Anti-D alloimmunization after D-mismatched allogeneic hematopoietic stem cell transplantation in patients with hematological diseases


In the case of a D-mismatched allogeneic haematopoietic stem cell transplantation (AHSCT), two situations can occur. A D-positive recipient of D-negative stem cells will produce D-negative red blood cells (RBC). In this situation, the patient could develop de novo anti-D alloantibodies, because D-positive RBC may persist in the blood-stream for at least 4 months. The opposite situation occurs when a D-negative recipient receives D-positive products. In this case, the recipient is unlikely to develop de novo anti-D, as the only lymphocytes capable of producing anti-D would have been destroyed by the myeloablative treatment. Transfusion of D-negative blood products has been recommended for the former situation, but anti-D immunisation has been little studied. The aim of the study by Cid and colleagues, carried out in the Bone Marrow Transplant Unit of Barcelona (Spain), was to analyse anti-D formation after D-mismatched AHSCT. Thirty patients, who underwent D-mismatched AHSCT, were retrospectively studied. Support therapy included RBC and platelet concentrates (PC) from whole blood donation or plateletpheresis. Before transplantation, D-negative recipients received only D-negative RBC, whereas D-positive recipients received either D-positive or D-negative RBC. After transplantation, all but two patients received D-negative RBC. In contrast, both before and after AHSCT, PC from D-positive donors were transfused into D-negative recipients, because of logistical constraints, without Rh immunoglobulins being administered. Antibody screening to detect anti-D antibodies was performed using a LISS indirect antiglobulin test.

From January 2000 to December 2004, 494 AHSCT were performed in patients with haematological diseases. In 30 cases (17.8%) a D-mismatch was present. The series comprised 12 (40%) men and 18 (60%) women. Fifteen D-positive patients received haematopoietic stem cells (HSC) from D-negative donors and 15 D-negative patients received HSC from D-positive donors. D-positive patients received 682 (83%) of 825 PC from D-positive donors and D-negative patients received 573 (85%) PC from D-positive donors. None of the 30 patients developed anti-D antibodies after a median follow-up of 32 weeks (range 4-310 weeks). In conclusion, anti-D alloimmunisation, after a D-mismatched AHSCT in patients with haematological diseases, is infrequent. D-positive patients who have undergone AHSCT from D-negative donors should be transfused with D-negative RBC, while, in the same patients, the use of PC from D-positive donors seems a reasonable and safe option.

Red blood cell antibodies after transfusion: factors influencing incidence and specificity


Subjects exposed to red blood cell (RBC) alloantigens through transfusion, pregnancy or transplantation may produce antibodies. The recipient's immune system reaction depends on several factors: genetic and acquired patient-related factors, dose and route of administration, and the immunogenicity of the RBC antigen, but the exact kinetics are still unknown. A 5-year retrospective multicentre study (from 1999 to 2003), analysing factors influencing the incidence and the specificities of alloimmunisation against RBC was carried out, by searching the computer databases from 19 hospitals within the Dutch Sanquin Blood Bank South West Region. Special attention was given to the time interval between the transfusion...
event and antibody detection. Clinically significant antibodies against antigens of the Rh, Kell, Duffy, Kidd, and MSs systems were included in the study, whereas auto-antibodies, anti-D antibodies, and Abs to low- and high-frequency antigens were excluded. New antibody specificities (n=2,177) were found in 1,778 patients: (the female-to-male ratio was 2.2, the mean age of the subjects was 64 years and the time interval after transfusion ranged from 1-101 days). Multiple new antibody specificities (2-5) were detected in 284 patients. Multivariant analysis, involving the 1,778 immunised subjects, revealed that the time interval between the transfusions and antibody detection was strictly correlated with the antibody specificity. As is well known, anti-Kidd antibodies (both anti-Jk^a and anti-Jk^b) tend to disappear within 2-3 months after the immunising exposure. For this reason, Kidd antibodies are frequently involved in delayed haemolytic transfusion reactions, in which a booster response is held responsible. In contrast, anti-K and anti-Fy^a were the most frequently encountered antibodies more than 5 years after transfusion. New antibodies were detected within 14 days in 299 patients (16.8%), and after more than 14 days in the other 1,479 patients (83.2%). During the 5-year study period, 16,126 patients received transfusions. Antibody screening was performed 1 to 2 days before scheduled transfusions in 20% of these patients and on the day of transfusion in the other 80%. Almost 50% of patients (8,004) were retested for alloimmunisation because of new transfusions, resulting in the detection of 165 new antibodies in 150 patients. In conclusion, the time interval between transfusion and a positive antibody test is strongly associated with the RBC antibody specificity. Antibody tests are rarely performed after transfusions, and in the case that antibodies are detected for the first time on the occasion of a new transfusion or have become undetectable, they put the recipient at risk of a delay in administering the transfusion or a delayed haemolytic reaction. Routine antibody screening at set intervals after transfusion would reduce these risks.

**Apelseth TO, Hervig TA, Wentzel-Larsen T, et al.**

*Cytokine accumulation in photochemically treated and gamma-irradiated platelet concentrates during storage* Transfusion 2006; 46: 800-10.

Platelet- and white blood cell (WBC)-derived mediators (cytokines) in the supernatant of platelet concentrates (PC) are involved in febrile non-haemolytic as well as allergic transfusion reactions and may contribute to pro-inflammatory or prothrombotic effects of platelet infusions. The levels of these cytokines are determined by the balance between mediator release (induced by active secretion combined with cell destruction) and their binding and/or degradation. Photochemical treatment for pathogen reduction of PC affects all cells containing DNA and/or RNA, and such treatment may affect the levels of cytokines. Apelseth *et al.* (from the Blood Bank of Bergen, Norway) compared mediator levels in photochemically treated (PCT), gamma-irradiated, and untreated PC.

Ten double-dose single-donor leukoreduced PC were split in two identical units. The study had two arms: arm A consisting of five PCT-PC and five untreated control PC; arm B consisted of five PCT-PC and five gamma-irradiated control PC. PCs with added PAS-III (Intersol) were treated with amotosalen and ultraviolet A light. Corresponding control PC, to which PAS-II (T-sol) was added, received no treatment or were gamma-irradiated before storage. The platelet-derived cytokines studied were: CCL5/RANTES, CXCL4/PF4, CCL3/MIP-1α, transforming growth factor (TGF)-β, CXCL8/interleukin (IL)-8, and IL-1β. The WBC-derived cytokines studies were: IL6, IL-10, IL-11, IL-12, tumour necrosis factor (TNF), and interferon-γ. The analyses were carried out, during storage for up to 12 days, using ELISA and cytometric bead assays. Independently of their prior treatment, it was observed that all PC showed low levels of WBC-associated mediators, while platelet-associated cytokines were detected at higher levels and showed significant increases during storage. Statistical analysis revealed that PCT-PC had lower platelet contents per unit, higher levels of activation variables, and higher levels and accumulation rate of CCL5, CXCL4, TGF-β, and CXCL8. In conclusion, platelets are the main source of cytokines released during storage of untreated, gamma-treated, and PCT-PC. Photochemical treatment may affect the levels of platelet-associated cytokines. No additional reduction of WBC-derived cytokines was observed after photochemical treatment of prestorage leukoreduced PC.

**Engelfriet CP, Reesink HW (Editors)**


In spite of the fact that allogeneic blood transfusion has become very safe as a result of the careful selection of donors and extensive testing for infectious markers, perioperative blood salvage is still widely used to reduce patients’ exposure to homologous blood. Potentially important reasons for avoiding allogeneic blood transfusion (an increased risk of recurrence of cancer and of
postoperative infections) are still controversial. Furthermore, there is some doubt regarding how much the use of salvaged blood does, in fact, reduce the amount of homologous blood transfused. For these reasons, it has been questioned whether there are still indications for the use of salvaged blood.

In their quality of editors of the Vox Sanguinis International Forums, Engelfriet and Reesink asked some experts in the field the following questions. 1. Do you think that the use of untreated (i.e. unwashed, non filtered) intraoperatively salvaged blood is still acceptable, and what are the indications for "treated" intraoperatively salvaged blood? 2. Do the same restrictions and indications apply to postoperatively collected salvaged blood? 3. Which of the methods to treat salvaged blood do you apply: washing, filtration, or both? 4. Do you think that the various factors (lipids, cell debris, red cell ghosts, activated coagulation factors, etc), which may be responsible for untoward reactions in the recipient, are sufficiently removed by the above-mentioned methods? 5. Do you apply a system of quality control (e.g. determining the plasma elimination rate)? 6. It has been reported that activated leucocytes and platelets are not removed by washing or filtration. Do you think that the various factors present in "treated" salvaged blood might induce adverse reactions of clinical importance, but such adverse effects had not been seen by the contributors.


Although commercial preparations of (relatively) pure fibrinogen are available, cryoprecipitates still widely used in the production of fibrin glue. Fibrin glue can be made by mixing cryoprecipitate and thrombin. The first potential application of fibrin glue was described in 1982 in the field of oral and maxillofacial surgery (Matras et al. J Oral Maxillofac Surgery 1982; 40: 617-22). Since then, this preparation has found wide application in plastic, orthopaedic and general surgery. Interest in this use of cryoprecipitates has been renewed recently, because of concerns about pathogen transmission from any pooled, commercial products. Furthermore, the more expensive commercial products contain aprotinin, which slows down the physiological absorption of fibrin glue. In some circumstances, in order to enhance the content of platelet–derived cytokines, platelets are added to the plasma before the preparation of the cryoprecipitate. In a study performed at the University of Ottawa (ON, Canada), Rock et al. compared the constituents of cryoprecipitates made from platelet-rich plasma (PRP) with those of cryoprecipitates made from platelet-poor plasma (PPP). Fresh plasma was collected by apheresis from the same donor to produce 250 mL of PRP and 250 mL of PPP. Cryoprecipitates were then produced, following the usual standards, and resuspended to a total volume of 8 mL, from which aliquots were removed and tested. Clot formation was measured using a thromboelastograph. The protein contents of the two
preparations formed from PRP and PPP were identical with regards to fibrinogen, factor VIII, and von Willebrand factor. In contrast, the concentration of platelet-derived growth factor (PDGF) was markedly higher in the cryoprecipitated from PRP (3,778±1,035 ng) than in that from PPP (45±50 ng). The delivery of PDGF in fibrin glue in circumstances such as maxillofacial surgery, in which the preparation is used to promote tissue regeneration, has considerable benefit, while in other circumstances, such as plastic surgery, in which the main requirement is for wound healing, the benefit is moderate. In conclusion, platelets do not significantly increase the concentration of the usual constituents of cryoprecipitates; however, the level of PDGF is markedly enhanced. Therefore, there are advantages from using cryoprecipitates produced from PRP to enhance the growth of new tissue.