

CASE REPORT

Hemolytic disease of the fetus and newborn in the sensitizing pregnancy where anti-D was incorrectly identified as RhIG

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Abstract

Background: Hemolytic disease of the fetus and newborn (HDFN) is a potentially fatal complication in Rh-incompatible pregnancies and rarely occurs in the sensitizing pregnancy. Distinguishing RhIG from true anti-D identified is challenging. A case of severe HDFN in which a sample drawn at 28 weeks showed anti-D antibody (3+ strength) attributed to RhIG is described. RBC antibody testing early in pregnancy was negative. At birth, the infant was severely anemic and maternal anti-D titer was 1:256. This case represents a clinically significant anti-D in the sensitizing pregnancy that was missed due to confusion with RhIG.

Methods: To determine if agglutination strength could be helpful, a retrospective chart-review using both electronic and paper medical records was performed on 348 samples identified as RhIG and 52 true anti-D samples. The agglutination strength of antibody was recorded for each sample.

Results: For RhIG, there was an even distribution between the weak to moderate agglutination strength (w+, 1+, and 2+) results (35%, 26%, and 33%, respectively) and just 6% had a 3+ strength. Agglutination strength in patients with high titer ($\geq 1:16$) anti-D showed they often (44.4%) have 1+ or 2+ agglutination reactivity.

Conclusions: These results show that agglutination strength alone does not provide reliable evidence to distinguish RhIG from high titer anti-D antibodies. We recommend that in cases where there is any uncertainty about whether the anti-D reactivity is due to RhIG, titers should be performed to rule out clinically significant anti-D antibody.

KEYWORDS

HDFN, RhIG, transfusion

1 | INTRODUCTION

Hemolytic disease of the fetus and newborn (HDFN) is a serious and potentially fatal complication due to the formation of

maternal alloantibodies following sensitization by a target antigen. Sensitization can occur either through exposure to paternally-derived antigens during pregnancy or secondary to transfusion of Red Blood Cell (RBC)-based products containing foreign antigens.

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HDFN severity can range from mild subclinical hyperbilirubinemia to severe anemia associated with fetal hydrops and dangerously elevated bilirubin in the newborn. While ABO incompatibility is the most common cause of mild symptoms associated with HDFN, severe HDFN is more often caused by the anti-D alloantibody. Sensitization in the latter occurs by maternal exposure to fetal blood during pregnancy or at the time of delivery. Under most circumstances, HDFN secondary to an anti-D alloantibody does not affect the sensitizing (initial pregnancy with antibody detected) pregnancy, but rather subsequent pregnancies.^{1,2} For women who have developed an anti-D alloantibody, titers are performed during pregnancy, most often monthly or biweekly. Once the maternal titer reaches 1:16, consultation is recommended with a Maternal-Fetal Medicine physician and biweekly ultrasound examinations are indicated to assess for fetal anemia with middle cerebral artery (MCA) Dopplers and fetal hydrops.³ Elevated MCA Dopplers consistent with severe anemia, >1.5 MoM, and/or fetal hydrops are indications for percutaneous umbilical blood sampling and intrauterine transfusion.⁴

In the US, Rh immunoglobulin (RhIG; 300 mcg/1500 IU) is given prophylactically to RhD negative pregnant women at 28 weeks gestation, following delivery (if the newborn is Rh-positive), and following any vaginal or uterine bleeding to prevent development of the anti-D alloantibody. Gel agglutination or solid phase antibody screens may be positive for anti-D due to passive transmission for up to 3–4 months following administration with strong agglutination (3+) with tube testing using PEG enhancement occurring within the first eight weeks after administration.⁵ Some sources found that that large doses may result in a positive anti-D for up to 6 months.^{6,7} For this reason, it is imperative to perform an antibody screen prior to RhIG administration to evaluate for a new anti-D alloantibody. In the rare circumstance that a true anti-D alloantibody has developed during pregnancy, it may be incorrectly reported as passive anti-D due to RhIG administration. When high suspicion exists for an anti-D alloantibody, a titer should be drawn. The patient should be treated as having a true anti-D antibody until a subsequent sample demonstrates a negative antibody screen or a decreasing anti-D titer, consistent with RhIG. Currently, there is no routine serological method in a single blood draw to differentiate between a true anti-D alloantibody and RhIG administration. Rather, historical documentation of RhIG administration and prior antibody screens prior to RhIG administration are used to make an inference about the cause of anti-D reactivity.

Here, we report a case of severe HDFN affecting what we believe is the sensitizing pregnancy (as evidenced by a negative antibody screen in the early pregnancy) that was missed following confusion with RhIG administration. This error likely resulted in severe anemia of the newborn at the time of delivery. Following this case, we investigated whether agglutination strength using polyethylene glycol (PEG) could help distinguish true anti-D alloantibodies from women who had received RhIG. Policy and methodology changes that have been implemented at our institution to minimize the risk of missing a true anti-D alloantibody are discussed.

2 | MATERIALS AND METHODS

IRB approval (#20191250) was obtained with a waiver of consent to perform a retrospective analysis of all patients with anti-D antibodies identified at our institution between October 2015 and December 2019. This approval included the case study presented here (mother and baby) which included review of the electronic medical record (EMR) information from our institution and faxed medical records from an outside institution for the mother. A dataset was generated from our EMR which contained all blood bank antibodies and titers reported during this time period. Standard blood bank testing include an antibody screen performed using solid phase testing (CaptureR) which is followed by tube testing using PEG enhancement if positive. This study focused on all antibodies reported as RhIG ($N = 348$) in which PEG tube testing had been completed and was positive. In addition, antibodies reported as anti-D that also had an antibody titer (performed in PEG) were also recorded ($N = 52$). The transfusion medicine physician on service at the time determined whether to report RhIG vs anti-D on a case-by-case basis. Of note, there is no “gold” standard to know with certainty that the antibodies reported represented RhIG or true anti-D antibodies and this represents one of the limitations of this study. Positive RBC antibody screens (solid phase testing/CaptureR) that come from RhD negative obstetric patients are reviewed by blood bank staff for RhIG administration in the previous 4 months. This information is provided to transfusion medicine physicians who sign out the reports and determine whether the antibody is most likely RhIG, in which case a titer is not performed or anti-D in which case a titer is performed if the mother is still pregnant.

2.1 | Antibody agglutination reactions

Agglutination strengths and titers were obtained from the EMR and the original antibody identification paper files which are stored in the blood bank. Antibody strength in tube testing using PEG enhancement was reviewed for all 348 samples over this time period. The strongest agglutination strength performed using PEG enhancement on R2R2 (ccDEE) cells was recorded from weak (w+) to 4+ for each sample. For positive type and screen samples from RhD negative obstetric patients, blood bank technologist review the medical record for evidence of RhIG administration. When the history of recent RhIG administration was identified, a mini panel containing just a single RhD homozygous positive cell was performed with PEG enhancement and the agglutination strength on this cell was recorded for this study.

Additionally, 52 true anti-D alloantibody samples with antibody titers were identified between October 2015 and December 2019 from 27 pregnant females that included an anti-D (33 total pregnancies). The strongest agglutination strength (generally the R2R2 cell) using PEG enhancement for each sample on which the titer was performed was recorded.

3 | CASE REPORT

A 24-year-old G2P0010 female presented to an outside institution with a newly confirmed pregnancy by urine pregnancy test and a history of a spontaneous first-trimester abortion about one year earlier. At her initial appointment at 8 weeks gestation, she was found to have a single viable intrauterine pregnancy. Her ultrasound was also notable for a subchorionic hemorrhage which was treated conservatively without medical intervention. At 10 weeks gestation, she was noted to be blood type O RhD-negative with a negative antibody screen. At her 20-week ultrasound appointment, her fetus was noted to have structural abnormalities (gastroschisis) which required consultation and follow-up at our tertiary institution with a neonatal intensive care unit (NICU). At her 28-week follow-up appointment, additional blood work revealed a positive antibody screen, with 3+ strength in tube-testing using PEG enhancement. RhIG was administered that same day and the blood bank reported the antibody was likely passive associated with RhIG administration. The patient presented at 33 weeks with a non-reactive non-stress test and biophysical profile in the setting of fetal growth restriction with subsequent spontaneous fetal decelerations. Urgent cesarean delivery was performed due to the findings on the fetal heart rate tracing. A screen for fetal cells (Rosette test from Immucor) was performed at delivery and was negative. The newborn was taken to the NICU due to prematurity and the associated structural abnormality. The infant was found to be severely anemic with a hemoglobin and hematocrit of 4.5 g/dl and 15%, respectively. Despite this level of anemia, no evidence of hydrops fetalis was noted on physical exam upon admission to the NICU. The child was noted to be type A RhD+, an antibody screen was positive and a direct antiglobulin test (DAT) was strongly positive. The mother's antibody screen was again positive, and an anti-D titer was performed which revealed an anti-D titer of 1:256. At that time, a detailed review of the medical records was performed and it was determined that the type and screen was drawn shortly before the RhIG was administered. Based on this information and the high titer, the antibody report was amended to indicate that the patient had an anti-D antibody instead of RhIG. The infant received five transfusions over about 6 weeks while an inpatient for over 2 months. The infant was also treated with IVIG after birth after the anti-D antibody was identified. The 28-week antibody screen results were reevaluated at this time, and it was determined that the type and screen sample was drawn prior to RhIG administration. The report was corrected to reflect a true anti-D alloantibody and we initiated the studies summarized here to determine how best to prevent errors such as this in the future. The timeline for this case is shown in Figure 1.

4 | RESULTS

In our analysis of records from women who had anti-D antibodies due to RhIG identified at our institution, there was a wide range of

Case 1

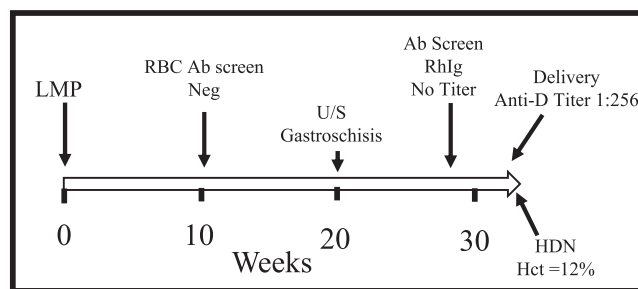


FIGURE 1 Subject timeline. The timeline for the subject in this report is shown. Weeks from the last menstrual period (LMP) are shown on the x-axis. Appointments prior to 20 weeks were at an outside facility and only limited information from these visits are available

agglutination strengths with the majority of samples (94%) showing strengths from w+ to 2+. A small proportion of samples had an agglutination strength of 3+ (6%) and none had 4+ agglutination. Agglutination strengths using PEG enhancement among samples identified as true anti-D alloantibodies also demonstrated a wide range of reactivity. For samples with low titers ($\leq 1:8$), 76% showed agglutination strength of W=+ or 2+. The rest (24%) had an agglutination strength of 3+. Of samples that had a clinically significant titer ($\geq 1:16$), the majority (96%) had an agglutination strength of 2–4+. Of the high titer samples only one sample (4%) had agglutination strength of 1+. These results are summarized in Figure 2.

5 | DISCUSSION

Prior to the introduction of RhIG in the late 1960s, maternal alloimmunization to the D antigen and subsequent HDFN was a common occurrence that resulted in fetal or neonatal death. Since the development and wide-spread implementation of RhIG, the cases of HDFN secondary to anti-D alloantibodies have dramatically declined but have not been eliminated.⁴ When RBC antibodies do develop, detection is critical to ensure proper surveillance and treatment in the form of intrauterine transfusion or early delivery when necessary.⁴ Unfortunately, when RhIG is administered, there is currently no serologic method for a single blood-draw to differentiate between a passive antibody secondary to RhIG and a true alloantibody.

Here, we describe a case of HDFN that was due to anti-D that is unique for several reasons. First, this case represents a rarely encountered HDFN occurring in a patient with an initial negative antibody screen. These rare cases have been postulated to be due to reemergence of a previous antibody from prior sensitization or from early exposure in current pregnancy.⁸ Regarding the latter possibility, initial sensitization is most commonly associated with ABO compatibility, as incompatibility is associated with removal of the mismatched RBCs before sensitization to the Rh antigen can occur.^{9,10} This case interestingly occurred despite the ABO incompatibility between the

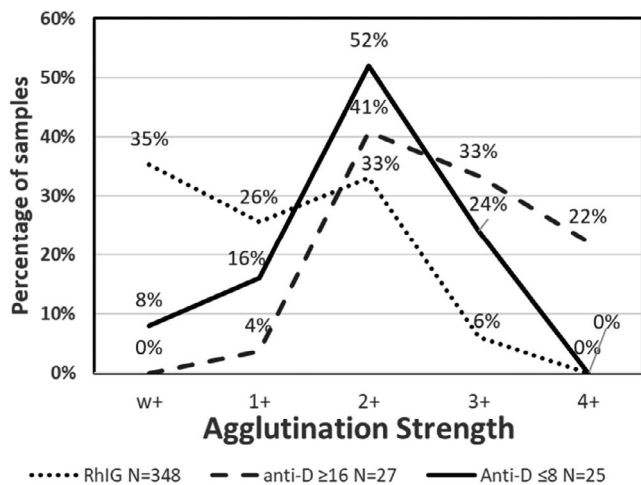


FIGURE 2 Agglutination strength from RhIG, low titer anti-D and high titer anti-D antibodies. The agglutination strength using PEG enhancement was determined as described in the materials and methods. The proportion of samples with the indicated strength is shown and the number of total samples in each data set is also indicated

mother and the fetus (maternal O-, newborn A+). Little clinical information about her prior abortion is available and the records of RhIG administration were not available. Allo antibodies can disappear over time with one study showing that 26% of allo-antibodies disappeared over time.¹¹ However, anti-D antibodies in this study only disappeared just 13% of the time.¹¹ Unfortunately, testing is not available to definitively determine if a true anti-D had formed during her first pregnancy but given the short time interval between pregnancies, we believe the anti-D didn't form until the second pregnancy. Whatever the cause of the anti-D development, this case illustrates the importance of distinguishing between RhIG and a true anti-D even when patients have a negative antibody screen earlier in that pregnancy.

The anti-D alloantibody in this case was initially missed due to a confusion about the timing regarding blood draw for antibody screen and RhIG administration. Because of the seriousness of this error, investigations were initiated and the blood bank changed their policy to perform titers on antibodies with RhD specificity when the sample is drawn on the same day as when RhIG was administered. Because all samples are tested in tubes using PEG enhancement following a positive antibody screen (solid phase screen), agglutination strengths were examined to determine the relationship between agglutination strength for anti-D antibodies due to RhIG. Because our investigations demonstrated that agglutination strength is *not* a reliable method to rule in or rule out a true alloantibody, this case and blood bank policies were discussed with the obstetrics department during their grand rounds to emphasize the importance of obtaining the type and screen prior to RhIG administration. In addition, the blood bank implemented a policy to perform an anti-D titer when an antibody with anti-D reactivity is identified on the same day

that RhIG is administered or whenever there is any question that the antibody could be an allo-antibody. While moderately high titer antibodies (1:16) have been described following the administration of RhIG,¹² in general antibody titers following RhIG administration are low and the result of the titer would be very unlikely to require close fetal monitoring.^{13,14} These policies aim to reduce human error in failing to correctly identify a clinically significant allo-anti-D antibody.

This case illustrates the importance of having policies in place regarding RhIG administration and blood-bank testing to minimize the likelihood that an anti-D antibody be mistaken for RhIG. In cases where there is any uncertainty regarding administration time of RhIG in relation to sample draw time for an antibody screen, a titer should be performed and the patient followed closely to assure a high titer antibody ($\geq 1:16$ for anti-D) is not identified.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to this manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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