Anti-D alloimmunisation following Rh-incompatible platelet transfusions

Saverio Misso¹, Bianca Feola¹, Luigi Paesano², Marcello D’Onofrio³, Giorgio Fratellanza³, Elio D’Agostino³, Mauro Nigro³, Antonio Minerva¹, Salvatore Formisano³

¹ Medicina Trasfusionale, AORN San Sebastiano, Caserta, Italia
² Immunoematologia, Medicina Trasfusionale e Immunologia dei Trapianti, Seconda Università di Napoli
³ Immunoematologia e Medicina Trasfusionale, Università Federico II, Napoli, Italia

Introduction

In recent years the transfusion of platelet concentrates (PC) has increased enormously as a consequence of both the chemotherapy regimens, that are ever more frequently used in onco-haematological patients, and the improvement of intensive therapy and anaesthetic techniques, that have increased the mean life span of patients. Transfusions of PC are indicated both for the prophylaxis of bleeding and for thrombocytopenic patients. PC can sometimes contain enough red blood cells to cause immunisation against antigens of the Rh system. When choosing platelets to transfuse it is opportune, whenever possible, to use units that are compatible with the recipient's ABO and Rh systems. The platelet membrane carries numerous structures with varying degrees of antigenic potential, including substances A and B absorbed from the plasma.

For these reasons, platelets are transfused according to the following criteria:

- platelets ABO-matched to the recipient;
- platelets according to compatibility of plasma or platelets resuspended in a cryoprecipitate solution.

Rh D+ PC should not be transfused to female Rh D- patients who could have children in the future (i.e. girls and women of child-bearing age). The aim of our study was to evaluate anti-D alloimmunisation caused by transfusions of incompatible platelets, produced from platelet-rich plasma, in both immunosuppressed and immunocompetent subjects.

Materials and methods

The 58 Rh D- patients with onco-haematological disorders did not have detectable irregular antibodies prior to transfusion and had not had other exposure to blood components.

All had undergone chemotherapy for their malignancy and some had received conditioning therapy for a subsequent bone marrow transplant.

Supportive therapy was based on transfusion of red blood cell concentrates selected for ABO and Rh compatibility and PC administered regardless of Rh compatibility.

The 45 surgical patients and the 12 paediatric patients, all Rh negative, received Rh-matched red blood cell (RBC) transfusions but unmatched PC (Table I).

The majority of our PC are obtained from single units.
of fresh whole blood through centrifugation and subsequent recovery of most of the platelets, which are then resuspended in 50-60 mL of plasma (Table II). These concentrates can, sometimes, be contaminated by red cells. Each platelet pool was formed of 5 or 6 units of platelets from single donors. The weight of each pool was recorded and then a full blood count was carried out using an automatic Sysmex SF-3000 cell counter (Dasit SpA, Cornaredo, MI, Italy).

The volume of red blood cells was calculated as follows:

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\text{volume of RBC (mL)} = \frac{\text{weight of bag in grams} - 60}{1,030} \times \frac{\text{RBC (x10}^{12})}{90 \text{ fL/1,000}}
\]

where the weight of the bag is the weight of the platelet pool, 60 grams is the weight of the transfer bag, 1,030 g per litre is the density of the platelets, RBC (x10\(^{12}\)) is the result of the full blood count, given by the automatic cell counter and 90 fl is the mean corpuscular volume of a red blood cell. The blood components for all the haematological patients and for the variably immunosuppressed patients were filtered at the bed-side. Fertile women were not excluded from receiving Rh incompatible transfusions, since these transfusions could not be delayed and compatible blood components were not available. Antigens of the ABO and Rh (CcDeEe) systems were determined using commercially available antisera (CLB Amsterdam, The Netherlands).

The antibody screen was carried out using a gel test (Ortho-Clinical Diagnostics, Raritan, NJ, USA and DiaMed AG, Cressiers/Morat, Switzerland).

### Results

The patients were transfused a total of 562 PC from single units of whole blood. ABO compatibility was respected in 43% of the cases. On average every transfusion episode consisted of 5 or 6 units of platelets (platelet pool). In all Rh negative patients an indirect antiglobulin gel-test was carried out at time 0, after 4 weeks and after 8 weeks.

The antibodies that developed were two anti-D (6.5%) and one anti-E (3.12%). Alloimmunisation became detectable by laboratory tests 37 ± 4 days after the first transfusion.

Of the D-negative patients affected by onco-haematological disorders, none became immunised by about 40 days after the first transfusion or by the 3-month follow-up. No neonate developed alloimmunisation.

### Discussion

The request for PC has increased enormously in recent years as a result of increased survival of patients receiving chemotherapy, that requires adequate transfusion support and the practice of carrying out major surgery (heart surgery, vascular surgery, etc.). However, given the lack of available blood, it is often necessary to transfuse platelets that are not matched to the recipient's blood group systems. In the absence of Rh D- PC, Rh D+ PC can be administered to women of child-bearing age, although it is recommended that such recipients are also given anti-D Ig10-15. It is not necessary, on the other hand, to administer anti-Rh D prophylaxis to males, menopausal females or women who are sterile16.

The red blood cells that contaminate PC can cause immunisation in D-negative recipients17-28. The incidence of anti-D alloimmunisation in immunosuppressed patients has been reported to range between 0-19%22,23. In accordance with the incidence reported by some authors14,27,28, we found no cases of
alloimmunisation (0%) among our immunosuppressed patients. This is because onco-haematological patients enrolled in our study received strongly immunosuppressive chemotherapy. The only exceptions were the patients with myelodysplastic syndromes, who did not receive chemotherapy, but whose disease itself was sufficient to cause a state of immune anergy\textsuperscript{29}. Another reason for the lack of alloimmunisation after transfusion of incompatible platelets is that new technology has produced a fairly substantial reduction in the volumes of red cells transfused with the PC, as described by Atoyebi \textit{et al.}\textsuperscript{14}. In our study, every platelet pool contained 0.42 mL of RBC. It is known that a single injection of 0.5-1 mL of D+ red blood cells is capable of causing anti-D in immunocompetent patients\textsuperscript{8}.

The incidence of alloimmunisation in our surgical patients (9.3%), who were immunocompetent, differed from that in the literature. In fact, it has been reported\textsuperscript{3,6-8} that the probability of immunisation, in particular anti-D isoimmunisation, is greater than 80% following incompatible transfusions. However, most of the studies generating these data were based on the observation of Rh-negative volunteers systematically and repeatedly immunised with Rh-positive red blood cells (for industrial production)\textsuperscript{10} or Rh-negative patients transfused numerous times with PC contaminated with Rh-positive red blood cells\textsuperscript{17-21}. The only report that adds some support to our work is a study by Frohn \textit{et al.}\textsuperscript{19}, in which immunisation occurred in 30.4% of 78 Rh D- patients. From our observations, despite the limited sample size, and from a review of the literature, we can draw the following conclusions: the risk of alloimmunisation from incompatible platelet is low, but does exist; this risk is correlated with the volume (>0.03 mL) of red cells contaminating the PC\textsuperscript{19-20}; and might be inversely related to immunomodulation due to massive or prolonged transfusion therapy\textsuperscript{21}. 

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References


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