterm birth occurred in 36 952 of 1 019 220 (3.6%) singleton live births. Mothers who had stillbirths or preterm deliveries were more likely to be older, have higher BMI, smoke, be nulliparous, have a history of transfusion prior to pregnancy and be born in a non-Nordic country. In total, 13 524 (1.3%) of all births occurred in RBC-alloimmunized mothers, with anti-D the most detected (2797, 0.3%), followed by anti-E (2281, 0.2%) and anti-Lea (2255, 0.2%).

Associations of antibodies with stillbirth

Associations of RBC antibodies with stillbirth are described in Table 2. Overall, only 68 of 3349 stillbirths were alloimmunized with any antibody (2.0%), and only 18 cases were alloimmunized with anti-D. Crude logistic regression models indicated that maternal alloimmunization with anti-D, -E, -C, -c and -Lea antibodies was associated with increased odds of stillbirth. For example, the odds ratio (OR) and 95% confidence interval (CI) for anti-D was 1.98 (1.24-3.15), whereas ORs for the other specific antibodies ranged from 1.85 to 2.63. These estimates did not meaningfully change after adjustment for maternal BMI, age, smoking, parity, country of birth or birth year (last column, Table 2).

Associations of RBC antibodies with preterm birth

Associations of RBC antibodies with preterm birth, stratified by parity, are described in Table 3. In primiparous women, 144 of 15 568 preterm births (0.9%) were alloimmunized with any antibody; in non-primiparous women, 1499 of 15 330 preterm births (3.3%) were alloimmunized with any antibody. Crude and adjusted logistic regression models indicated that alloimmunization with anti-D and antibodies in the Kell system was associated with an increased odds of preterm birth in firstborn children, with adjusted ORs (95% CI) of 1.41 (1.00–1.98) and 1.81 (1.00–3.25), respectively. The crude and adjusted ORs were larger for subsequent births. Anti-D, -E, -C, -c and -Kell antibodies were associated with increased risk, with adjusted ORs ranging from 2.10 (1.53–2.87) for Kell antibodies to 3.41 (2.92–3.98) for anti-D antibodies.

Sensitivity analyses

We repeated our analyses after removing from the study sample all women with a transfusion history prior to pregnancy, or pregnancies with gestational hypertensive disorders. Neither of these analyses resulted in any meaningful change in the risk estimates (results not shown). We also examined whether there was evidence that antibodies were associated with the outcomes, independent of the other antibodies, from models with anti-D, non-anti-D Rh antibodies, Lewis antibodies, MNS antibodies and Kell antibodies. The adjusted ORs from these analyses were all attenuated (Table 4) but still indicated that: anti-D and non-anti-D antibodies in the Rh system were associated with increased odds of both stillbirth and preterm birth; antibodies in the Lewis system were associated with increased odds of stillbirth; and antibodies in the Kell system were associated with increased odds of preterm birth.

Discussion

In a Swedish national sample of over one million singleton births, we found that maternal alloimmunization with red blood cell antibodies was associated with increased odds of

Table 1. Characteristics of 1 022 569 singleton pregnancies, Sweden, 1987–2002

Characteristics	*N* (%) of stillborn (*n* = 3349)	*N* (%) of liveborn (*n* = 1 019 220)	*N* (%) of preterm (*n* = 36 952)	*N* (%) of non-preterm (*n* = 982 268)
Maternal BMI at first antenatal visit				
<20	171 (5.1)	77 334 (7.6)	3030 (8.2)	74 304 (7.6)
20–<25	998 (29.8)	380 172 (37.3)	12 462 (33.7)	367 710 (37.4)
25–<30	589 (17.6)	155 224 (15.2)	5340 (14.5)	149 884 (15.3)
≥30	331 (9.9)	58 527 (5.7)	2459 (6.7)	56 068 (5.7)
Missing	1260 (37.6)	347 974 (34.1)	13 661 (37.0)	334 302 (34.0)
Maternal age at delivery				
<20	60 (1.8)	22 660 (2.2)	1066 (2.9)	21 594 (2.2)
20–<25	532 (15.9)	182 124 (17.9)	7084 (19.2)	175 040 (17.8)
25–<30	1041 (31.1)	365 867 (35.9)	12 364 (33.5)	353 503 (36.0)
30–<35	994 (29.7)	301 731 (29.6)	10 216 (27.7)	291 515 (29.7)
35–<40	575 (17.2)	123 365 (12.1)	4998 (13.5)	118 367 (12.1)
≥40	147 (4.4)	23 473 (2.3)	1224 (3.3)	22 249 (2.3)
Maternal country of birth				
Sweden	2718 (81.2)	862 151 (84.6)	31 350 (84.8)	830 801 (84.6)
Other Nordic	127 (3.8)	36 112 (3.5)	1302 (3.5)	34 810 (3.5)
Non-Nordic	504 (15.0)	120 957 (11.9)	4300 (11.6)	116 657 (11.9)
Maternal smoking at first antenatal visit				
No smoking	2237 (66.8)	790 238 (77.5)	26 024 (70.4)	764 214 (77.8)
1-9 cigarettes/day	385 (11.5)	107 636 (10.6)	4619 (12.5)	103 017 (10.5)
≥10 cigarettes/day	316 (9.4)	59 235 (5.8)	3011 (8.2)	56 224 (5.7)
Missing	411 (12.3)	62 111 (6.1)	3298 (8.9)	58 813 (6.0)
Parity				
1	1602 (47.8)	427 051 (41.9)	19 372 (52.4)	407 679 (41.5)
2	961 (28.7)	367 586 (36.1)	10 117 (27.4)	357 469 (36.4)
≥3	785 (23.4)	224 583 (22.0)	7463 (20.2)	217 120 (22.1)
Missing	1 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
History of transfusion pre-pregnancy				
No	3225 (96.3)	996 393 (97.8)	35 682 (96.6)	960 711 (97.8)
Yes	124 (3.7)	22 827 (2.2)	1270 (3.4)	21 557 (2.2)
Sex of fetus				
Male	1714 (51.2)	523 339 (51.3)	19 901 (53.9)	503 438 (51.3)
Female	1631 (48.7)	495 881 (48.7)	17 054 (46.1)	478 830 (48.7)
Missing	4 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Maternal ABO group				
O	1252 (37.4)	383 930 (37.7)	13 376 (36.2)	370 554 (37.7)
A	1498 (44.7)	461 823 (45.3)	17 041 (46.1)	444 782 (45.3)
B	417 (12.5)	119 281 (11.7)	4444 (12.0)	114 837 (11.7)
AB	182 (5.4)	54 186 (5.3)	2091 (5.7)	52 095 (5.3)
Maternal Rh group				
Rh+	2815 (84.1)	859 900 (84.4)	31 110 (84.2)	828 790 (84.4)
Rh−	534 (15.9)	159 320 (15.6)	5842 (15.8)	153 478 (15.6)

stillbirth and preterm birth. These associations remained after potential confounding factors relevant to mother and fetus were taken into account. To our knowledge, this is the first population-based epidemiological study of associations of maternal RBC alloimmunization with stillbirth and preterm birth.

With the exception of the Lewis antibodies, the associations reported here are generally consistent with the results of the case studies in the literature. In the largest case study to date, including 629 alloimmunized women in central Sweden, 7 had a stillbirth and 3 additional mothers had a premature delivery,[23] although measures of association were not reported. Pregnancies with non-anti-D Rhesus antibodies were described in a case report of a woman alloimmunized with anti-c in her first and second pregnancies, both of which terminated in spontaneous

Table 2. Associations between stillbirth and maternal red blood cell alloimmunization with antibodies against specific antigens and antigen systems in 1 022 569 singleton births in Sweden, 1987–2002

Alloimmunization	*N* (%) of stillborn exposed	*N* (%) of live born exposed	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
Anti-D	18 (0.5)	2779 (0.3)	**1.98 (1.24–3.15)**	**1.81 (1.14–2.88)**
Non-anti-D antibodies in Rh system	20 (0.6)	3291 (0.3)	**1.85 (1.19–2.88)**	**1.82 (1.17–2.83)**
Anti-E	14 (0.4)	2267 (0.2)	**1.88 (1.11–3.19)**	**1.86 (1.10–3.15)**
Anti-C	8 (0.2)	932 (0.1)	**2.62 (1.30–5.25)**	**2.48 (1.23–4.98)**
Anti-c	6 (0.2)	694 (0.1)	**2.63 (1.18–5.89)**	**2.52 (1.13–5.65)**
Antibodies in Lewis system	17 (0.5)	2729 (0.3)	**1.90 (1.18–3.07)**	**1.85 (1.14–2.98)**
Anti-Lea	17 (0.5)	2238 (0.2)	**2.32 (1.44–3.74)**	**2.25 (1.39–3.63)**
Antibodies in MNS system	2 (0.1)	1306 (0.1)	0.47 (0.12–1.86)	0.48 (0.12–1.90)
Antibodies in Kell system	6 (0.2)	927 (0.1)	1.97 (0.88–4.40)	1.81 (0.81–4.06)

[a]Adjusted for maternal BMI, age, smoking, parity, maternal country of birth, and birth year. Bold face indicates significance.

Table 3. Associations between preterm birth and maternal red blood cell alloimmunization in firstborn and later-born singleton live births in Sweden, 1987–2002

Alloimmunization	*N* (%) of preterm exposed	*N* (%) of non-preterm exposed	Crude OR (95% CI)	Adjusted OR[a] (95% CI)
First born *N* = 406 500				
Anti-D	35 (0.2)	574 (0.1)	**1.52 (1.08, 2.13)**	**1.41 (1.00, 1.98)**
Non-anti-D antibodies in Rh system	23 (0.1)	513 (0.1)	1.11 (0.73, 1.69)	1.10 (0.72, 1.67)
Anti-E	18 (0.1)	386 (0.1)	1.16 (0.72, 1.86)	1.15 (0.71, 1.84)
Anti-C	7 (0.0)	169 (0.0)	1.03 (0.48, 2.19)	0.99 (0.46, 2.11)
Anti-c	2 (0.0)	62 (0.0)	–[b]	–[b]
Antibodies in Lewis system	31 (0.2)	751 (0.2)	1.03 (0.72, 1.47)	1.01 (0.71, 1.45)
Anti-Lea	23 (0.1)	619 (0.2)	0.92 (0.61, 1.40)	0.91 (0.60, 1.38)
Antibodies in MNS system	15 (0.1)	353 (0.1)	1.06 (0.63, 1.77)	1.04 (0.62, 1.75)
Antibodies in Kell system	12 (0.1)	158 (0.0)	**1.89 (1.05, 3.40)**	**1.81 (1.00, 3.25)**
Later-born *N* = 580 367				
Anti-D	181 (1.2)	1850 (0.3)	**3.60 (3.09, 4.20)**	**3.41 (2.92, 3.98)**
Non-anti-D antibodies in Rh system	192 (1.3)	2438 (0.4)	**2.90 (2.50, 3.36)**	**2.77 (2.38, 3.21)**
Anti-E	105 (0.7)	1666 (0.3)	**2.31 (1.89, 2.81)**	**2.21 (1.81, 2.70)**
Anti-C	80 (0.5)	631 (0.1)	**4.64 (3.68, 5.86)**	**4.46 (3.53, 5.64)**
Anti-c	42 (0.3)	555 (0.1)	**2.76 (2.02, 3.78)**	**2.59 (1.89, 3.55)**
Antibodies in Lewis system	60 (0.4)	1771 (0.3)	1.24 (0.96, 1.60)	1.14 (0.88, 1.47)
Anti-Lea	49 (0.3)	1478 (0.3)	1.23 (0.92, 1.63)	1.13 (0.85, 1.50)
Antibodies in MNS system	31 (0.2)	843 (0.2)	1.34 (0.94, 1.92)	1.37 (0.96, 1.97)
Antibodies in Kell system	42 (0.3)	670 (0.1)	**2.29 (1.68, 3.13)**	**2.10 (1.53, 2.87)**

[a]Adjusted for maternal BMI, age, smoking, maternal country of birth, and birth year.
[b]Not estimated due to small numbers (< 5) of exposed cases.
Bold face indicates significance.

abortion in the first trimester.[12] The same paper reported on a further four anti-c-alloimmunized pregnancies (one of which also had anti-E detected) which were delivered by Caesarean section between 29 and 35 weeks of gestation, due to fetal distress. Such severe cases of alloimmunization, resulting in Caesarean section or induced delivery, would account for only a few cases, and so cannot explain the increased risk observed in our study. However, our results suggest that antibodies in the MNS system were not associated with the risks of stillbirth and preterm birth, although cases of premature delivery and fetal death *in utero*[14,15,24] have been reported for mothers with some antibodies in the MNS system.

Of note, whereas alloimmunization with anti-D and -Kell antibodies was associated with increased risk of preterm birth in firstborn children, alloimmunization with

Table 4. Associations of stillbirth and preterm birth with maternal red blood cell alloimmunization in Sweden, 1987–2002

Alloimmunization	Stillbirth		Preterm	
	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)	Model 1[a] OR (95% CI)	Model 2[b] OR (95% CI)
Anti-D	**1.73 (1.06–2.81)**	1.61 (0.99–2.622)	**2.33 (2.01–2.70)**	**2.26 (1.97–2.60)**
Non-anti-D antibodies in Rh system	**1.59 (1.00–2.53)**	**1.60 (1.01–2.54)**	**1.78 (1.54–2.06)**	**1.80 (1.56–2.07)**
Antibodies in Lewis system	**1.87 (1.16–3.02)**	**1.83 (1.13–2.96)**	1.08 (0.87–1.33)	1.09 (0.90–1.32)
Antibodies in MNS system	0.45 (0.11–1.78)	0.46 (0.11–1.84)	1.12 (0.83–1.50)	1.20 (0.91–1.57)
Antibodies in Kell system	1.80 (0.80–4.03)	1.68 (0.75–3.77)	**1.77 (1.34–2.34)**	**1.78 (1.37–2.30)**

[a]Model 1 adjusted for every other antibody or antibody system shown.
[b]Model 2: adjusted for every other antibody shown and for maternal characteristics (BMI, age, smoking, parity, country of birth, and birth year).
Bold face indicates significance.

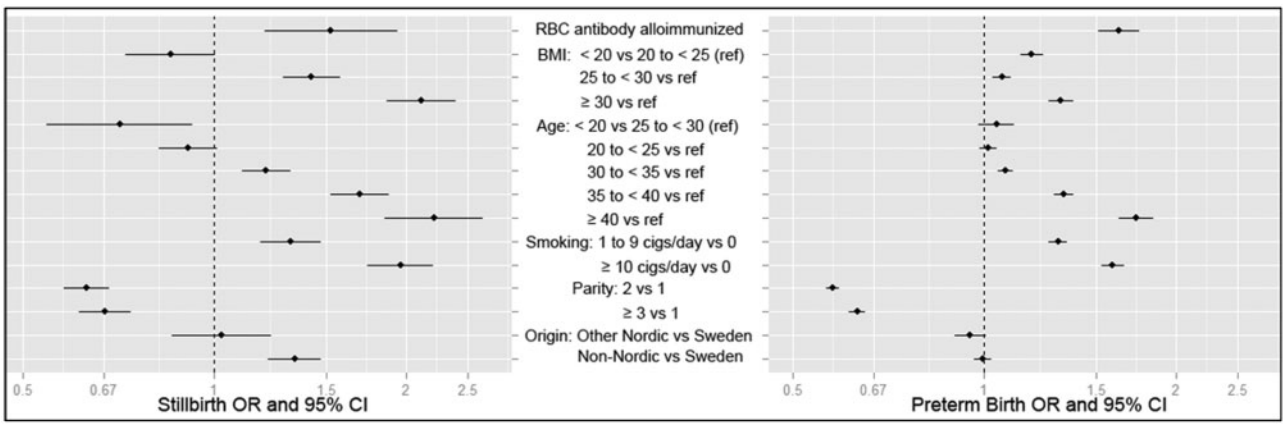

Figure 1. Odds ratios and 95% confidence intervals for associations of stillbirth and preterm birth with maternal red blood cell alloimmunization and other maternal risk factors. All estimates are derived from logistic regression models adjusted for all covariates shown, in addition to a categorized year of birth (not shown in figure).

these and other Rhesus antibodies posed greater risks in subsequent births. Two possibilities underlie this finding. First, it is understood that deliveries of non-primiparous women are more affected by alloimmunization since the immunization event is often the previous delivery. In contrast, whereas immunization can and does occur in the first pregnancy, a large proportion of immunizing events occur in the third trimester, resulting in a less severely affected child.[25] In addition, it is also possible that due to the low frequency of exposed cases of preterm birth in first pregnancies, our ability to detect an association was limited.

A major strength of our study is that it provides results based on a large population-based cohort with almost complete data on maternal antibody screening results for estimating the effects of maternal alloimmunization on pregnancy outcome. However, even with the large sample size, not all RBC antibodies in the database could be studied with respect to stillbirth and preterm birth, due to the small numbers of exposed pregnancies. An important limitation of the study is that, at the present time, further detail on titre information is not coded in the database. Although risk estimates were adjusted for factors such as maternal BMI, age, parity and smoking, we cannot exclude the possibility of some residual confounding of the associations between antibody status and pregnancy outcome. Our database also had no information on delivery onset and therefore we could not investigate whether antibody status had different effects on spontaneous and medically indicated preterm birth, respectively. In addition, we did not have information on neonatal care and could not explore whether certain antibodies were associated with specific neonatal morbidities, their treatment and the duration of care. Therefore our conclusions should be interpreted cautiously regarding the causality of the association with the outcomes studied. Another limitation is that of generalizability. The risk of RBC alloimmunization is known to depend on ethnic/genetic background, so that any associations (or lack thereof) found in the Swedish population may not be relevant to other populations. A striking example is the high prevalence of anti-Mi and almost zero

prevalence of anti-D in the Hong Kong Chinese population, consistent with the reported frequencies of the Mi and D antigens in Chinese populations.[26]

Several considerations are warranted in interpretation of the associations presented here. Anti-D antibody is one of the most well-known risk factors for adverse fetal outcomes, and routine use of RhD prophylaxis has effectively reduced much of the morbidity and mortality associated with anti-D alloimmunization.[27] In our study sample, anti-D antibody occurred at a frequency comparable to other antibodies (e.g. anti-Lea, anti-E) that are not as well studied and which do not have prophylactic treatments. However, the relative risk of stillbirth and preterm birth associated with these less well-known antibodies was of similar magnitude to that found for anti-D. Moreover, the relative risks associated with the antibodies are of similar magnitude to those of recognized risk factors such as advanced maternal age and maternal smoking (Figure 1) which have received significantly more attention in the medical literature. Thus, the associations we report, especially those for non-anti-D alloimmunization, deserve further investigation.

The associations reported here do not necessarily provide any insight regarding potential mechanisms linking RBC alloimmunization with stillbirth and preterm birth, although presumably haemolytic disease and fetal anaemia are involved. Fetal blood sampling and fetal transfusion could lead to preterm birth[29] or fetal death,[28] but these procedures are likely to be implemented only in a small number of unusually severe cases. In addition, it must be noted that induction of labor or a Caesarean section may be considered upon impression of possible fetal haemolytic disease. However, this is not routinely performed before 37 completed weeks of gestation in the Swedish population. Anti-D and anti-c have frequently been reported to be associated with severe haemolytic disease and anti-K with early suppression of erythropoiesis,[29] whereas -C, -E, and -MNS antibodies have been infrequently associated with severe haemolytic disease.[30] The findings concerning Lewis antibodies are surprising, as they are not associated with haemolytic disease since they are almost exclusively IgM antibodies and thus do not cross the placental barrier.[31] In addition, Lewis antigens are not expressed on fetal RBCs. Therefore, the biological plausibility for an association between Lewis antibodies and stillbirth requires identification of an immunological mechanism other than attack of fetal RBCs.

In conclusion, specific RBC antibodies in alloimmunized mothers are associated with increased risks of stillbirth and prematurity. The current screening programmes for many RBC antibodies lack valid population-based evidence and are generally reliant on small case studies. Our findings provide population-based evidence that alloimmuninzation may increase the risks of stillbirth and preterm birth. These findings should be further investigated and, if validated, should be considered in optimizing the screening and monitoring of pregnant women.

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References

1. WHO. *Preterm Birth*. 2012. Fact sheet No. 363: http://www.who.int/mediacentre/factsheets/fs363/en/ (2 April 2014, date last accessed).
2. Blencowe H, Cousens S, Oestergaard MZ *et al*. National, regional and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. *Lancet* 2012;**379**:2162–72.
3. Cousens S, Blencowe H, Stanton C *et al*. National, regional, and worldwide estimates of stillbirth rates in 2009 with trends since 1995: a systematic analysis. *Lancet* 2011;**377**:1319–30.
4. Lawn JE, Yakoob MY, Haws RA, Soomro T, Darmstadt GL, Bhutta ZA. 3.2 million stillbirths: epidemiology and overview of the evidence review. *BMC pregnancy and childbirth* 2009;**9(Suppl 1)**:S2.
5. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet* 2008;**371**:75–84.
6. Wei SQ, Fraser W, Luo ZC. Inflammatory cytokines and spontaneous preterm birth in asymptomatic women: a systematic review. *Obstet Gynecol* 2010;**116**:393–401.
7. Hulthen Varli I, Petersson K, Kublickas M, Papadogiannakis N. Both acute and chronic placental inflammation are overrepresented in term stillbirths: a case-control study. *Infect Dis Obstet Gynecol* 2012;**2012**:293867.
8. Urbaniak SJ, Greiss MA. RhD haemolytic disease of the fetus and the newborn. *Blood Rev* 2000;**14**:44–61.
9. Moise KJ. Fetal anemia due to non-Rhesus-D red-cell alloimmunization. *Semin Fetal Neonatal Med* 2008;**13**:207–14.
10. Singla S, Kumar S, Roy KK, Sharma JB, Kachhawa G. Severe hydrops in the infant of a Rhesus D-positive mother due to anti-c antibodies diagnosed antenatally: a case report. *J Med Case Rep* 2010;**4**:57.
11. Murki S, Kandraju H, Devi SA. Hemolytic disease of the newborn- anti c antibody induced hemolysis. *Ind J Pediatr* 2012;**79**:265–66.
12. Babinszki A, Berkowitz RL. Haemolytic disease of the newborn caused by anti-c, anti-E and anti-Fya antibodies: report of five cases. *Prenat Diagn* 1999;**19**:533–36.
13. Chandrasekar A, Morris KG, Tubman TR, Tharma S, McClelland WM. The clinical outcome of non-RhD antibody affected pregnancies in Northern Ireland. *UlsterMed J* 2001;**70**:89–94.
14. Kanra T, Yuce K, Ozcebe IU. Hydrops fetalis and intrauterine deaths due to anti-M. *Acta Obstet Gynecol Scand* 1996;**75**:415–17.

15. Wikman A, Edner A, Gryfelt G, Jonsson B, Henter JI. Fetal hemolytic anemia and intrauterine death caused by anti-M immunization. *Transfusion* 2007;**47**:911–17.
16. Berkowitz RL, Beyth Y, Sadovsky E. Death in utero due to Kell sensitization without excessive elevation of the delta OD450 value in amniotic fluid. *Obstet Gynecol* 1982;**60**:746–49.
17. McKenna DS, Nagaraja HN, O'Shaughnessy R. Management of pregnancies complicated by anti-Kell isoimmunization. *Obstet Gynecol* 1999;**93**:667–73.
18. Bowman JM, Pollock JM, Manning FA, Harman CR, Menticoglou S. Maternal Kell blood group alloimmunization. *Obstet Gynecol* 1992;**7**:239–44.
19. Lee BK, Ploner A, Zhang Z, Gryfelt G, Wikman A, Reilly M. Constructing a population-based research database from routine maternal screening records: a resource for studying alloimmunization in pregnant women. *PLoS One* 2011;**6**:e27619.
20. Edgren G, Hjalgrim H, Tran TN *et al*. A population-based binational register for monitoring long-term outcome and possible disease concordance among blood donors and recipients. *Vox Sang* 2006;316–23.
21. Lee BK, Zhang Z, Wikman A, Lindqvist PG, Reilly M. ABO and RhD blood groups and gestational hypertensive disorders: a population-based cohort study. *BJOG* 2012;**119**:1232–37.
22. Laughon SK, Albert PS, Leishear K, Mendola P. The NICHD Consecutive Pregnancies Study: recurrent preterm delivery by subtype. *Am J Obstet Gynecol* 2014;**210**:131.
23. Filbey D, Hanson U, Wesstrom G. The prevalence of red cell antibodies in pregnancy correlated to the outcome of the newborn: a 12 year study in central Sweden. *Acta Obstet Gynecol Scand* 1995;**74**:687–92.
24. Buerki U, Degnan TJ, Rosenfield RE. Stillbirth due to anti-U. *Vox Sang* 1964;**9**:209–11.
25. Tiblad E, Westgren M, Pasupathy D, Karlsson A, Wikman AT. Consequences of being Rhesus D immunized during pregnancy and how to optimize new prevention strategies. *Acta Obstet Gynecol Scand* 2013;**92**:1079–85.
26. Wong KF, Tse KT, Lee AW, Mak CS, So CC. Is antenatal antibody screening worthwhile in Chinese? *Br J Haematol* 1997;**97**: 917–19.
27. Moncharmont P, Juron Dupraz F, Vignal M, Rigal D, Meyer F, Debeaux P. Haemolytic disease of the newborn infant. Long term efficiency of the screening and the prevention of alloimmunization in the mother: thirty years of experience. *Arch Gynecol Obstet* 1991;**248**:175–80.
28. Howard H, Martlew V, McFadyen I *et al*. Consequences for fetus and neonate of maternal red cell allo-immunisation. *Arch Dis Child Fetal Neonatal Ed* 1998;**78**:F62–66.
29. Vaughan JI, Manning M, Warwick RM, Letsky EA, Murray NA, Roberts IA. Inhibition of erythroid progenitor cells by anti-Kell antibodies in fetal alloimmune anemia. *Engl J Med* 1998;**338**:798–803.
30. Moise KJ Jr. Non-anti-D antibodies in red-cell alloimmunization. *Eur J Obstet Gynecol Reprod Biol* 2000;**92**:75–81.
31. Barss VA, Moise KJ Jr. Significance of minor red blood cell antibodies during pregnancy. In: Basow DS (ed). *UpToDate*. Waltham, MA: UpToDate, 2009.