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Red Blood Cell Alloimmunization in the Pregnant Patient

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ABSTRACT

Alloimmunization to red blood cell (RBC) antigens represents a challenge for physicians caring for women of child bearing potential. Exposure to non-self RBC antigens may occur during transfusion or pregnancy leading to the development of antibodies. If a subsequent fetus bears that antigen, maternal antibodies may attack the fetal red blood cells causing red cell destruction and clinically significant hemolytic disease of the fetus and newborn (HDFN). In the most severe cases, HDFN may result in intrauterine fetal demise due to high output cardiac failure, effusions and ascites, known as "hydrops fetalis". This article reviews strategies for management and prevention of RBC alloimmunization in women of child bearing potential.

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Alloimmunization to red blood cell (RBC) antigens may occur following a blood transfusion, fetal maternal hemorrhage (FMH) during pregnancy or parturition, or through other blood exposure. The exposed patient may develop antibodies to any non-self RBC antigen. Though patients are tested for ABO and RhD type to avoid incompatible blood transfusion, rates of alloimmunization in the general population range from 1–10% of transfusions [1, 2]. The incidence of alloimmunization may be as high as 60% in chronically transfused patients with underlying hemoglobinopathies, hematologic malignancies, renal failure or organ transplant [3]. Women of childbearing potential represent a challenging population for transfusion services to manage, as alloimmunization may have devastating consequences for the fetus, the most severe of which his hydrops fetalis, however transfusion matching for every foreign RBC antigen is logistically difficult [4]. Further, RBC transfusion of the mother during or after delivery due to bleeding is complicated by RBC alloimmunization, particularly when the mother has an antibody to a high frequency RBC antigen. This article reviews strategies and outcomes for the testing and management of an alloimmunized mother with an affected fetus, as well as prevention strategies to avoid RBC sensitization.

Detection of Alloimmunization

Most Western countries have implemented screening programs for detection of RBC alloimmunization in pregnancy; however, the frequency and timing of those screening programs vary [5, 6]. In the United States, routine blood bank testing to assess maternal blood type (ABO), RhD and for any unexpected RBC IgG antibodies using an indirect antiglobulin test (IAT) is recommended for all pregnant women. The American College of Obstetrics and Gynecology (ACOG) recommends testing mothers at their first prenatal visit [7].

First trimester screening has been shown to be approximately 77.8% sensitive for clinically significant, RBC antibodies resulting in hemolytic disease of the fetus and newborn (HDFN). However, the sensitivity varies by antibody specificity. For example, first trimester screening

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for clinically significant anti-E was only 57.1% sensitive in one study [8]. Blood group antibodies differ in their risk of causing clinically significant HDFN. Up to 50% of RBC antibodies detected by screening may be clinically insignificant as they are antibodies against antigens that are poorly expressed on fetal RBCs, such as Lewis antibodies, or because they are IgM antibodies, which will not cross the placental barriers, such as anti-N [9, 10]. The most common clinically significant alloantibodies causing HDFN include anti-D, anti-E, anti-c and anti-K; however, over 50 non-ABO blood groups have been implicated in HDFN [11].

Additional antibody testing is recommended by ACOG for RhD negative mothers between 28–29 weeks gestation prior to administration of RhD immunoglobulin (RhIg) [7, 12]. This may detect RhD sensitization due to early FMH. Some studies have questioned the utility of additional screening in RhD positive mothers [13-15]. However, up to 27% of severe HDFN cases occur unexpectedly in RhD positive mothers with negative first trimester RBC antibody screens. Risk factors for late alloimmunization included a history of blood transfusion, increasing parity and amniocentesis or chorionic villus sampling with the current pregnancy [16]. The most severe fetal outcomes occurred in fetuses of mothers who developed anti-c, indicating that mothers who are Rhc negative may particularly benefit from additional testing later in pregnancy to allow for timely interventions [16, 17]. In the United Kingdom, screening is recommended at initial prenatal visit and at 28 weeks gestation in all mothers, regardless of RhD status [5].

Prevalence of Alloimmunization

Reported rates of alloimmunization in women of childbearing potential vary greatly depending upon the period and manner in which the data were collected. In the United States, a representative series of 22 102 blood samples from women of reproductive potential (age 15–44 years) identified RBC antibodies in 1.15%, of whom 18% had multiple antibodies [18]. Luckily, rates of clinically significant HDFN are much lower at 3/100000 to 80/100000 live births [19]. In modern cohorts in the Netherlands, RBC alloimmunization detected by first trimester screening occurred in 1232/100000 pregnancies. Of these, 400/100000 were clinically significant, with the most common specificities being anti-D, anti-E, anti-K and anti-c [8]. In Sweden, 0.4% of 78 145 pregnancies were complicated by non-ABO alloimmunization [6]. In Africa, the prevalence of RhD negativity is less common in the population; however, due to barriers to prenatal testing and care, rates of anti-D in women of child bearing potential are as high as 2–12% [20-22].

The possibility of alloimmunization varies between populations based on the prevalence of blood group antigens within the population. For example, the frequency of RhD negativity is estimated at 15–17% among people of European/North American ancestry. This falls to 3–8% in people of African and Indian ancestry. In Asian populations, RhD negativity may be as low as 0.1–0.3% of the population [23, 24]. The prevalence of other RBC antigens may vary widely between populations, resulting in varied rates of alloimmunization.

Where ABO incompatibility occurs between mother and fetus, studies have shown a protective effect against further RBC alloimmunization. Studies prior to routine administration of RhIg prophylaxis showed that 16% of RhD negative mothers pregnant with RhD positive fetuses became sensitized; however, rates of sensitization decreased to 2% when there was ABO incompatibility in addition to RhD incompatibility [19]. Therefore, population frequencies of ABO blood types may exert further effects on maternal RBC sensitization.

In addition to ABO discrepancy, other maternal factors may influence the risk of alloimmunization. Prior major surgery, RBC or platelet transfusion, multiparity, prior male child or operative removal of a prior placenta have been associated with RBC alloimmunization [25]. Maternal risk factors for RhD sensitization despite RhIg prophylaxis include conditions related to FMH or insufficient RhIg dose, such as assisted vaginal delivery, caesarian section, post-maturity (>42 weeks), maternal age or maternal red blood cell transfusion; however, none of these was present in 43% of RhIg failures [26]. Other factors that have been significantly associated with rates of sensitization to RBC antigens within the general population include age at time of transfusion and numbers of transfusions received [27, 28]. Female gender has variably been associated with increased rates of RBC alloimmunization; however, that risk is obviated when controlled for numbers of transfusion exposure events [29]. Women who are HLA-DRB1*15 positive also represent a group that is at increased risk for forming RBC antibodies [30, 31].

Murine models of RBC alloimmunization to transfused cells are shedding light on additional risk factors for alloimmunization that have yet to be fully studied in humans [32, 33]. In mouse models, RBC alloimmunization has been associated with donor or product specific factors, including longer storage duration [34] and inversely related to the efficacy of leukoreduction and platelet reduction [32]. Alloimmunization has also been associated with recipient factors, such as faster rate of RBC clearance [35] and heightened recipient inflammatory state at the time of exposure [36], which has been confirmed in human studies [37]. Historically, all of these models have relied on transfusion of RBCs; however, novel murine models of pregnancy-related alloimmunization to human RBC antigens have been developed [38]. These novel models will allow for further mechanistic studies of maternal sensitization not possible in humans [39].

Monitoring and Management of the Sensitized Mother and Fetus During Pregnancy

Once a mother is identified as having a clinically significant RBC alloantibody, further monitoring and evaluation is required. For first pregnancies affected by maternal anti-D, antibody titers may be predictive of disease severity [19]; however, blood banks may have different critical titer thresholds, often 1:16–32 [9]. Unfortunately, titers are less predictive in subsequent pregnancies or with other antibodies, such as anti-Kell, which has been shown to cause significant HDFN even at low titers [40, 41]. Titer thresholds and management strategies have been proposed for maternal antibodies other than anti-D and anti-Kell; however, the evidence is limited as to whether they predict or mitigate clinical outcomes [42]. In addition, historic titer thresholds are based on the titers being performed using conventional tube methods. It is unclear if other methodologies, such as gel-based platforms, are equivalent [43, 44]. Even with standardized procedures, titer proficiency testing shows wide variability between centers and methodologies, so serial titers should be performed at the same institution to facilitate interpretation [5, 45].

Titer alone may not be the single-best predictor of clinical potency of maternal antibodies. The degree of fucosylation of IgG antibodies has been shown to influence the pathogenicity in HDFN. For RhD IgG antibody, less fucosylation predicts increased phagocytosis on monocyte-based antibody dependent cellular cytotoxity testing and correlates with fetal hemoglobin levels [46]. However, the influence of IgG fucosylation when directed against other RBC antibodies is variable [47]. Further research is necessary to apply the clinical implications of these findings.

If paternal identity is confirmed, fetal risk of carrying the implicated antigen should be determined by assessing paternal zygosity [48]. For RhD, this requires paternal RHD genetic testing which is usually available at reference laboratories. For antigens such as Kell/k, routine blood bank antigen phenotyping of the father may determine if the fetus has a 50% or 100% chance of carrying the implicated antigen. Non-invasive, high throughput testing platforms testing cell-free DNA, fetal DNA circulating in maternal plasma, for RhD have been developed and implemented in Europe with excellent effect [49-51]. As a screening test for potentially affected pregnancies, they have been shown to be >99.3% sensitive at 10–11 weeks gestation, allowing for very early monitoring and intervention [50]. When implemented as a routine test at 24–26 weeks gestation for RhD negative mothers, the sensitivity of

RhD detection was 99.9% and pregnancies with RhD negative fetuses avoided unnecessary RhIg administration [52, 53]. In Norway, the Netherlands, the UK, and Finland, RhD negative mothers with negative initial antibody screens are routinely offered cell-free fetal DNA testing for RhD at 14–29 weeks, limiting unnecessary RhIg administration [54-57]. Cell-free fetal DNA testing is feasible as early as 9 weeks gestation in alloimmunized mothers, and has been used to test for other RBC (RhC/c, RhE, Kell) and platelet antigens [56, 58]. Currently, this testing is available through a reference laboratory in the United States and has been shown to be accurate in all three trimesters of pregnancy [59]. Some authors question if routine screening using cell-free fetal DNA in populations that are of non-European descent is ready. However, with advances in genomic testing technology, this technique has the potential to be widely applicable for testing multiple RBC antigens in diverse populations [60].

As the pregnancy progresses, serial RBC antibody titers may be used. Once a critical titer threshold is reached, and the fetus is determined to be at risk of carrying the antigen by paternal zygosity testing or more direct measurements or the fetal result is unknown, the fetus must be assessed for clinical anemia. In 1953, Bevis, et al. identified that bilirubin levels in amniotic fluid correlated with degree of fetal hemolysis and anemia [61]. Liley, et al. expanded on that observation and defined three levels of risk of HDFN based on gestational age and amniotic fluid deviation in optical density at 450 nm (ΔOD_{450}) [62]. The Liley curve was valid beginning at 27 weeks of gestation which limited its ability to predict severely affected fetuses early in the pregnancy, so later investigators extrapolated the risk curves down to 16 weeks gestation to allow for earlier identification and intervention [63]. In 1993, Queenan, et al. used the same ΔOD_{450} measurement to classify four zones of threat to the fetus and provided recommendations on monitoring and intervention at the different levels of severity based on gestational age [64]. All of these techniques rely on the availability of amniocentesis, an invasive procedure that carries an inherent risk to the mother and fetus; therefore, investigators sought to identify a non-invasive predictor of fetal anemia.

In the early 2000s, a landmark study demonstrated the utility of intrauterine monitoring of fetal middle cerebral artery peak systolic velocity (MCA-PSV) as a non-invasive surrogate marker of fetal anemia in affected fetuses [65]. Current guidelines recommend fetal assessment at 16–24 weeks gestation with cerebral MCA-PSV measurements [7]. These velocities may be serially monitored throughout the pregnancy with limited risk to the fetus or mother, unlike repeated amniocentesis. If the velocity is >1.5 multiples of the median for gestational age, that is predictive of moderate to severe anemia and further invasive assessment of fetal anemia with a potential for intervention is required [65-70]. Algorithms for management define specific timeframes in which to obtain and follow MCA velocity depending upon gestational age [48, 71].

After a fetus is determined to be at risk of significant anemia based on MCA-PSV, they should be offered in-utero blood sampling and possible intrauterine transfusion. These invasive techniques require specialized expertise and have a 1–3% risk of fetal adverse events [72, 73]. Historically, transfusions have been delivered into the peritoneal cavity of the fetus, though modern techniques of ultrasound guided cordocentesis allow for direct testing of fetal hemoglobin and infusion of blood products through the umbilical vein at the placental insertion or intrahepatic vein. Most centers perform in-utero transfusions through 35 weeks gestation, with anticipation of induction of labor at 37–38 weeks gestation depending upon the degree of fetal anemia [74]. Coordination of this procedure takes a multidisciplinary approach, including obstetricians, maternal-fetal-medicine specialists, transfusion medicine and pediatricians, as there is a risk of urgent need for pre-term delivery should there be a procedural complication [75].

In severely affected pregnancies, the fetus may be affected early in gestation, prior to technical feasibility of in-utero transfusions. The use of pooled intravenous immunoglobulin (IVIg) given to mothers, which has been shown to have efficacy in the synonymous neonatal alloimmune thrombocytopenia, has been used in high doses early in gestation to delay or limit the need for in-utero transfusions [76-79]. Plasma exchanges on the mother have also been used to reduce antibody titer in cases when a mother had a significantly affected previous pregnancy [80]. The combination of IVIg and plasma exchange used in severely affected mothers is supported by case reports demonstrating fetal safety and tolerability [81-86]. There are technical challenges to the use of plasma exchange and IVIg in mothers, such as calculations of total blood volume and the need for adequate IV access; however, these therapies may offer opportunity for fetal survival when maternal antibody titer and previously affected pregnancies predict a potential for fetal demise.

If the maternal antibody is to a high frequency RBC antigen, obtaining rare blood units for in-utero transfusion as well as for maternal transfusion at delivery may require special care and coordination. In circumstances where antigen negative blood is not available, maternal blood has been collected and successfully used for in-utero transfusion and in preparation for postpartum hemorrhage [87-89]. A failure to take into consideration those antibodies in the mother may result in an acute hemolytic transfusion reaction. When limiting transfusion is of paramount importance, supportive therapy to maximize maternal red blood cell mass through the use of iron and erythropoietin has been used, in addition to banking of autologous blood for delivery [90].

Prevention of Alloimmunization

The majority (83%) of severe HDFN cases are due to previous pregnancy, thus the discussion about prevention pertains only to strategies aimed at preventing alloimmunization caused by transfusion, which cause the minority of cases [91]. However, a number of strategies exist to prevent RBC alloimmunization due to transfusion from occurring in women of child bearing potential. Prior to any non-emergent transfusion, all patients should have their ABO and RhD type determined, as well as testing for any unexpected RBC antibodies [9]. For women of childbearing potential, the use of RhD negative blood in situations where RhD type is unknown prevents sensitization to this highly immunogenic antigen. In some countries, standard blood bank practice includes matching for Kell (K1) to prevent sensitization in women of childbearing potential [92-94]. In women requiring transfusion with underlying hemoglobinopathies, rates of alloimmunization may be as high as 30–60% [95, 96]; however, the use of prophylactic matching at RhD/CE and Kell has been successful in limiting alloimmunization rates [97]. In studies of surgical patients, extended matching for multiple antigens (RhD, Rhc/C, RhE, Kell, Fya, Jka and S) reduced alloimmunization rates by 64% [4]; however, this is not a standard practice.

Women of childbearing potential may particularly benefit from the application of molecular techniques for RBC antigen matching, allowing for rapid and accurate matching beyond ABO, RhD/CE and Kell to further prevent sensitization from occurring [98, 99], but challenges and barriers remain. One of the major barriers is cost. Molecular RBC matching has yet to be shown to be cost-effective even in a highly transfused population of patients [100, 101]; however, the cost of the testing is constantly decreasing due to technological advances allowing for increased automation and higher throughput testing. Another barrier is implementation. In a retrospective study designed to assess the influence of extended matching at the time of blood transfusion on the development of clinically significant red cell antibodies associated with HDFN, 49% of mothers who received blood products from a center that provided extended matching also received transfusions outside of that center [91]. Though few mothers in this study were sensitized due to transfusion, for an extended matching strategy to be successful, it must be adopted universally.

RhIg, which prevents active sensitization to RhD after the mother has been exposed through pregnancy, has been critical in decreasing

the frequency of HDFN due to anti-D. Traditional RhIg is a highly purified, polyclonal product derived from sensitized plasma donors. However, novel recombinant preparations are being developed [102, 103]. Current standards in the United States recommend the administration of RhIg in mothers who are RhD negative at 28 weeks gestation and again at parturition, as well as within 72 h of other expected exposure to fetal antigens (ectopic pregnancy, termination, chorionic villus sampling, abdominal trauma, amniocentesis, etc.) [104, 105]. The idea of passive antibody to prevent active sensitization came from the observation that fewer RhD negative mothers who were ABO incompatible with their fetuses became sensitized to RhD, as compared to mothers were ABO compatible with their fetuses, thought to be due to early clearance of the RhD positive cells because of ABO incompatibility. The actual mechanism is likely more complex and multifactorial [106, 107]; however, the clinical benefit is well recognized. Initially only given at parturition, mothers were still found to be sensitized, so earlier dosing at 28 weeks was recommended to further decrease sensitization rates. With adequate implementation and dosing, rates of RhD negative mothers becoming sensitized during pregnancy with an RhD positive fetus is less than 1% [108-114]. Recent Cochrane review of the efficacy of RhIg found few direct benefits to the mother or fetus; however, it concluded that the degree of protection afforded subsequent pregnancies could not be assessed and further longitudinal research is needed [115].

Dosing of RhIg at parturition is reliant upon accurate measurements of the degree of FMH. Maternal blood is screened for FMH using an erythrocyte rosette test which will cause agglutination visible with light microscopy if RhD positive fetal cells are present in maternal circulation [9]. If negative, a standard dose of RhIg is delivered. If positive, further quantitation using the Kleihauer-Betke test to calculate of the degree of FMH is necessary to determine how much RhIg should be administered. The Kleihauer-Betke test uses acid elution, to which cells replete with hemoglobin F are resistant, to quantitate the degree of FMH. This technique relies on subjective quantitation of the number of cells containing hemoglobin F. It may also be inaccurate in instances where mothers have conditions that allow for persistence of hemoglobin F in circulation [9]. This may result in under dosing or over dosing RhIg as one 300mcg dose of RhIg is considered appropriate for 15 mL of fetal RBCs in maternal circulation. More objective testing for FMH can be performed using flow cytometric methods, directly measuring the concentration of RhD antigen or hemoglobin F in maternal circulation [116, 117]. Using anti-F antibodies and flow cytometry, circulating fetal hemorrhage of <2.0 mL is detectable [118], and can distinguish maternal cells carrying hemoglobin F from fetal cells [119]. These techniques can be readily adapted to detect other discordant antigens that may be present on fetal cells, and not maternal cells [120]. Unfortunately, the use of flow cytometric detection and quantitation of FMH is limited due to long-standing national standards of care and equipment and staffing requirements [121].

Maternal obesity, defined as a BMI ≥ 30, may also affect efficacy of RhIg prophylaxis as standard dosing calculations for RhIg are based on a maternal blood volume estimate of 5000 mL. In obese mothers, 5000 mL is likely an underestimate of total blood volume (TBV), resulting in under dosing RhIg [122]. Obese mothers also face challenges in RhIg administration, which is recommended to be intramuscular (IM). Due to concerns about poor absorption of RhIg from the subcutaneous tissue, intravenous administration should be considered in mothers with significant adiposity that precludes IM administration [123]. Because of these challenges, concern has been raised that the rates of anti-D HDFN will rise along with the obesity epidemic unless providers accurately dose and administer RhIg.

Recent focus has shifted to avoid unnecessary RhIg exposure to mothers who type as RhD negative, but who may be one of the common forms of weak RhD. RhD is a large, multi-pass protein present on RBCs that exhibits a lot of genetic variation. Certain genetic mutations, often coding for the transmembrane or intracellular trafficking portions of the RhD protein, result in weakened RhD expression on RBCs [124]. These mutations do not affect the extracellular epitopes expressed by RhD; therefore, individuals with the most common of these weak RhD mutations, type 1, 2 and 3, are not at risk to form an anti-D. Historically, mothers who demonstrate weakened D expression were given RhIg as though they were RhD negative. However, with specialized genotyping for weak D type 1, 2 and 3, those mothers can be excluded from receiving unnecessary doses of RhIg [125]. Evidence suggests that this is not only cost-neutral for care, weighing the cost of molecular testing against the savings of avoided RhIg doses, but also clinically beneficial [126]. Current ACOG guidelines address the potential to avoid unnecessary RhIg administration in mothers who demonstrate decreased RhD expression using standard blood banking techniques and molecularly type as weak D type 1, 2 and 3. However, they recommend RhIg administration in the appropriate clinical scenarios until further cost–benefit analysis is performed [12].

Previously sensitized mothers with severely affected fetuses may be exposed to additional RBC antigens at the time of in-utero transfusion, due to the risk of bleeding associated with the procedure. Up to 25% of mothers who receive in-utero transfusion form a new alloantibody, which may impact future pregnancies [127, 128]. Further sensitization to RhCE and Kell are thought to be due to fetally derived cells; however, sensitization to Duffy, Kidd and S antibodies are often due to exposure to the transfused unit [127]. Further matching of the donor for in-utero transfusions to maternal antigens may decrease further alloimmunization to those antigens by 60%; however, it is not protective against further sensitization to fetal antigens [129]. Units that require extended matching to the maternal antigens require particular coordination with the blood bank, but may protect future pregnancies from additional antibody exposure.

In summary, care of the mother and fetus during a sensitized pregnancy requires a multidisciplinary approach that includes obstetricians, maternal-fetal-medicine specialists and transfusion medicine specialists. Detection of antibodies and assessment of titers may be relevant to predict clinical course. Mothers who are sensitized require frequent monitoring and fetal assessments, including MCA-PSV Dopplers. In-utero transfusions may be required for severely affected fetuses. Subsequent pregnancies may be more severely affected and early interventions with IVIg and maternal plasma exchange may be beneficial.

To prevent transfusion-caused RBC sensitization from occurring in women of childbearing potential preventative transfusion strategies could be universally adopted. Novel murine models of transfusion and HDFN are helping to further elucidate recipient and donor factors that influence rates of RBC sensitization; such has inflammatory status and product storage duration. Secondary prophylaxis with accurate determination of FMH and RhIg administration has been shown to be highly effective in preventing RhD sensitization. The use of molecular techniques to assess paternal zygosity, fetal antigen carriage and maternal weak D typing, are allowing for more precise, personalized medicine and prevent over-use of unnecessary RhIg doses. Highly specialized blood units that are multiply antigen negative may be required for in-utero transfusion, necessitating clear communication and coordination between clinicians and their transfusion services.

Conflict of Interest Statement

Jennifer Webb; Meghan Delaney - None.

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