D+ platelet transfusions in D– patients: cause for concern?

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Patients whose RBCs are D– may produce anti-D if they are exposed to D on donor RBCs. Except in emergency situations, patients whose RBCs lack D are transfused with only D– RBCs. Platelets carry no Rh antigens, but platelet units may be contaminated by RBCs that could carry D when these units are collected from D+ donors. The purpose of this study was to determine whether our policy of allowing D+ platelets to be transfused to patients whose RBCs type as D–, without the use of prophylactic Rh immunoglobulin (RhIG), results in D alloimmunization. The transfusion records of all patients who received platelet transfusions from December 2004 to March 2007 were reviewed. Transfusion recipients were evaluated with pretransfusion ABO and D typings, and an antibody screen. Recipients were reevaluated in the same manner before subsequent transfusions. Transfusion records of 114 D– patients were analyzed. Overall, 104 patients received D+ platelets; 67 had repeat antibody screening after transfusion. No patients were shown to make anti-D after platelet transfusion. There was no evidence of D alloimmunization as a result of transfusion of D+ platelets in any D– patient during this study. The data do not support the practice of restricting D– patients to receiving only D– apheresis platelets, even among patients with chronic transfusion requirements. Prophylactic use of RhIG for D+ apheresis platelet transfusions in D– patients also appears to be unnecessary.

Key Words: platelets, Rh alloimmunization, transfusion

We retrospectively reviewed the transfusion records of all patients who received platelet transfusions at University Medical Center (Tucson, AZ) from December 26, 2004, to March 29, 2007. Transfusion recipients were evaluated with pretransfusion ABO and D typings and an antibody screen. All patients who were D– and were transfused with D+ platelets were included in the study regardless of disease status. The data were later censored for patients who only received D– platelets, had no repeat testing after transfusion, died shortly after transfusion, or had repeat testing performed less than 5 days after transfusion. ABO group, D type, and an antibody screen test were performed on all patients before transfusion and again every 3 days if additional transfusions were requested. ABO and D antigens were detected using commercially available reagents following the manufacturer’s instructions. D– patients who were pregnant were also tested with another reagent to detect weak D. The antibody screen test to detect the presence of anti-D was performed by tube method using LISS-IAT and a three-cell screen. For some patients beginning in February 2006, ABO and D typings and four-cell antibody screens were performed by the Galileo instrument (all reagents previously listed and the Galileo instrument were supplied by Immucor/Gamma, Norcross, GA).

Patient age, clinical diagnosis, source of platelets, dates of transfusion, number of units transfused, results of antibody testing, and duration of the period between an incompatible transfusion and the date of the last antibody screen were recorded. Institutional Review Board approval was obtained for all data collection in this study.
Results

One hundred fourteen D– patients received 1085 platelet transfusions during this study. Overall, 104 of the 114 D– patients received D+ platelets. Eighteen of the 104 recipients of D+ platelets had no repeat antibody screen after transfusion, 9 had antibody screens performed less than 5 days after transfusion, and 10 others died within days of receiving the transfusion. Sixty-seven patients who received a total of 866 platelet transfusions had repeat antibody screening after D platelet transfusions. Thirty patients were female and 37 were male. The mean age was 46.5 years (median, 52 years; range, 0.58 months to 90 years), including 13 pediatric patients, who were 0.58 months to 16 years of age. The mean number of transfused platelet units per patient was 12.9 (median, 6; range, 1 to 109). Six of the 67 patients received random donor platelets: 4 received one pool, 1 received five pools, and 1 received three pools. Clinical diagnosis at the time of transfusion varied, including hematologic disorders or malignancies (n = 31), trauma (n = 10), heart conditions (n = 15), organ failure or transplant (n = 9), and other malignancies (n = 2). None were shown to make anti-D.

Currently, the entire platelet inventory at this institution is irradiated; thus all patients receive only irradiated platelet units. During this study, most platelet transfusions given were prestorage leukoreduced apheresis units. Random donor platelets given earlier in the study were not leukoreduced. Only 8 patients of the 114 examined received a total of 14 pools of platelet concentrates prepared from whole-blood donors according to the buffy coat method. Each pool contained five platelet units and was considered the equivalent of one apheresis platelet transfusion. Seven of these 8 patients had an antibody screen performed after the transfusion. Of these 7, 1 had only one antibody screen performed less than 5 days after the transfusion. Therefore only 6 patients receiving 12 random donor platelet transfusions were included in the study population.

It is not possible to estimate the quantity of RBCs in the platelet transfusions administered in this study, but a published report that counted the residual RBCs in similar apheresis platelet units suggested that these units contain $2.7 \times 10^7$ RBCs per apheresis unit or fewer than 90 RBCs per microliter. None of the 67 included patients were obstetric patients, so pregnancy status was not known. As a consequence, none of the patients were tested for weak D.

Three recipients were found to have anti-D that were not thought to be related to platelet transfusions, as described later. A pregnant woman received prenatal RhIG, and afterward she was found to have a positive antibody screen attributable to anti-D before transfusion of platelets. She was not included in the final patient group because her last antibody screen was done the day after her last platelet transfusion. Another woman presented with anti-D and anti-C before any transfusion at this institution. Finally, a D– boy received D+ granulocytes donated by family members, along with prophylactic RhIG. An antibody screen performed 3 months later was positive for true (i.e., not infused) anti-D, despite the use of RhIG.

Discussion

There is little agreement as to whether RhIG immunoprophylaxis is necessary in D– patients receiving apheresis platelet transfusions. This is especially true in infants and women of childbearing age, and few conclusive studies have been published as to the necessity of immunoprophylaxis in these patients. Our data suggest that RhIG immunoprophylaxis is not necessary in D– patients receiving D+ apheresis platelet transfusions. After transfusing more than 800 D-incompatible platelet units during a 27-month period at our institution, we detected no cases of primary alloimmunization. Ten D– patients received only D– platelets. Of the 67 patients included in the final analysis, 65 had subsequent antibody screens that were negative, thus demonstrating no new antibody formation in response to the transfusions. Two patients did have positive antibody screens that demonstrated anti-D, but these antibodies could easily be explained by causes other than the platelet transfusions. Most patients received few platelet transfusions (the median number of transfusions was six), but 5 patients received more than 50 platelet transfusions. Of these, only the patient who also received granulocyte transfusions made anti-D.

Although it is known that D is not expressed on platelets, it is present on RBCs that contaminate units of platelets. This contamination varies depending on the method by which the platelets are prepared. The dose of RBCs required to induce an immune response to D varies among individuals and is influenced by many factors. Studies attempting to quantify the immunogenic dose are limited in size and number. One early trial tested the immune response in healthy volunteers who were treated with small doses of D+ blood. Volumes as low as 0.05 mL were found to be immunogenic in some susceptible individuals. Additional studies on platelet transfusions from the 1970s and 1980s showed that alloimmunization resulting from incompatible platelet transfusions based on older methods of platelet preparation (poled random donor and early apheresis methods) ranged up to 19 percent in patients with malignant disease. Platelet concentrates prepared by early apheresis methods as used in that study contained up to 3 mL of RBCs.

By current methods, whole-blood–derived platelet concentrates contain 0.3 to 0.5 mL of RBCs, and platelet concentrates derived from modern apheresis methods contain trace RBC contamination (0.0002 to 0.007 mL). The rate of seroconversion after low-dose exposure is unknown, but anecdotal evidence suggests it is much lower than what has been observed with larger transfusions. Therefore, contaminant RBCs in modern apheresis platelet units are present in quantities that may be insufficient to incite an alloimmune response. Atoyebi et al. studied serology for the presence of anti-D after D-incompatible platelet transfusions in patients with hematologic and nonhematologic
The patients were transfused with platelets from either whole blood or apheresis methods or both. None of the patients were given prophylaxis with anti-D. Of the patients without hematologic disease, 68 percent received apheresis platelets, with the remainder receiving pooled platelets. Of those with hematologic disorders, 75 percent received apheresis platelets. Thirteen percent of patients without hematologic disease and no patients with hematologic disease formed anti-D. They concluded that the risk of alloimmunization after D-incompatible platelet transfusion using concentrates prepared by modern technical methods appeared to be small in patients with hematologic diseases, but was significant in immunocompetent patients.

A later case report by Haspel et al. described a D− infant who received two D-mismatched whole blood−derived platelet units at 17 weeks of age and was subsequently found to have anti-D on retesting 13 months later. The infant was exposed to less than 0.6 mL of D+ RBCs in these units. Another study by Molnar et al. examined all D-incompatible platelet transfusions administered to pediatric oncology patients during a 1.5-year period, which included 42 patients with various diagnoses. No cases of D alloimmunization were detected. They concluded that D immunoprophylaxis is generally unnecessary in pediatric oncology patients receiving D-incompatible single-donor platelets not visibly contaminated by RBCs. As well, a smaller study by Cid et al. examined 22 immunosuppressed patients receiving D-incompatible pooled platelet transfusions. None of them developed detectable anti-D after a median follow-up of 8 weeks. Thus, in studies that used only prestorage leukoreduced apheresis platelet units, there is no evidence that D− patients developed anti-D when transfused with D+ platelet units.

The most important reason for administration of prophylactic RhIG in D− women or girls with childbearing potential who are receiving D+ transfusions is to prevent HDN with a D+ fetus in future pregnancies. Secondarily, it reduces the need for additional pretransfusion testing owing to positive antibody screens in patients who would otherwise have made anti-D. The clinical practice guidelines of the American Society of Clinical Oncology on platelet transfusions in patients with cancer state, “anti-D prophylaxis should be considered for Rh-D-negative children and for women of child bearing age.” They give this recommendation a “Grade D,” however, which is categorized as “little or no systematic empirical evidence.”

Although generally not life threatening, the complications of RhIG administration include infection and hemotoma formation. Some patients experience low-grade fever, chills, headache, myalgias, flushing, or nausea and vomiting. Depending on the method of production (i.e., cold alcohol fractionation), RhIG may be contaminated with other plasma proteins, including IgA, which may put IgA-deficient patients at risk for developing hypersensitivity reactions, or with aggregates of IgG polymers, which are capable of activating the complement cascade. The formulations created using ion-exchange chromatography have been associated with hepatitis C virus outbreaks in the past. All formulations now include additional virus inactivation steps in their processing to minimize the risk of such outbreaks. One formulation also contains high levels of maltose, which can interfere with blood glucose testing, giving falsely high readings.

In the past, concern had been raised about the levels of thimerosal, a mercury-containing compound, in vials of RhIG. Since 2001, all of the brands of RhIG sold in the United States have been made without thimerosal, so concerns about this compound should no longer be an issue. The risks and benefits of RhIG administration should be discussed with the patient’s clinician. Based on our observations, we propose that there is little benefit to the patient of administering RhIG for platelet transfusions given to D− men or D− women who are incapable of conceiving. This is specially true when quality control measures are taken to ensure the RBC content of apheresis platelets is less than the average quality control dose of less than 0.1 mL RBCs per adult therapeutic dose.

We found no evidence of D alloimmunization caused by transfusion of D+ platelets in any D− patient during this study. Some limitations to our study include small sample size, retrospective analysis, incomplete knowledge of immune status, and lack of serial antibody screening in all patients. A prospective study of sensitization to D by exposure of D− patients to D+ platelets is needed in the future. However, our data do not support the practice of restricting D− patients to D− apheresis platelets, even among patients with chronic transfusion requirements. This restriction may further lead to continued unnecessary D− platelet inventory supply problems. Prophylactic use of RhIG for D+ apheresis platelet transfusions in D− patients also appears to be unnecessary. We have adopted and use this policy in our own patient population; however, we realize other institutions with different patient populations and platelet inventories may prefer a more conservative approach. We invite others to perform additional studies and to examine their current policies.

References


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