

Research & Reviews on Pregnancy and Women's Health

Chapter 4

Red Cell Alloimmunization in Pregnancy : An Update

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Abstract

The transfusion of blood and components has become an integral part of patient management in modern medicine. As a result, the blood transfusion services play an important role and are responsible for ensuring sufficient quality and safe blood supply. Blood transfusion support is vital to the management of patients with hematologic disorders and malignancies. Many such patients require blood transfusion during their illness or may be lifetime.

It is well-known that alloimmunization to red blood cell antigens resulting from the genetic disparities between donor and recipient is one of the risks of blood transfusion. The antibody screening cells are used to detect unexpected antibodies. The risk of alloimmunization is higher in patients who have received multiple blood transfusions such as thalassemia, other hematological disorders, renal failure patients on dialysis who receive blood transfusions, and females having multiple pregnancy.

Keywords: Alloimmunization; Pregnancy; Blood transfusion; Red blood cells

1. Introduction

Alloimmunization resulting from contact with nonautogenous antigens concerns just a minority of patients, [1] and when it happens, it depends on various factors. Thus, antigen systems on red blood cells (RBCs) are very important systems: in addition to the carbohydrate AB0-system and its naturally occurring immunoglobulin M antibodies (AB0-isoagglutinins), several hundred RBC antigens, mostly protein antigens, exist [1]. The most immunogenic RBC protein antigen is Rhesus-D, which leads to an immune response for up to 70% of Rhesus-D-negative individuals. In the case of pregnancy, it can cause hemolysis in the fetus, and thereby, in the worst case, fetal death [2] After delivery, maternal antibodies also can cause hemolytic

disease of the newborn (HDN). This occurs if a Rhesus-D-negative woman carries a Rhesus-D-positive fetus and has preexisting antibodies, which developed during a prior pregnancy or after transfusion of Rhesus-D-positive RBCs. The symptoms range from mild (eg, anemia) to severe (eg, hydropsfetalis and stillbirth). In 1969, postdelivery application of anti-D-immunoglobulin was introduced in the United Kingdom to prevent this complication.⁵At this time, the prevention of HDN consists of two intramuscular applications of anti-Rhesus-D-immunoglobulin in the 28th week of gestation, sometimes followed by another application at the 34th week. If a woman gives birth to a Rhesus-positive newborn, she needs a further application of anti-D. Different countries recommend different dosages of anti-D, ranging from 500–1,500 IU per application. After introduction of immunoprophylaxis, sensitization risk decreased from 10% to 0.1%–2% [2].

Other Rhesus antigens, as well as Kidd, Duffy, MNS, and Kell antigens, may be clinically important as well, even though their immunogenicity is much less than the immunogenicity of Rhesus-D (from 0.03%–10%) [3]. With the only exception of Anti-N, all alloantibodies against these antigens can cause hemolysis when the patient has a second contact with the foreign antigen. In most transfusion departments, all these important antibodies can be detected by pretransfusion screening of the recipient's blood, using a RBC panel with at least three cells. To specify a certain antibody, panels with more cells are used. Under normal circumstances, compatibility testing is performed for all patients by testing the recipient's blood against a screening cell panel (antibody screening), as well as against the red cells of blood components for transfusion (cross-match testing). If an antibody has been specified, the corresponding antigen should be considered when selecting appropriate RBC components for transfusion. The early detection of newly formed antibodies depends on the necessity of early reinvestigation for patients who repeatedly require RBC transfusions. Otherwise, some alloantibodies can drop under the detection limit if the investigation occurs after a longer period [3].

2. Red Blood Cells

Alloimmunization in transfusion medicine is a well-known complication that occurs when the recipient's immune system reacts to a donor's antigens. Alloimmunization problems vary, depending on the different involved antigens and reach from HDN, hemolysis, NAIT, and PLT refractoriness over TRALI to autoimmune neutropenia [4]. Ways to reduce this medical problem lie on the recipient's side as well as on the donor's/product's side. In practice, RBCs are generally transfused compatible in the ABO-system. In practice, RBCs are generally transfused compatible in the ABO- system. Matching in the antigen systems D, C, c, E, e, and Kell is also useful. To avoid alloimmunization in other antigen system, especially when alloimmunization has already occurred, antigen matching (eg, HLA, HPA, and HNA matching) is widely accepted [4].

Transfusion of blood components, transplantations, and exchange of blood between

mother and child during pregnancy or at birth can lead to alloimmunization. Because of its clinical relevance, this review brings into focus alloimmunization against red blood cells, human platelet antigens, human leukocyte antigens, and human neutrophil antigens. In principle, an individual is able to develop antibodies after exposure to nonautogenous antigen, but these cells actually induce alloimmunization only for a minority of patients. An individual producing alloantibodies after having contact with foreign antigens depends on various factors, such as genetic predisposition, underlying diseases, the patient's immune status, and clinical immune modulation.

The discovery of the ABO blood group system by Karl Landsteiner greatly reduced the fatalities associated with blood transfusion. Introduction of the indirect antiglobulin test (IAT) by Coombs added a new dimension to the safety of blood transfusion with the identification of alloantibodies causing hemolytic diseases of the newborn or transfusion reactions. This led to the discovery of about 300 blood group antigens as per International Society of Blood Transfusion [5]. Blood groups are antigens and alloimmunization occurs when an incompatible antigen introduced in an immunocompetent host evokes an immune response. Clinically significant red blood cell (RBC) alloantibodies develop in 6-36% of multi-transfused patients and can pose major problems in long-term transfusion therapy.

It is well-known that alloimmunization to RBC antigens resulting from the genetic disparities between donor and recipient is one of the risks of blood transfusion. The risk depends on the recipient's exposure to the foreign antigen and its immunogenicity. Immunization may also be influenced by the number and frequency of the transfusion as well as the recipient's sex, age, and underlying disease.

Red blood cell (RBC) alloimmunization in pregnancy continues to occur despite the widespread use of both antenatal and postpartum Rhesus immune globulin (RhIG), due mainly to inadvertent omission in administration as well as antenatal sensitization prior to RhIG given at 28 weeks gestation. Additional instances are attributed to the lack of immune globulins to other RBC antigens. Evaluation of the alloimmunized pregnancy begins with the maternal titer. Once a critical value (32 for anti-Rh(D) and other irregular antibodies; 8 for anti-K and-kl) is reached, fetal surveillance using serial Doppler ultrasound measurements of the peak velocity in the fetal middle cerebral artery (MCA) is standard. In the case of a heterozygous paternal phenotype, amniocentesis can be performed to detect the antigen-negative fetus that requires no further evaluation. MCA velocities greater than 1.5 multiples of the median necessitate cordocentesis, and if fetal anemia is detected, intrauterine transfusion therapy is initiated [6].

Patients with high antibody titers and recurrent perinatal loss in the second trimester have few options other than artificial insemination with RBC antigen-negative donor semen, surrogate pregnancy, or preimplantation diagnosis. Future therapy will probably involve

selective manipulation of the maternal immune system. In vitro data and clinical case reports suggest that maternal alloantibodies to paternal leukocytes may result in an Fc blockade, thereby protecting the fetal RBCs from hemolysis in cases of Rh (D) alloimmunization. In a rabbit model for HDFN, alloimmunization to paternal leukocytes resulted in fetal hemoglobin levels that approached normal in does that had been previously sensitized to RBCs [6]. As an alternative strategy, four peptides have been associated with the proliferation of T-helper cells involved in the development of antibody of the RhD antigen; therapeutic administration of these peptides might ameliorate an established anti-D response, preventing severe HDFN in a subsequent pregnancy.

Major advances in diagnostic techniques continue to be realized for the evaluation of the patient with red cell alloimmunization in pregnancy. Noninvasive assessment of the fetal blood type through the use of free fetal DNA in maternal plasma in conjunction with middle cerebral artery Doppler ultrasound the detection of fetal anemia will likely make amniocentesis obsolete in the near future. Although currently intrauterine transfusion remains the mainstay of fetal treatment once anemia has been detected, selective manipulation of the maternal immune system will probably replace this technique in the coming years.

3. Antibodies of the ABO System

3.1. Anti-A and anti-B

These naturally occurring antibodies are predominantly IgM, react at room temperature and activate the complement. They are present in sera of people lack the corresponding antigen from their red cells, detected first at an age of three months and increase in titre gradually. In neonates, ABO antibodies are usually IgG and of maternal origin. Anti-A and anti-B may be IgM, IgG, or IgA, whereas some sera may contain the three classes. Transfusion of ABO incompatible red cells results in symptoms of haemolytic transfusion reaction, also anti-A and anti-B are responsible for rejection of incompatible transplants. Although ABO is very rare, hydropsand cases needed an exchange transfusion are occasionally found [7]

3.2 Anti-H

Pure anti-H is a very uncommon antibody; it is found in subjects of the very rare phenotype Oh as a haemolysin, and as an agglutinin which is almost active at 0°C. Although usually IgM, it may be partly IgG. In rare cases, anti-H may also be found in A1 subjects

4. Rh Blood Group System

Rh system is the most complex blood group systems, comprising 46 antigens numbered RH1 to RH53 with seven numbers obsolete. The Rh antigens are encoded by two homologous, closely linked, genes on the short arm of chromosome 1: RHD, producing the D antigen, and

RHCE, producing the Cc and Ee antigens. In 1940, Landsteiner and Wiener made an antibody by injecting rhesus monkey red cells into rabbits (and later guinea pigs). The antibody, called anti-Rh, agglutinated rhesus monkey red cells, but also agglutinated the red cells from human. In the same year, an antibody appeared to be the same anti-Rh has been identified in the sera of patients who had transfusion reactions after receiving ABO compatible blood. Later, a difference between animal and human anti-Rh identified and the antigen defined by animal anti rhesus called LW in honour of Landsteiner and Wiener. The clinical importance of the Rh blood group system stems from the fact that the antigen D of the system is highly immunogenic; if a unit of D positive blood is transfused to a D-negative recipient, the recipient forms anti-D in some 90% of cases and thereafter cannot safely be transfused with D-positive red cells. Moreover, if a D-negative woman becomes pregnant with a D-positive (ABO-compatible) infant, the passage of red cells across the placenta from fetus to mother induces primary immunization to D in about one in six cases, unless the mother receives anti-D Ig. In a subsequent pregnancy with a D-positive infant, secondary immunization may be induced leading to hemolytic disease in the infant. Rh is also involved [8]

4.1. Rh antigens

Rh antigens are glycosylated polypeptides (i.e. without carbohydrates attached to the protein). Although Rh antigens are fully expressed at birth, they are present on red cells only and are not detectable on leukocytes or other tissues. The first discovered and clinically most important antigen is D. It is present on red cells of about 85% of white people and more frequent in Africans and Asians. Before the introduction of the anti-D immunoglobulin prophylaxis programme, anti-D was a common cause of severe HDN. D antigen expression varies quantitatively from the greatly enhanced expression to the very weak D (DEL). In a very rare phenotype Rh null, red cells express none of the Rh antigens. Even among the common phenotypes, there is detectable quantitative variation of D [9]. The term weak D, weakly reacting form of D (formerly referred to as Du), defines any D phenotype where the expression of D antigen is quantitatively weaker than normal, whereas 'partial D' defines a D phenotype qualitatively different from normal D. The importance of determining whether a D variant phenotype is present on the red cells of a donor relates to whether or not the red cells will be immunogenic if transfused to a D-negative patient (or a patient with a different D variant). In addition, anti-D in women with partial D antigens has been the cause of HDN [9].

4.2. C, c, E and e

C and c, E and e, represent two pairs of antigens controlled by RHCE gene. C and c differ from one another in four amino acid positions, whereas one amino acid differentiates E from e [10].

4.3. Rh antibodies

Rh antibodies are usually produced in response to red cell immunization resulting from blood transfusion or pregnancy, although 'natural occurring' Rh antibodies are not unknown [10]. Most of Rh antibodies are IgG immunoglobulins, react optimally at 37°C or following antiglobulin testing. Exposure to less than 0.1 ml of Rh-positive red cells can stimulate antibody production in an Rh-negative person. These antibodies can cross the placenta to coat fetal (Rh-positive) RBCs; therefore they should be considered potential agents of HDN and of HTR. Clinically, anti-D is the most important after anti-A and B. It is mostly IgG with IgG1 and IgG3 as predominant subclasses. Naturally occurring IgG anti-D that were only detectable in an autoanalyser, were reported to be relatively common.

Anti-C, c, E, e shared many of the characteristics of anti-D. Anti-c is clinically the most important Rh antibody after anti-D and may cause severe HDN. Rh antibodies may be involved in autoimmune haemolytic anaemia, these Rh auto antibodies are commonly anti-e but anti-c, E, D and C also occur [10].

5. The Kell and KX systems

This is a most clinically important blood group system as its corresponding antibodies involved in HTR and HDN more frequently than any other antibody outside the ABO and Rh systems [11].

5.1. Kell antigens

There are five sets of antigens in the Kell system: K and k; K_{pa}, K_{pb} and K_{pc}; J_{sa} and J_{sb}; K₁₁ and K₁₇ (W_{ka}); and K₁₄ and K₂₄. There are an additional seven high frequency antigens, K₁₂, K₁₃, K₁₈, K₁₉, K₂₂, T_{OU} and R_{AZ} and three low frequency antigens, U_{1a}, K₂₃ and V_{LAN}¹¹K clinically the most important; is expressed on red cells by about the tenth week of life. Amongst the other Kell antigens, K_p^a, K_p^b, K_{pc}, J_{sa}, J_s are known to be clinically important [11]. The KX antigen (the only antigen in the K system) is expressed most strongly on red cells that lack Kell antigens, i.e. KO red cells.

5.2. Kell and KX antibodies

Anti-K is the commonest immune red cell antibody outside the ABO and Rh systems, accounts for almost two thirds of non-Rh immune red cell alloantibody. It is usually IgG, reactive in the antiglobulin phase, and has been implicated in severe HTR and HDN. Naturally occurring IgM

examples of anti-K are rare and have been associated with bacterial infections [12].

Anti-k is rare due to the rarity of k-negative subjects. It is associated with mild HDN.

Other antibodies of the K system (anti—Kp^a and Kp^b) are rare but some may be found as autoantibody [12].

5.3. The Lewis system

The Lewis system differs from most of the other human blood group systems. It is a system of soluble antigens in saliva and plasma. Red cells acquire their Lewis phenotype by adsorbing its substances from the plasma.

There is an association between Lewis substances and the ABH secretor status [13]. The Lewis system is not considered a significant system in transfusion medicine, it has significance at the tissue level for the establishment of a biologic relationship between blood group antigens and diseases, e.g. Leahas receptors for *Helicobacter pylori*, a micro organism causes a variety of diseases including gastric and duodenal ulcers [13].

6. Haemolytic Disease of the Fetus and Newborn (HDFN)

HDFN is a condition in which the life span of the infant's red cells is shortened by the action of specific antibodies derived from the mother by placental transfer [14]. The disease begins in intra-uterine life and may result in death in utero. In a liveborn infant, the haemolytic process is maximal at the time of birth, however, jaundice and anaemia become more severe after birth. HDN was first described in a set of twins in 1609 by a French midwife. One was hydropic and stillbirth; the other became intensely jaundiced and died at three days of age, undoubtedly of kernicterus [14].

The transfer of antibodies from mother to fetus takes place only via the placenta. The only immuno globulin transferred is IgG, which is bound and transported by an FC receptor. Virtually all antibodies reactive in an indirect antiglobulin test (IAT) have been implicated in HDN Whenever an IAT-reactive antibody is detected during pregnancy, a cord sample should be tested by a direct antiglobulin test (DAT), and, if positive, the haemoglobin and bilirubin levels should be monitored to diagnose HDN.

7. The Pathophysiology of HDN

Occurrence of HDN as a result of red blood cell alloimmunization secondary to pregnancy involves three key stages. First, a paternally derived red blood cell antigen foreign to the mother must be inherited by the foetus. Second, the volume of foetal red cells that gain access to the maternal circulation must be sufficient to stimulate an immune response in the particular individual. Finally, maternal antibodies to foetal red cells must gain transplacental access and cause immune destruction of sensitized red cells by Fc receptor-bearing effector cells in the foetus and neonate [15]. HDN is often classified into three categories, on the basis of the specificity of the causative IgG antibody: D haemolytic disease caused by anti-D

alone or, less often, in combination with anti-C or anti-E, other haemolytic disease caused by antibodies against other antigens in the Rhesus system or against antigens in other systems; anti-c and anti-K are most often implicated, and ABO HDN caused by anti-A or anti-B [15].

7.1. Rh-D HDN

In 1939, Levine and Stetson first described the involvement of the Rhesus factor in erythroblastosis fetalis.¹⁶ Despite the use of Rh immunoglobulin, anti-D is still a common antibody identified in alloimmunized women [16].

1. If an Rh-positive foetus is ABO compatible with its mother the risk of Rh immunization is 16% and if ABO incompatible the risk is 1.5–2% [17]. Concurrent ABO incompatibility offers the mother protection against immunization presumably because leaked foetal red cells are promptly coated by circulating isohaemagglutinins (IgM) and probably also by complement, and then removed from the circulation by the mononuclear phagocyte system (MPS), mainly in the liver, which is less immuno responsive than the spleen and therefore is less likely to stimulate antibody production.

2. The likelihood of anti-D appearing in the maternal circulation depends on the size of transplacental FMH and the Rh phenotype of the foetal blood. Although the average FMH occurring at delivery is less than 1 ml of wholeblood, approximately 50% of all women with ABO-compatible pregnancies have detectable circulating foetal red cells [17]. By the end of the third trimester, anti-D may be detected in sera from less than 1% of D-negative women bearing D-positive foetuses. After the birth of a first D-positive infant to a D-negative mother, the chance of maternal anti-D formation can be related to the number of foetal red cells demonstrable in the mother's circulation at the time of delivery. When no foetal cells are detectable, anti-D is found in only about 3% of cases, whereas when the amount is 0.1 ml or more anti-D is found in about 31% of cases. In the absence of Rh prophylaxis, about 16% of Rh negative women will become immunized as a result of their first Rh-positive ABO-compatible pregnancy. Of those women who become immune, about half have a detectable anti-D about 6 months after delivery, and half mount a risk secondary response in a subsequent Rh-positive pregnancy, indicating that primary immunization had occurred [17].

3. Infants with R2r phenotype are more effective in sensitizing their mothers to RhD than are infants of other phenotypes, since the R2 phenotype expresses most D antigen [18].

4. The pregnant mother's immune responsiveness influences the immune response to RhD-positive cells. Some women produce potent anti-D in a first pregnancy sufficient to cause severe haemolytic disease but usually no first child of an RhD-negative woman will be affected, unless the mother has been sensitized as a result of a prior miscarriage or abortion or, rarely, by a sensitizing event earlier in the pregnancy [18]. The Rh-positive firstborn is not

affected because the mother has not yet being immunized. After immunization, all subsequent offspring inheriting the D antigen will be affected.

7.2. Clinical Manifestations and degrees of severity

The disease due to anti-D shows a wide spectrum of severity. Not all D-positive infants born to mothers with anti-D in their serum are affected by HDN. About 50% of Rh positive newborn infants with Rh (D) HDN are somildly affected that they require no treatment [19]] Approximately 25% will be born near term, in good condition, but without treatment will become extremely jaundiced and either die of kernicterus (90%) or will be left severely damaged with neurosensory

deafness, spastic choreoathetosis, and some degree of intellectual retardation(10%). The remaining 20 to 25% are so severely affected that they become hydrotic in utero, about one half before 34 weeks gestation, occasionally as early as 17 to 18 weeks gestation; the other half between 34 weeks and term.

In early 1940's before any treatment measures were available, the perinatal mortality rate from Rh-D HDN was 50% [19].

7.3. Predicting the severity of HDN

Several techniques that measure or characterize antibodies in the maternal circulation have been reported to predict the disease severity. These assays may be serological, quantitative, or cellular.

7.4. Serological assays

Up to 1961, antibody titers and history of HDN in previous pregnancies were the only arameters available to predict severity of HDN before delivery, although they are only 62% accurate in predicting severity of HDN [20].

7.5. Quantitative assays

Levels of maternal anti-D are quantified by different assays such as auto analyzer, radiometric antiglobulin test, flow cytometry, and enzyme linked immunosorbent assay (ELISA). These assays, although they are correlating with disease severity better than antibody titration, fail to distinguish between mild and severe HDN

7.6. Cellular assays

Cellular assays, that generally measure the efficiency of the maternal antibody to react with mononuclear phagocytes, include antibody-dependent. cytotoxicity (ADCC), monocyte

monolayer (MMA), and chemiluminescent (CLT) assays.

It now seems generally accepted, based on the results of several comparative studies, that the ADCC test and CLT offer the best predictive value [20].

8. Prevention of Rhesus D alloimmunization

Immunization of the D antigen can be prevented by the administration of Rh immunoglobulin there before or shortly after exposure to Rh-positive cells. This dose of immunoglobulin has three mechanisms of action: antigen blocking (i.e. competitive inhibition) by attaching to or covering antigenic sites on the Rh-positive red cells; clearance and antigen deviation; central inhibition by the generation of antigen-specific suppressor cells. Despite this; some investigators believed that the precise mechanism is still unclear. The percentage of anti-D immunization decreased to 0.7-2.5% in the various countries after the introduction of anti-D immuno prophylaxis. Initial studies proved that the postpartum administration of a single dose of anti-D immune globulin to susceptible RhD-negative women within 72 h of delivery reduced the alloimmunization rate by 90% [20]. Rh immunoglobulin (RhIg) is a concentrate of predominantly IgG anti-D derived from pools of human plasma. A full dose of anti-D (300- μ g-1500IU) is sufficient to counteract the immunizing effects of 15 ml of D-positive red cells; this corresponds to approximately 30 ml of fetal whole blood.

RhIg prophylaxis is administered to unimmunized Rh-negative women following events that might allow fetal red cells to enter the maternal circulations, i.e. delivery, spontaneous or therapeutic abortion, ectopic pregnancy, amniocentesis, chorionic villus sampling, cordocentesis, antepartum haemorrhage, blunt abdominal trauma, and fetal death (Engelfriet CP et al 2003). Antepartum RhIg at or after 28 weeks is also recommended [21]. Massive FMH can lead to immunization as the standard dose of RhIg fails to cover this excess of amount. A screening test such as the rosette technique should be used, and, if positive, quantification of the haemorrhage must be done by Kleihauer-Betke test or by flow cytometry [21]. The failure of anti-D Ig prophylaxis related to increased FMH and/or insufficient anti-D Ig levels.

8.1. HDN due to antibodies other than anti-D

Although anti-D was once the major etiology of HDN, the widespread adoption of antenatal and postpartum RhIg has resulted in a marked decrease in the prevalence of alloimmunization to the RhD antigen. Maternal alloimmunization to other red cell antigens continues to play a role as the cause of fetal disease since no prophylactic immunoglobulins are available to prevent the formation of these antibodies. It is concluded that the number of pregnant women with antibodies other than anti-D exceeded those with anti-D. Severe HDN caused by antibodies other than anti-D is associated with anti-K, and anti-c.

An increasing incidence of anti-K has been noted in the United States, a trend not seen in the other countries.

8.2. Other Rh antibodies

Anti-c: Of non-anti-D, anti-c is associated with most neonatal morbidity it was found in 177 of 280000 pregnancies during a period of 8 years of these, there was one neonatal death, two infants severely anaemic, eleven required an exchange transfusion. women during the period 1953-1973 had anti-c in their sera were pregnancies alone responsible for the immunization and some affected infants had a serious degree of haemolytic disease.

In a more recent study from the USA, 55 pregnancies with anti-c managed at the Ohio State university from 1967 to 2001 for anti-c isoimmunization. Of the 55 pregnancies, 46 had fetuses with positive direct antiglobulin test, 12 had serious HDN [22].

Anti-C: A study of maternal blood samples from 280000 pregnancies in an 8-years period had shown 38 examples of anti-C (without anti-D) sensitization. Among these; two required exchange transfusion for hyperbilirubinaemia and five cord blood samples had a positive direct antiglobulin test [23].

Anti-CW: Anti-CW is relatively common, occurring in about 1 in 1100 pregnant. Anti-CW HDN although not severe, may end in kernicterus with brain damage or neonatal death unless it detected promptly and treated appropriately. Byers and coworkers reported a severe case of anti-CW HDN that required multiple transfusions [23].

Anti-E: Anti-E allo-immunization can cause HDN requiring prenatal intervention. A total of 283 pregnancies were identified with anti-E in a period from June 1959 to April 2004. Of these, five of 32 (15%) fetuses had Hb less than 10g/dl and 1 fetus had hydrops fetalis due to anti-E allo-immunization

Anti-e: rare case of severe HDN associated with maternal antibody to Rh e was described by McDams and associates. In addition to severe anaemia, the infant developed thrombocytopenia and conjugated hyperbilirubinaemia

Anti-Rh29: Anti-Rh29 implicated in a severe case of HDN when a Brazilian woman (gravida 4, Para 2) was delivered of severely anemic child with strongly positive DAT and requiring two exchange transfusions within 24h of birth [23].

Anti-R17: In a case described by Denomme and associates, a group B RhD positive woman with a history of HDN due to anti-R17 presented to the obstetrical clinic at 12 weeks gestation for management of her third pregnancy. Her serum contained strongly reactive anti-R17

8.3. Kell antibodies: Kellallo immunization is the second major cause of fetal anaemia, with a reported and still increasing incidence. In the fetus, Kell antibodies cause suppression of Kell-positive erythroid precursor cells, a mechanism different from D haemolytic disease where the A antibodies cause destruction of RBCS (Marije M.K *etal*2008). Another feature of anti-K HDN is a poor correlation between the severity of the disease and the titer of antibody in the mother serum [23].

Anti-K: Maternal anti-K was found in 127 of 127076 pregnancies during a 16 year period. Thirteen of them ended with a Kell-positive newborn infant, five had a poor perinatal outcome (hydrops, intrauterine or neonatal death, Hb less than 7.9 gm, congestive heart failure) In a 10-year period, 407 of 350000 pregnancies showed maternal anti-K immunization, 88% of these gave history of previous transfusion

Anti -k: Bowman and coworkers reported erythroblastosis fetalis produced by anti-K so severe that three intrauterine fetal transfusions were required

8.4. Other antibodies of the Kell system: Gorlin JB and Kelly L reported a case of clinically significant hemolytic disease of the newborn due to Kpballo-immunization requiring obstetric intervention. Severe HDN caused by anti-Jsb reported by Gordon *etal*.1995. A patient with high titred anti-Jsb causing fetal hydrops in one pregnancy followed by a pregnancy treated with multiple intrauterine fetal transfusions [24].

8.5. Antibodies belong to the other blood group systems

8.5.1. Anti-Fya: Greenwalt and colleagues (1959) described 11 cases of anti-Fya HDN In a review of recent eriences, 68 pregnancies with anti-Fyawere detected. Three were identified where the fetus was severely anemic; in two cases the fetus received intrauterine transfusions. [24].

8.5.2. Anti-M: Stone and Marsh (1959) described a severe haemolytic disease due to anti-M affecting twins. One child was still born and the other responded to exchange transfusions [24]. Another severe anti-M HDN case described by Matsumoto H *etal*. A woman with repeated still births had a high tittered anti-M antibodies formed during incompatible pregnancy and crossed the placenta.

8.5.3. Anti-U: A case has been reported of death in utero associated with maternal immunization to the U antigenic determinant

8.5.4. Anti-PP1Pk: A newborn infant suffering from haemolytic disease was born to another of the very rare genotype pp. The disease was severe enough to require exchange transfusion.

8.5.5. Anti-JKa: In 1959, Matson GA and coworkers described a case of severe anti-JKa HDN,

where a baby developed kernicterus and was given an exchange transfusion. His mother had five previous pregnancies and gave no history of having received transfusions [25].

8.6. Antibodies to low frequency antigens (700 series): Some antibodies in this series have caused HDN. A severe HDN caused due to anti-ELo needed an exchange transfusion has been reported

8.7. Antibodies to high frequency antigens (901 series): Of these, anti-MAM has caused severe HDN. Three women have been identified with an antibody to a high frequency antigen (MAM)

8.8. Multiple maternal antibodies : Pregnancies complicated by more than one antibody may suggest a worse clinical situation, i.e. significantly increase the need for intrauterine fetal transfusions. Evaluation and management of patients with multiple maternal antibody immunization is unclear. The presence of anti-D appears to be the most significant factor guiding the course of isoimmunization with multiple antibodies

8.9. ABO haemolytic disease: ABO haemolytic disease of the newborn is limited to mothers with blood group type O whose babies are group A or B. Maternal ABO IgG antibodies have the ability to cross the placenta leading to fetal red cells destruction but rarely leading to severe anaemia. The mother's history of prior transfusions or pregnancies seems unrelated to the occurrence and severity of the disease,

thus ABO HDN may occur in the first pregnancy and in any subsequent pregnancies [25]. There seem to be two main reasons for low incidence and severity of ABO HDN despite considerable foeto-maternal ABO incompatibility; first, the A and B antigens are not fully developed at birth, and second, A and B substances are not confined to their red cells so that only a small fraction of IgG anti-A and anti-B which cross the placenta combines with the infant's red cells. Prenatal screening for maternal ABO antibodies can demonstrate the presence of IgG antibody but do not correlate well with the extent of fetal RBCS destruction. Therefore, detection of ABO HDN is best done after birth.

9. Diagnosis

9.1. Obstetric history: Information about previous pregnancies or blood transfusions is essential as previous severe disease and poor outcome predict similar findings in the current pregnancy. For women with a history of an infant with hydrops fetalis due to anti-D, there is a 90% or more chance of a subsequent fetus being similarly affected [26].

9.2. Maternal serology: All pregnant women should be tested for ABO, Rh-D typing and for irregular serum antibodies at the initial antenatal visit preferably in the first trimester and repeated at 28 weeks' gestation (prior to administration of prophylactic anti-D). A woman should be classified as Rh-D positive if the test for either D or either weak D is positive.

For antibody screening; at least two separate reagent screening cells covering all common blood group antigens should be used. The test conditions should be able to detect clinically significant IgG alloantibodies that react at 37°C and in the antiglobulin phase. An antibody-enhancing medium such as polyethylene glycol (PEG) or low ionic strength saline (LISS) can increase sensitivity of the assay. If the antibody screen is reactive, the antibody specificity must be determined. Follow-up testing will depend on the antibody specificity.

Cold reactive IgM antibodies such as anti-I, -IH, Lea, Leb, and P1 can be ignored. Other than anti-D, the most common and most significant antibodies are anti-K, -E, -c, -C, and -Fya [26].

The relative concentration of all antibodies capable of crossing the placenta and causing HDN must be determined by antibody titration. This is serially diluted and tested against appropriate RBCs to determine the highest dilution at which a reaction occurs. The method must include the indirect antiglobulin phase using anti-IgG reagent. The true significance of an antibody titer in maternal serum is controversial because some studies show poor correlation between the level of the titer and effects on the fetus.

On the surface of platelets (PLTs), different antigenic systems exist. For clinical medicine, polymorphic structures located on the membrane of PLTs, so-called human PLT antigens (HPAs), are important. Until now, 33 different PLT antigens have been known, of which twelve are grouped in the biallelic systems HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15. HPAs represent single-amino-acid polymorphisms and are immunogenic, and are therefore able to provoke an immune response in pregnancy and after transfusion [26].

In pregnancy, the immune response is based on maternal and fetal HPA disparity. Maternal alloantibodies can cross the placenta and cause neonatal alloimmune thrombocytopenia (NAIT). These antibodies attack fetal PLTs and are responsible for their shorter survival and an associated bleeding tendency ante- and postnatal. The main cause for NAIT and post transfusion purpura among whites is an incompatibility in the HPA-1 system, whereas among Asian populations, the incompatibility is mainly found in the HPA-4 system. It has been shown that in 10.6% of the HPA-1a-negative mothers, which carry a HPA-1a-positive fetus, an antibody is detectable. Possible antenatal treatments are the injection of intravenous immunoglobulin to inhibit anti-HPA-immunoglobulin G, together with or without steroids and intrauterine PLT transfusions. PLT donor concentrates need to be washed, and thereby plasma-reduced, to avoid volume overload of the fetus. In addition, Kjeldsen-Kragh et al. recommended a screening program to identify immunized HPA-1a-negative pregnant women and promote cesarean delivery for these women as a way of delivery 2–4 weeks before term.

The knowledge of HPA gene frequency in different populations might be helpful for avoiding alloimmunization and the risks involved. Perhaps it should be considered whether

PLT transfusion should only be performed in the same ethnic group and whether a screening program for women should be developed in the context of prenatal care, taking into account ethnic group. In the future, new serological techniques and molecular typing strategies will hopefully report HPAs unknown until now, and therefore provide better treatment and detection options for alloimmunized patients.

Antigens of the human leukocyte antigen (HLA) system are expressed on white blood cells (WBCs), as well as on PLTs. Alloimmunization in this system can lead to PLT refractoriness, to a lower survival rate, and to an impaired in vivo function of transfused PLTs. The majority of HLA antibodies is 80%–90% directed against HLA class 1 antigens. HLA class 1 antigens are named A and B antigens [26].

Other causes for PLT refractoriness are alloimmunization against HPA, alloimmunization against HLA and HPA together, and autoimmune causes. However, 80% of the major causes are non-immune factors such as disseminated intravascular coagulation, bleeding, sepsis, or fever. An immune response to HLA is provoked by contact with foreign antigens; for example, by fetomaternal blood transfusion during pregnancy, by transplantation, or by transfusion of blood components containing WBCs. Why HLA alloimmunization occurs is not completely understood. The failure or success of these processes depends on the transfused product and the recipient's immune status [26].

Different ways to reduce HLA alloimmunization exist. The most important practice is the reduction or inactivation of WBCs contaminating cellular blood components. This can be achieved by filtration or ultraviolet B irradiation. The Trial to Reduce Alloimmunization to Platelets study showed the benefit of the leukocyte reduction, upon which, in 2008, 19 countries implemented leukoreduction of cellular blood components. However, van de Watering et al. found that two thirds of the patients who had immunoglobulin G antibodies against HLA class 1 in their blood before transfusion developed additional antibodies. Patients having immunoglobulin M or other antibodies not directed against HLA class 1 meant that this problem did not occur. In addition, the authors could not support the widespread opinion that alloimmunization could be prevented by WBC reducing.

An additional method to further minimize the probability of HLA alloimmunization is to use PLT collected from single donors instead of pooled PLT concentrates. However, this procedure is not generally recommended [26].

10. Conclusion

Multi-transfused patients are always at higher risk of alloimmunization and this creates difficulty in their pretransfusion testing. Our data show that alloimmunization to minor erythrocyte antigens and erythrocytes alloimmunization of variable clinical significance, are

frequent findings in Indian patients. We recommend including antibody screening test in routine pretransfusion testing protocols atleast for the patients who are higher risks of alloimmunization and require long-term transfusion dependence. This test may not be cost-effective for all the patients currently in our country. However, it will definitely add significant value in blood safety.

Extensive antigen matching before transfusion of patients should be a routine practice for such patients. Being costly exercise, reference centers facility should be created to provide antigen negative blood to such patients and pool of regular voluntary donors should be screened and developed to provide antigen negative blood to such patients. Considering the different ethnicity and huge population in India, we need regular monitoring of alloimmunization in our patients.

11. References

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