OBSTETRICS

Hemolytic disease of the fetus and newborn due to multiple maternal antibodies

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OBJECTIVE: The objective of the study was to determine whether women with combinations of red blood cell antibodies are more likely to develop significant hemolytic disease of the fetus and newborn than those with single antibodies.

STUDY DESIGN: A retrospective exposure cohort study was conducted of pregnant women with red blood cell antibodies. The development of significant hemolytic disease of the fetus and newborn was then compared between patients with single antibodies and those with multiple antibodies. Data analysis was limited to pregnancies delivering since the year 2000.

RESULTS: Thirteen percent of the patients referred to our program had multiple red blood cell antibodies. Odds of developing significant hemolytic disease of the fetus and newborn for patients with anti-Rh(D) combined with at least 1 additional red blood cell antibody were 3.65 times the odds for women with anti-Rh(D) antibodies in isolation (95% confidence interval, 1.84—7.33). In the setting of multiple antibodies including anti-Rh(D), Rh-positive fetuses/neonates have an increased odds of developing significant hemolytic disease even if the fetus is negative for the other corresponding red blood cell antigen.

CONCLUSION: Women with multiple red blood cell antibodies are more likely to develop significant hemolytic disease of the fetus and newborn than those with a single antibody especially in the presence of anti-(Rh) D. This pathophysiology may suggest a more aggressive immune response in women who develop more than 1 red blood cell antibody.

Key words: alloimmunization, hemolytic disease of the fetus and newborn, red blood cell antibodies

Cite this article as: Markham KB, Rossi KQ, Nagaraja HN, et al. Hemolytic disease of the fetus and newborn due to multiple maternal antibodies. Am J Obstet Gynecol 2015;213:68.e1-5.

Maternal red blood cell alloimmunization is an important cause of morbidity and mortality in the antepartum and neonatal periods. In the United States, 35 per 10,000 live births are at risk for hemolytic disease of the fetus and newborn (HDFN) because of red blood cell alloimmunization, 20% of which may become severely affected.[1,2] Of those who are severely affected by anti-D, approximately half are sufficiently mature to be delivered and receive neonatal care, whereas the other half require antenatal intervention for survival.[1]

With the introduction of Rh immune globulin, the incidence of Rh(D) alloimmunization has decreased, leading to a relatively increased proportion of red blood cell alloimmunization because of other antibodies.[1] A significant number of these red blood cell antibodies have well-recognized associations with HDFN, including anti-K, anti-c, anti-E, anti-Fya, and anti-Jka.[1-7]

Further complicating matters, a significant number of patients produce more than 1 red blood cell antibody during pregnancy. Filbey et al[8] demonstrated that 8.2% of pregnancies complicated by HDFN in Sweden had multiple red blood cell antibodies. It has been proposed that such pregnancies affected by multiple red blood cell antibodies are at greater risk for HDFN. Such an effect was seen by Spong et al[9] in 2001, who found an increased need for intrauterine fetal transfusions (IUTs) in pregnancies affected by anti-D in combination with other red blood cell antibodies.

The objective of our study was to further evaluate the effect of multiple red blood cell antibodies on the development of HDFN. To do so, we have compared the fetal and neonatal outcomes of pregnancies with multiple maternal red blood cell antibodies with those with single antibodies. We chose to limit our data analysis to pregnancies delivered since the year 2000 to reflect modern techniques for monitoring women with elevated red blood cell titers via middle cerebral artery Doppler assessment.[10]

Materials and Methods

The Ohio State University Maternal Alloimmunization Program has maintained a computerized database of pregnancies complicated by alloimmunization since 1959. This database includes patients from our institution as

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Received Oct. 28, 2014; revised Dec. 19, 2014; accepted Jan. 29, 2015.

The authors report no conflict of interest.

Presented as 2 separate posters ([1] Hemolytic disease due to antibody combination including anti-D and [2] Hemolytic disease due to antibody combinations including anti-c or anti-K) at the 30th annual meeting of the Society for Maternal-Fetal Medicine, Chicago, IL, Feb. 1-6, 2010.

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0002-9378/$36.00

http://dx.doi.org/10.1016/j.ajog.2015.01.049

TABLE 1
Demographic data

Variable	Anti-Rh(D) alone (n = 138) (13.6%)	Anti-Rh(D) in combination (n = 54) (5.3%)	Single other antibody (n = 744) (73.4%)	Other antibody combinations (n = 78) (7.7%)	*P* value
Maternal age, y[a]	Mean (SD) 28.7 (5.0) Median (range) 28 (17–39) Unknown: 15	Mean (SD) 29.0 (5.4) Median (range) 29 (20–44) Unknown: 6	Mean 28.7 (6.1) Median (range) 28 (15–49) Unknown: 100	Mean 28.7 (5.1) Median (range) 28 (20–40) Unknown: 6	.9836[a]
Maternal race					
White	47 (34.1)	30 (55.6)	121 (16.3)	20 (25.6)	< .0001
Black	16 (11.6)	2 (3.7)	53 (7.1)	8 (10.3)	
Other	8 (5.8)	1 (1.85)	25 (3.4)	4 (5.1)	
Unknown	67 (48.6)	21 (38.9)	545 (73.3)	46 (59.0)	
Parity (>20 wks)					
0	26 (18.8)	12 (22.2)	241 (32.4)	16 (20.5)	.0054
1	50 (36.2)	25 (46.3)	217 (29.2)	26 (33.3)	
2	36 (26.1)	12 (22.2)	150 (20.2)	22 (28.2)	
>3	26 (18.8)	5 (9.3)	136 (13.3)	14 (18.0)	
Fetal sex					
Female	37 (26.8)	18 (33.3)	110 (14.8)	16 (20.5)	< .0001
Male	36 (26.1)	12 (22.2)	139 (18.7)	13 (16.7)	
Unknown	65 (47.1)	24 (44.4)	495 (66.5)	49 (62.8)	

[a] *P* value is from 1-way analysis of variance (ages normally distributed); rest of *P* values are based on the Pearson χ^2 test.

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well as referrals from central and southeastern Ohio and neighboring regions and was used to obtain patient data for this study.

All laboratory testing was performed at The Ohio State University Medical Center using guidelines established by the American Association of Blood Banks.[11] These guidelines were updated frequently to remain consistent with medical care over the years. Techniques were routinely used to rule out anti-G as a mimicker of anti-D in combination with anti-C. Permission to retain and review patients' data was obtained from our local institutional review board before proceeding with this study.

This was a retrospective exposure cohort study including women managed in our alloimmunization program from January 2000 through May 2013. Data collected included, but were not limited to, maternal pregnancy history, paternal antigen testing, maternal indirect antiglobulin tests (antibody identification), fetal direct antiglobulin test, fetal antigen type, and fetal hematocrit. Fetal antigen information was obtained prior to the performance of the first IUT of the pregnancy. Neonatal data included gestational age at delivery, delivery hemoglobin, cord blood direct antiglobulin test results and red blood cell antigen status, and necessary treatment for HDFN. Not all data were available for each case.

We identified all women with relevant red blood cell antibodies (those known to place patients at risk for HDFN), analyzing only the first documented pregnancy in our system for each individual patient. Patients were categorized into the following 4 groups according to their antibodies: (1) anti-Rh(D) only, (2) anti-Rh(D) in combination with another red blood cell antibody, (3) other relevant red blood cell antibodies in isolation, and (4) other relevant red blood cell antibody combinations without anti-Rh(D).

For the purpose of this study, clinically significant HDFN was defined as the following: (1) fetal demise, (2) the development of hydrops fetalis, (3) a requirement for intrauterine transfusion, (4) a birth or umbilical cord blood hemoglobin of 10 g/dL or less, or (5) the need for neonatal blood transfusions.

Patient demographics and clinical characteristics are described and compared between the 4 groups. Comparisons of the categorical data were made using the likelihood ratio χ^2 test, and trends were assessed by the Cochran-Armitage trend test. Maternal mean ages were compared using a 1-way analysis of variance. Logistic regression models with multiple predictors were constructed in a step-wise process starting with only the individually

significant predictors. Only the significant predictors were retained in the final multiple predictor model. Confidence intervals for odds ratios were estimated using this logistic regression model, and 95% confidence intervals are reported. All *P* values are 2 sided and considered significant if the value is $P = .05$. All analyses were performed in SAS JMP, version 10 (SAS Institute, Cary, NC).

Results

A total of 1014 patients with pregnancies complicated by red blood cell alloimmunization were managed at The Ohio State University Wexner Medical Center between January of 2000 and May 2013, a period of more than 13 years. Of these, 132 (13.0%) had more than 1 red blood cell antibody. Demographic data comparing these patients are depicted in Table 1, whereas Table 2 depicts the HDFN-defining categories for each group.

Anti-Rh(D) was the most commonly encountered red blood cell antibody, with 138 cases affected by anti-Rh(D) alone. Of these, 30 pregnancies were complicated by significant HDFN (21.7%). As expected, the odds of developing HDFN for women with anti-Rh(D) alone were 12.40 times the odds for women with other single antibodies (95% confidence interval [CI], 6.52–24.51). Such other isolated antibodies included anti-E (203 cases), anti-K (97 cases), anti-c (52 cases), anti-Jka (44 cases), anti-Fya (23 cases), and anti-C (19 cases).

As noted, 132 women in our population had multiple red blood cell antibodies. Of these, 54 had a combination of antibodies that included anti-Rh(D). The most common combinations were anti-Rh(D) plus anti-C (33 cases) and anti-Rh(D) plus anti-E (7 cases). Of the combinations including anti-Rh(D), 27 (50.0%) developed significant HDFN, resulting in an OR of 3.65 (95% CI, 1.84–7.33) in comparison with patients with anti-Rh(D) alone (Table 3). Significant HDFN was more common among women with antibody combinations including anti-Rh(D) than those with combinations that did not include this antibody (OR, 5.24; 95% CI, 1.95–18.27).

The most common antibody combinations without anti-Rh(D) included anti-c plus anti-E (8 cases), anti-K pkus] anti-Fya (3 cases), anti-K plus anti-E (5 cases), and anti-E plus anti-Jka (4 cases). In all, only 20 pregnancies had fetal/neonatal red blood cell antigen typing confirming the presence of 2 or more antigens corresponding to the maternal antibodies, 10 of whom developed significant HDFN (50%).

Of the patients with anti-Rh(D) only, 81 fetuses/newborns were proven to be Rh positive, 24 of whom developed significant HDFN (29.63%). In the setting of anti-Rh(D) in combination with other red blood cell antibodies, 27 fetuses/neonates were Rh positive, 19 of whom developed significant HDFN (70.37%). The addition of at least 1 additional antibody was therefore associated with a 5.64-fold increased odds of HDFN (95% CI, 2.24–15.36; $P = .0002$). In 4 of these antibody combination cases, the fetus/neonate was proven to be Rh positive but was negative for the other corresponding antigen, with significant HDFN occurring in 3 patients (75%).

The small number of such cases prevented statistical analysis, so we evaluated this further by including all patients managed in our program since it was established in 1959. Using this larger dataset, 13 antibody combination cases were found to have a fetus/neonate that was Rh positive but was negative for the other corresponding antigen, with significant HDFN occurring in 9 patients (69.2%). Even in the absence of the corresponding fetal/neonatal antigen, the odds of developing significant HDFN in the setting of an additional antibody were 4.99 times the odds of disease in pregnancies with anti-(Rh)D alone (95% CI, 1.60–18.68; $P = .0037$).

Comment

The presence of multiple red blood cell antibodies is associated with an increased odds for the development of significant HDFN. Similar results were reported by Spong et al, [9] who studied a cohort of 24 pregnancies with

TABLE 2
HDFN-defining categories for pregnancies complicated by single and multiple red blood cell antibodies

Category	Other antibody, n, %	Other antibody combination, n, %	Anti-Rh(D) alone, n, %	Anti-Rh(D) in combination, n, %	*P* value[a]
Pregnancies, n	744	78	138	54	
No HDFN	729 (98.0)	74 (94.9)	108 (78.3)	27 (50.0)	< .0001
HDFN total[b]	15 (2.0)	4 (5.1)	30 (21.7)	27 (50.0)	< .0001
Fetal demise	1 (0.1)	0 (0)	0 (0)	1 (1.9)	.2924
Hydrops fetalis	1 (0.1)	0 (0)	3 (2.2)	6 (11.1)	< .0001
IUT required	7 (0.9)	1 (1.3)	11 (8.0)	16 (29.6)	< .0001
Neonatal anemia	3 (0.4)	0 (0)	4 (2.9)	6 (11.1)	< .0001
Neonatal transfusion	10 (1.3)	4 (5.1)	24 (17.4)	19 (35.2)	< .0001

HDFN, hemolytic disease of the fetus and newborn; *IUT*, intrauterine fetal transfusion.

[a] *P* values comparing groups and trends: calculations are based on the likelihood ratio test (for equality of proportions) and on Cochran-Armitage trend test and were both $P < .0001$ in all cases; [b] Fetuses may fall into more than 1 HDFN-defining category.

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TABLE 3
Comparison of the occurrence of HDFN in the setting of single and multiple red blood cell antibodies

Variable	OR for HDFN (95% CI)[a]	*P* value
Anti-Rh(D) alone compared with other single antibodies	12.40 (6.52–24.51)	< .0001
Anti-Rh(D) in combination compared with anti-Rh(D) alone	3.65 (1.84–7.33)	.0002
Anti-Rh(D) in combination compared with other single antibodies	45.24 (21.71–98.13)	< .0001
Anti-Rh(D) alone compared with other antibody combinations	5.24 (1.95–18.27)	.0005
Anti-Rh(D) in combination compared with other antibody combinations	19.12 (6.64–70.05)	< .0001
Other antibodies in combination compared with other single antibodies	2.37 (0.66–6.76)	.1691

CI, confidence interval; *HDFN*, hemolytic disease of the fetus and newborn; *OR*, odds ratio.

[a] After controlling for parity effect.

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multiple red blood cell antibodies, finding that 50% required intrauterine transfusion compared with a baseline of 25% of pregnancies with Rh(D) alloimmunization alone who required IUT during the same time period. In our population, 21.7% of pregnancies with isolated anti-Rh(D) antibodies developed significant HDFN compared with 50% of patients with anti-(Rh)D antibodies in combination with 1 or more additional red blood cell antibody (OR, 3.65; 95% CI, 1.84–7.33).

The etiology for this increase in occurrence of clinically significant HDFN in the presence of multiple red blood cell antibodies is unknown, but we propose the following 2 possible theories: (1) a cumulative effect involving increased hemolysis secondary to binding of the multiple antibodies to more fetal red blood cells and (2) a more aggressive immune response in women who are prone to developing multiple antibodies.

It is possible that both of these proposed mechanisms are involved in this worsening disease process. The increased odds of significant HDFN in the setting of multiple antibodies but only 1 corresponding fetal/neonatal antigen suggests, though, that the enhanced immune response may play a more important role in this pathophysiology.

The main strength of this study is the overall large number of patients included in our database. We chose to limit our analysis to pregnancies delivered since the year 2000, approximately corresponding with the introduction of middle cerebral artery Dopplers as a monitoring tool for HDFN in women with high red blood cell antibodies. By doing so, we also eliminated confounders related to changes in referral practices to our alloimmunization program and technological advances in the laboratory detection of antibodies. Temporally limiting our analysis, though, potentially reduced our ability to identify statistically significant findings and prevented us from having sufficient patient numbers to permit the evaluation of specific antibody combinations.

It should be noted that our institution is a tertiary care center, and the patient population may therefore be at an increased risk of having severe disease or confounding complications.

Another limitation of our study is the incomplete demographic data. In particular, we were unable to include antibody titers in our analysis. Furthermore, close examination of demographics revealed that 18.8–32.4% of women in our database were nulliparous, suggesting sensitization at the time of spontaneous miscarriage or elective termination of pregnancy and perhaps indicating missed opportunities for administration of Rh immune globulin. Of note, we opted not to include kernicterus, hyperbilirubinemia, and/or the need for phototherapy in our definition of HDFN. These data would be difficult to obtain and, more importantly, elevated bilirubin levels are not specific to a diagnosis of HDFN because they can occur related to prematurity, ABO incompatibility, sepsis, breast-feeding, and numerous other causes.

Based on our experience, HDFN is more likely to occur in the presence of multiple red blood cell antibodies, especially in the presence of anti-(Rh)D. Heightened awareness of the increased potential for significant HDFN in the presence of multiple red blood cell antibodies may prove to be helpful to the clinician, permitting anticipation and more aggressive antenatal management of these patients. ■

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