R E V I E W

# Prevention and management of transfusion-induced alloimmunization: current perspectives

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**Abstract:** 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 a 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. When alloimmunization has occurred, it could lead to problems for future transfusions or transplantations.

**Keywords:** transfusion, alloimmunization, prevention

## Red blood cells

Alloimmunization resulting from contact with nonautogenous antigens concerns just a minority of patients,[1] and when it happens, it depends on various factors.[2,3] 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.[4] 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.[4] 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, hydrops fetalis and stillbirth). In 1969, postdelivery application of anti-D-immunoglobulin was introduced in the United Kingdom to prevent this complication.[5] At 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.[5] 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%.[6]

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

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immunogenicity of Rhesus-D (from 0.03%–10%).[7,8] 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.

Alves et al discovered that alloimmunization risk does not depend on patients' gender, whereas Santos et al pointed out a higher alloimmunization rate for females.[9,10] By researching literature, Verduin et al figured out that only women with sickle cell disease develop more RBC antibodies than men, whereas in thalassemia, both genders have the same risk.[11] On the basis of these data, the authors saw no reason for a gender gap in transfusion policy.[11] Alves et al did not find a relationship between alloimmunization risk, age and blood type of the AB0 system;[10] however, development of an alloantibody is more likely when a patient already has an existing antibody.[1] Furthermore, a higher risk of alloimmunization exists when more transfusions are made.[10] This is a major problem for the therapy of patients who are reliant on periodically recurrent transfusions of RBCs, such as patients with sickle cell disease. In this group, the mean alloimmunization rate is approximately 25%.[4]

It is known that in this patient group, alloimmunization against antigens D, C, c, E, e, and Kell occurs frequently.[12] A possibility for decreasing alloimmunization risk after RBC transfusion is to match these antigens as well. However, Chou et al recently found that matched transfusions for D, C, E, and K did not reduce Rhesus alloimmunization when using primarily blood from black donors.[12] Patients who have already formed RBC alloantibodies are at a higher risk of developing hemolytic transfusion reactions because it is sometimes remarkably difficult to identify a second, third, or fourth alloantibody. Future strategies to prevent alloimmunization in this group of patients may include cytokine blockade or the application of immune cell-depleting agents.[13] In general, different precautions to avoid alloimmunization should be taken: the blood group of a patient should be determined as precisely as possible, especially for recipients with preexisting alloantibodies. Before every transfusion, a screening test for alloantibodies should be performed. Recipients must not get RBCs that bar an antigen to which the patient has already formed alloantibodies, even if it is only a weak alloantibody. Furthermore, it is extremely important to examine a patient's transfusion history, especially in the case of polytransfused patients.

## Human platelet antigens

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.[14] Until now, 33 different PLT antigens have been known,[15] of which twelve are grouped in the biallelic systems HPA-1, HPA-2, HPA-3, HPA-4, HPA-5, and HPA-15.[16] HPAs represent single-amino-acid polymorphisms and are immunogenic, and are therefore able to provoke an immune response in pregnancy and after transfusion.[4]

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.[17] The main cause for NAIT and posttransfusion 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.[15,18] 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.[17] Possible antenatal treatments are the injection of intravenous immunoglobulin to inhibit anti-HPA-immunoglobulin G, together with or without steroids and intrauterine PLT transfusions.[19] PLT donor concentrates need to be washed, and thereby plasma-reduced, to avoid volume overload of the fetus.[19] 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.[17]

After transfusion or transplantation, HPA alloantibodies can cause posttransfusion purpura, PLT transfusion refractoriness, passive alloimmune thrombocytopenia, or transplantation-associated alloimmune thrombocytopenia.[14,20] In these settings, patients become

immunized if they are exposed to donor cells bearing a certain HPA the recipients PLTs do not bear. It is known that 8% of the PLT transfusion recipients develop detectable antibodies against PLT antigens after transfusion.[4] When these antibodies lead to a PLT refractoriness, and if this refractoriness is severe, future PLT transfusions may not be helpful for patients with thrombocytopenia.[4] To avoid these complications, it is necessary to avoid contact with foreign antigens.[4] Theoretically, antigen matching between the donor and the recipient in PLT transfusion should be performed. However, until now, no special screening programs have existed. Only already immunized patients are transfused with matched PLTs from selected donors because it is known that frequencies of HPA vary between different populations and ethnic groups, as many studies about the incidence of HPA have been performed.[14,21] 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.[15]

## Human leukocyte antigens

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.[22] The majority of HLA antibodies is 80%–90% directed against HLA class 1 antigens.[22] HLA class 1 antigens are named A and B antigens.[22]

Other causes for PLT refractoriness are alloimmunization against HPA, alloimmunization against HLA and HPA together, and autoimmune causes.[22] However, 80% of the major causes are non-immune factors such as disseminated intravascular coagulation, bleeding, sepsis, or fever.[22] 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.[22] 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.[22]

Surgical patients can develop HLA antibodies after RBC transfusions.[22,23] In these studies, patients received RBCs that were WBC-reduced by filtration or buffy coat depletion. Both methods are precautions to prevent alloimmunization against HLA.[23] However, filtration is much more efficient to reduce WBCs than buffy coat depletion.[23] In RBC transfusion, the length of storage of the RBC component before transfusion is important. Whereas fresh donated RBCs seem to induce HLA alloimmunization, RBCs stored for at least 15 days seem to induce immune tolerance against foreign HLA antigens.[23,24] Mincheff found that 15 days of storage leads to a full disintegration of granulocytes.[24] Storage in protein-free media for 5–7 days results in functional impairment of donor T cells.[24]

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.[25] 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.[22,25] 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.[23] In addition, the authors could not support the widespread opinion that alloimmunization could be prevented by WBC reducing.[23]

An additional method to further minimize the probability of HLA alloimmunization is to use PLT collected from single donors instead of pooled PLT concentrates.[22] However, this procedure is not generally recommended.[22]

When PLT refractoriness has developed, patients with bone marrow failure and a PLT count 10,000/μL are in a life-threatening situation.[26] In this situation, the patient's immune system can be modulated by administration of high-dose immunoglobulins to slow down PLT sequestration by the reticuloendothelial system.[26] On the product side, there is the possibility of using HLA-matched products.[22] This can be done by searching for donors bearing the identical HLA-A and HLA-B antigens as the PLT recipients. An alternative strategy is to specify the HLA antibodies of the PLT recipient and to search for donors not bearing a corresponding HLA antigen. The latter strategy is able to identify much better-suited donors than the former.[27,28]

All in all, HLA matching needs a large typed donor pool and, sometimes, cooperation between different blood donation services.[22]

## Human neutrophil antigens

The next system, which is important in transfusion medicine, is the system of the human neutrophil antigens (HNAs). They are polymorphic structures located in the membrane of neutrophils.[29] Up to now, five HNA-systems (HNA-1–HNA-5) have been characterized.[30,31] In the HNA-1 system, three antigens (HNA-1a, HNA-1b, and HNA-1c) have been described. They are located on the Fc-γ-receptor IIIb glycoprotein.[32] HNA-2–HNA-5 are biallelic systems. It is well known that HNA antibodies are able to provoke transfusion reactions and autoimmune neutropenia.[33] Autoimmune neutropenia is a consequence of autoantibodies in the patients' blood being directed against their own neutrophils, which occurs mostly in infancy.[33]

Transfusion reactions are caused by HNA-specific alloantibodies. One of the most serious adverse events after the transfusion of blood components is the transfusion-related acute lung injury (TRALI). It is caused by HLA antibodies or HNA antibodies in the plasma of different blood components, but also by transfused biologically active lipids, other soluble factors, or transfused leukoagglutinin.[34] Most often, fresh frozen plasma or PLT concentrates are involved in TRALI cases. However, TRALI can also be caused by small amounts of residual plasma in RBC concentrates, most often by anti-HNA-3.[35] The clinical picture of TRALI is defined as noncardiogenic pulmonary edema.[34] Associated symptoms are fever, dyspnea, hypotension or hypertension, and cough.[34] The real incidence of TRALI is difficult to estimate because symptoms were often put on patient's volume overload by given transfusions.[34] In addition, TRALI is almost exclusively seen in severely ill patients, who may have many other causes for acute respiratory failure. To prevent this complication, some blood centers have stopped collecting plasma from female donors, or at least excluded female donors with a history of pregnancy.[36] HNA antibodies can also lead to febrile transfusion reactions, refractoriness to granulocyte transfusion, and neutropenia after stem cell transplantation.[37] They can furthermore be responsible for neonatal neutropenia as a consequence of maternal antibodies against fetal antigens.[37,38] The probability of developing this rare immune response depends, among other factors, on the HNA of mother and child. Frequencies of HNA antigens in different ethnic groups have been examined, and significant differences have been shown.[29,39]

## Summary

Alloimmunization in transfusion medicine is a well-known complication that occurs when the recipient's immune system reacts to a donor's antigens.[22] Alloimmunization problems vary, depending on the different involved antigens and reach from HDN, hemolysis, NAIT, and PLT refractoriness over TRALI to autoimmune neutropenia. Ways to reduce this medical problem lie on the recipients' side as well as on the donor's/product's side. In practive, RBCs are generally transfused compatible in the AB0-system. Matching in the antigen systems D, C, c, E, e, and Kell is also useful.[12] To avoid alloimmunization in other antigen systems, especially when alloimmunization has already occurred, antigen matching (eg, HLA, HPA, and HNA matching) is widely accepted. In addition, a widely accepted, and in some countries implemented, way to avoid alloimmunization on product's side is the universal prestorage leukoreduction of cellular blood components.[22,23] On the patient's side, prevention of alloimmunization is to influence the immune system in several ways.[13,19,26] Because blood antigens differ in different ethnic groups, a future way to reduce immunization may be to transfuse in the same ethnic groups.[21,40]

## Disclosure

The authors report no conflicts of interest in this work.

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