A retrospective study of positive antibody screens at delivery in Rh-negative parturients

Une étude rétrospective portant sur les dépistages d'anticorps positifs lors de l'accouchement de parturientes Rh négatives

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Abstract

Purpose Rhesus- (Rh-) negative women receiving anti-D antibodies antenatally often have a positive antibody screen at delivery. We investigated the incidence of positive antibody screens at delivery in this population and examined how the presence of positive antibody screens affected the time required to obtain type and screen or type and crossmatch results.

Methods Records of parturients who had type and screen or type and crossmatch done upon presentation for delivery from June to October 2007 were examined to determine estimated gestational age at admission, Rh-status, the presence of positive antibody screens, and the time interval from receipt of specimen in the blood bank to the availability of antibody screen results.

Results Of the 480 specimens sent for type and screen or type and crossmatch, 20% of parturients were Rh-negative, with 57% of those demonstrating a positive antibody screen compared with 4% of the Rh-positive parturients ($P < 0.01$). In the Rh-negative group, 100% (95% CI 98–102) of positive antibody screens were anti-D antibodies. There was a longer median laboratory time for Rh-negative vs Rh-positive parturients (146 vs 65 min), for antibody positive vs antibody negative parturients (243 vs 65 min) ($P < 0.001$ for both), but not for Rh-positive/antibody positive vs Rh-negative/antibody positive patients (312 vs

218 min) ($P = 0.09$). The antibody screen was positive in 100% of Rh-negative parturients until 37 weeks gestation, after which there was a decline.

Conclusions Rh-negative parturients who receive anti-D antibodies antenatally have a higher incidence of positive antibody screens at delivery than Rh-positive parturients due to the presence of anti-D antibodies.

Résumé

Objectif Les femmes de facteur Rhésus (Rh) négatif recevant des anticorps anti-D avant l'accouchement ont souvent un résultat positif au dépistage d'anticorps à l'accouchement. Nous avons exploré l'incidence de dépistages d'anticorps positifs à l'accouchement dans cette population et examiné la manière dont la présence de dépistages positifs d'anticorps a affecté le temps nécessaire avant d'obtenir les résultats de typage et de dépistage ou les résultats de typage et de compatibilité croisée.

Méthode Les dossiers de parturientes pour lesquelles des tests de typage et de dépistage ou de typage et de compatibilité croisée ont été réalisés lors de leur arrivée à l'hôpital pour un accouchement entre juin et octobre 2007 ont été examinés afin de déterminer l'âge gestationnel estimé à l'admission, le statut rhésus, la présence de dépistages positifs d'anticorps et l'intervalle entre la réception du spécimen à la banque de sang et la disponibilité des résultats de dépistage des anticorps.

Résultats Parmi les 480 spécimens envoyés pour un test de typage et de dépistage ou de typage et de compatibilité croisée, 20 % des parturientes étaient Rh négatives, dont 57 % ont démontré un dépistage positif d'anticorps comparativement à 4 % des parturientes Rh positives ($P < 0,01$). Dans le groupe Rh négatif, 100 % (IC 95 %, 98–102) des dépistages positifs d'anticorps visaient des

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anticorps anti-D. Il a fallu un temps médian plus long au laboratoire pour tester les parturientes Rh négatives que pour les parturientes Rh positives (146 vs 65 min); pour les parturientes positives aux anticorps que pour celles négatives aux anticorps (243 vs 65 min) ($P < 0,001$ pour les deux); mais pas pour les patientes Rh positives / positives aux anticorps que pour celles Rh négatives / positives aux anticorps (312 vs 218 min) ($P = 0,09$). Le dépistage des anticorps était positif chez 100 % des parturientes Rh négatives jusqu'à 37 semaines de gestation, et un déclin a été observé après cette date.

Conclusion *Les parturientes Rh négatives qui reçoivent des anticorps anti-D avant l'accouchement manifestent une incidence plus élevée de dépistages positifs d'anticorps à l'accouchement que les parturientes Rh positives en raison de la présence d'anticorps anti-D.*

The occurrence of rhesus- (Rh-) isoimmunization during pregnancy has become a rare clinical event in obstetrics, with the latest United States National Center for Health Statistics data from 2003 reporting 6.8 cases of Rh-isoimmunization per 1,000 live births.¹ One of the primary factors responsible for this low rate is the widespread administration of antenatal anti-D antibodies during the 28 to 34 weeks estimated gestational age.^{A,2,3} Administration of anti-D antibodies during this time period has been shown to reduce the rate of Rh-isoimmunization to 0.1–0.2%.^{4,5} If there is no evidence of anti-D alloimmunization in the Rh-negative woman, current American College of Obstetrics and Gynecology guidelines suggest that she should receive 300 µg of anti-D antibodies at 28 weeks of gestation.^A

Based on these recommendations, virtually all Rh-negative women in the United States receive anti-D antibodies during pregnancy at approximately 28 to 34 weeks gestation.^A Later, when these women undergo a routine “type and screen” or “type and crossmatch”, a “positive antibody screen” is often reported.⁶ The time course of this phenomenon is not well understood, either in terms of the duration of the effect or its prevalence when they present at or near term for delivery. In most cases, the positive antibody represents the previously administered anti-D antibodies, and this is of little clinical significance, even in the rare circumstances where blood products are required, provided that there has been an appropriate crossmatch. However, the time required for detection and identification of these antibodies may postpone the availability of blood

products for transfusion or, in an emergency, necessitate transfusion of uncrossmatched O-negative blood.

Therefore, we investigated the incidence of positive antibody screens at delivery in Rh-negative parturients who have been treated antenatally with anti-D antibodies, and we examined the impact of positive antibody screens on the timely availability of type and screen or type and crossmatch results compared with those patients who did not receive antenatal anti-D treatment. We hypothesized that maternal antibodies would be detected at a higher rate in parturients who received anti-D antibodies antenatally than in those who did not, and that the presence of the positive antibody screen in those patients would lead to delays in obtaining antibody screen results.

Methods

This study was approved by the Institutional Review Board of the Office for the Protection of Research Subjects of Northwestern University. We identified patients in an institutional quality assurance transfusion database who had a type and screen or type and crossmatch performed upon presentation to the labour and delivery unit for delivery during June 1, 2007 to September 30, 2007. At our institution, the decision to screen or crossmatch blood is based on the results of a prior institutional retrospective analysis: those patients who have an estimated risk of transfusion of 5–10% based on pre-existing obstetric or medical diagnoses undergo a type and screen, and those patients with an estimated risk > 10% undergo a type and crossmatch. Examples of conditions that require a type and screen or type and crossmatch at our institution include vaginal trial of labour, placenta previa, placental abruption, chorioamnionitis, placenta accreta, or history of positive antibody screen. Overall, we have found that use of this algorithm results in approximately 9.6% of patients receiving screening and 1.3% receiving crossmatching.

Identified subjects' records were examined to determine each patient's Rh-status, allowing us to stratify parturients based on this parameter. The presence of a positive antibody screen and the type of antibody identified were noted, as was the time interval from when the specimen arrived in the blood bank to when the final results of the antibody screen became available to the providing clinicians—of note, all blood samples sent for type and screen or type and crossmatch from the labour and delivery unit are sent “stat”, based on institutional protocol. Estimated gestational age at admission was recorded, and those patients < 28 weeks estimated gestational age were excluded from analysis. Subjects with incomplete medical records or laboratory results were also excluded from analysis.

^A American College of Obstetricians and Gynecologists. Prevention of RhD alloimmunization. ACOG Practice Bulletin no. 4. Washington, DC: American College of Obstetricians and Gynecologists, 1999.

Data were analyzed to determine the incidence of positive antibody screenings based on Rh-status and the proportion of anti-D and non-anti-D antibodies in each group. The time intervals from specimen receipt in the blood bank to antibody screen result availability were compared between groups based on Rh-status and antibody screen status. Finally, the Rh-negative group was analyzed to determine if there was a relationship between the estimated gestational age and the incidence of positive antibody screens due to anti-D antibodies. Seven 16-day groupings were created (30 2/7 – 32 3/7 weeks, 32 4/7 – 34 5/7 weeks, etc.) based on the estimated half-life of anti-D antibodies of 16 days.⁷ The number of anti-D positive and anti-D negative parturients within each grouping was determined. Of note, the time period from 28 to 30 and 2/7 weeks gestational age was not included in analysis due to a lack of patients with positive antibody screens during this time period.

The blood bank at our institution uses an automated solid-phase testing (Galileo model, Immucor, Norcross, GA, USA) that has a minimum test cycle time of 40 min. This is followed up as needed by antibody identification in automated solid-phase or in manual tube antiglobulin testing (polyethylene glycol or low-ionic-strength saline).

Based on the number of deliveries at Prentice Women's Hospital in the first five months of 2007 (10,000 per annum in 2007) and our rate of blood bank testing, we estimated that there would be approximately 454 samples in our database that would be sent for antibody status determination. Using a Rh-D negative phenotype rate of 15%, a 95% rate of detecting anti-D antibodies, and a 1% incidence of a positive antibody screen in a Rh-positive mother, we estimated that approximately 70 samples would undergo antibody determination. Assuming a standard screening time of 120 min for notification of screening results and a standard deviation of 60 min, the study sample would achieve 93% power to detect a 40-min difference in the time for notification with a standard deviation of 90 min using a two-sided Mann-Whitney test at an alpha of 0.05.

Categorical data were analyzed using the Fisher's exact test. The time interval to notification was analyzed for groups based on Rh-phenotype and antibody presence using the Kruskal-Wallis H test. Post hoc comparisons were made using the Mann-Whitney U test with Bonferroni correction for multiple comparisons. Estimates of exact *P* values were determined for the Mann-Whitney test using a Monte Carlo method with 10,000 samples and confidence limits of 99%. Statistical analyses were performed using PASS 2008 version 8.0.13 release date January 14, 2010 (NCSS Inc., Kaysville, UT, USA) and PASW Statistics 18 version 18.0.2 release date April 2, 2010 (SPSS Inc., Chicago, IL, USA).

Results

A type and screen or type and crossmatch was ordered upon presentation to the labour and delivery unit in 557 of 3,874 (14.4%) parturients from June 2007 to October 2007. Of these, 480 were included for analysis. The remaining 77 patients were excluded from analysis due to incomplete medical records, missing blood bank information, duplication in patient information, blood bank testing orders entered in error, or estimated gestational age at presentation < 28 weeks. Of the included 480 parturients, 96 (20%) were Rh-negative. The median estimated gestational age on admission for delivery was 39 weeks (interquartile range 37-39 weeks), with no difference between the Rh-negative and the Rh-positive groups.

Parturients who were Rh-negative had a higher incidence of a positive antibody screen than those who were Rh-positive, and the positive antibody screens in the Rh-negative group were due more often to the presence of anti-D antibodies (Table). The median time interval from blood bank specimen receipt to antibody presence result was 243 min (interquartile range 152-375 min) for antibody positive patients compared with 65 min (interquartile range 54-80 min) for antibody negative patients ($P < 0.001$) (Fig. 1). When comparing patients based on Rh-status, Rh-negative patients had a median time interval of 146 min (interquartile range 68-251 min) compared with an interval of 65 min (interquartile range 54-82 min) for Rh-positive patients ($P < 0.001$). Rh-positive patients who were antibody positive had a median time to antibody result interval of 312 min (interquartile range 200 to 544 min) compared with 218 min (interquartile range 150-335 min) for Rh-negative/antibody positive patients ($P = 0.09$).

When examining the incidence of positive antibody screens in relation to gestational age, the screen was anti-D positive in 100% of Rh-negative patients until 37 weeks gestational age, but declined thereafter (Fig. 2).

Discussion

The important finding of this retrospective study is that the majority of Rh-negative patients have a positive antibody screen when they present for delivery, and the detected

Table Incidence of positive antibody screen and anti-D antibody

	Rh-Negative	Rh-Positive
Positive antibody screen proportion (%)	55/96 (57%)*	14/384 (4%)
Presence of anti-D Antibody proportion (%)	55/55 (100%)*	0/14 (0%)

* $P < 0.01$ compared with Rh-Positive group

Fig. 1 Box plot of time intervals for return of blood type and antibody screen results. Median presented as the solid line within the box. The box is the 25th and 75th percentile of the data and the whiskers the 10th and 90th percentiles. † $P < 0.001$ vs antibody negative in either Rh type group

Fig. 2 Number of Rh-negative patients with positive and negative anti-D test results as a function of estimated gestational age at time of presentation for delivery. w = weeks

antibody was anti-D in all cases. This is in contrast to the Rh-positive parturients who had a positive antibody screen due to antibodies other than anti-D, which is to be expected since they would not have received anti-D antibody administration antenatally. These results would be of little clinical importance, except that the presence of the antibody leads to longer time intervals for the blood bank to complete an antibody screen. In fact, compared with Rh-positive patients, it took over an hour longer to obtain these results if the patient was Rh-negative, simply because of the time needed to perform antibody identification due to the presence of anti-D antibodies. This delay could have important clinical implications, especially during

management of an unexpected hemorrhagic emergency. However, if a patient was Rh-positive/antibody positive, the time interval to obtain an antibody screen report was not longer than if the patient was Rh-negative/anti-D antibody positive.

The onset of developing a positive antibody screen from antenatally administered anti-D antibodies and the duration of this positive antibody screen are not known with certainty. Although the half-life of anti-D antibodies is thought to be from 15.6 to 17.6 days, anti-D antibody titers have been detected in women up to nine weeks following intramuscular administration, with clinical suppression of Rh-isoimmunization for approximately 12 weeks.⁸ Furthermore, mean peak concentrations after intramuscular administration of anti-D antibodies have been shown to occur within two to seven days.⁸ Based on these data, it would seem reasonable to expect that a certain percentage of Rh-negative parturients would present at the time of delivery with a positive antibody screen due to the presence of the previously administered anti-D antibodies. The results of this study suggest that the majority of Rh-negative parturients will be positive for anti-D antibodies at delivery. However, those Rh-negative parturients who present after 37 weeks gestational age have a lower likelihood of having a positive antibody screen from the previously administered anti-D antibodies. Moreover, these results highlight the potential importance of re-administration of anti-D antibodies if the parturient has not delivered by 40 weeks gestation, as recommended by some experts to prevent Rh-isoimmunization.⁹

During each pregnancy, all women should be tested for clinically significant unexpected serum antibodies, preferably at their first visit to the obstetrician.¹⁰ If this initial antibody screen is negative, it is highly unlikely that a Rh-negative parturient would convert to a positive antibody screen due to antibodies other than anti-D, unless there was a history of blood transfusions in the interim. A retrospective review of 17,568 pregnancies by Heddle, *et al.*¹¹ found 42 cases (0.24%) in which new antibody production occurred during pregnancy with no clinically significant neonatal sequelae occurring due to the newly formed antibodies. A second retrospective analysis of 1,265 parturients by Lurie, *et al.*¹² found one case (0.9%) in Rh-negative parturients having a positive third trimester antibody screen with a negative first trimester screen. Yet, one should not assume that a positive antibody screen in this patient population is exclusively due to anti-D antibodies. Although there were no Rh-negative parturients with a positive antibody screen from non-D antibodies in this study, our 95% confidence interval suggests that the occurrence of this scenario could be as high as 2%. Although the incidence of positive antibody screen other than anti-D is rare, its detection is clinically important to

avoid hemolytic transfusion reactions. Therefore, it is important to wait for the identification of the detected antibodies in Rh-negative parturients before proceeding with an elective case. If there is any further question regarding the presence of non-D antibodies after anti-D antibody administration, Shulman, *et al.*¹³ suggest the use of selected D-red blood cells for testing.

Communication and planning may prevent delays in beginning elective Cesarean deliveries, and, more importantly, may positively affect the timely availability of obtaining blood products. For instance, Rh-negative parturients, or even patients with known positive antibody screens, ideally should receive a second antibody screening shortly before a planned delivery. Additionally, the threshold for sending a type and screen or type and crossmatch should be lower in Rh-negative parturients.

Based on the results of this study, we have changed practice at our institution. All Rh-negative parturients now receive at least a type and screen prior to elective Cesarean delivery. To avoid delays, we routinely advise our obstetrical colleagues to have their patients undergo a type and screen (or crossmatch if the clinical history warrants it) one to three days prior to their scheduled Cesarean delivery. For unscheduled Cesarean deliveries, we send a type and screen for Rh-negative patients as soon as the decision to proceed with Cesarean delivery is made. Due to the lower blood loss and risk of transfusion compared with patients undergoing Cesarean delivery, we do not mandate this testing for Rh-negative patients undergoing vaginal delivery.

There are several limitations to this study. First, it was not powered adequately to determine the incidence of antibodies other than anti-D. Second, there exists an inherent selection bias due to the retrospective design of this study, which was potentially exacerbated by the exclusion of 14% of the subjects. Third, we were unable to obtain individual dates of administration of anti-D antibodies. As our patient population generally consists of women who receive regular prenatal care, we assumed that all Rh-negative parturients received anti-D antibodies in the recommended 28 to 34 week gestational age interval. If this assumption was not the case, our data may be skewed regarding the time course of positive antibody screen after treatment. Our results may not be extrapolated to other patient populations who receive their anti-D antibodies at other gestational ages. Additionally, the blood bank methods used can affect the sensitivity for anti-D detection, so our results may not apply in institutions that use testing other than solid-phase methods.

For this study, we used the results of a prior institutional retrospective analysis to determine those patients who underwent a type and screen or type and crossmatch. However, the use of this algorithm may underestimate the true incidence of positive antibody screens due to anti-D

antibodies, as not all Rh-negative parturients underwent a type and screen or type and crossmatch. Also, since only a select number of all parturients underwent blood bank testing, the time interval for laboratory testing may be skewed for the Rh-positive/antibody-positive group.

The final limitation of this study is that we measured the time interval from when the specimen was received in the blood bank to when the final results of the antibody screen became available, and we did not measure the time required for blood products to become available for transfusion. Since the majority of patients did not in fact receive transfusion, the time required for blood product availability is not known. However, blood product availability is likely to be delayed for patients in whom antibody screening takes significantly longer, and this delay has clinical implications, especially for management of hemorrhagic emergencies.

In conclusion, due to previous administration of anti-D antibodies for antenatal prophylaxis of Rh-isoimmunization, the incidence of a positive antibody screen in parturients who present for delivery is higher in those who are Rh-negative than in those who are Rh-positive. The presence of anti-D antibodies in Rh-negative parturients also resulted in a longer time interval to obtain an antibody screen report compared with those who were Rh-positive, regardless of antibody status. This longer time interval likely has clinical implications regarding timely availability of blood products.

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Competing interests None declared.

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