CORRESPONDENCE

Red cell transfusion in alloimmunised allogeneic stem cell transplant patients

To the Editor,

Despite high transfusion rates during induction treatments for haematological malignancy, preceding clinically significant alloimmunisation is a rare complication affecting stem cell transplantation. This is due to the low statistical likelihood of a transplant recipient possessing a rare red cell phenotype and a clinically significant antibody. There are few cases reported of non-D Rhesus alloantibodies,^{1,2} and this along with the unpredictability of peri-transplant red cell immunogenicity results in minimal published evidence around optimal management of such cases.

Routine extended phenotype matching for Rh and Kell antigens for all haematology patients receiving chemotherapy is adopted in many centres, including ours. The goal of this practice is to minimise allo-antigen exposure and thus antibody development in patients requiring recurrent transfusion, or who may proceed to allogeneic transplant in the future. However, this does not overcome the issue of pre-existing antibodies, seen most commonly in multiparous women or those who have been transfused prior to a haematological diagnosis.

ABO blood group mismatched transplants are commonly performed, as blood group compatibility is a secondary consideration in donor selection for allogeneic stem cell transplant in haematological malignancy. The impact of ABO compatibility on outcome has diminished in the recent years due to the decreased use of bone marrow grafts.³ Blood group testing is routinely performed for ABO and D antigens; however, it is not routine to perform an extended phenotype on donor's red cells due to the low incidence of recipient antibodies. Routine extended phenotype matching for Rh and Kell antigens for all haematology patients receiving chemotherapy is considered the standard of care in Australia.⁴ This is performed to minimise antigen exposure and antibody development in patients requiring recurrent transfusion related to their cancer treatment.

The rate of clinically significant, predominantly Rhesus group, alloantibodies amongst pregnant women has been estimated in large population studies to be ~0.7–0.75%.⁵ Haematology patients generally receive extended phenotype matched blood which markedly reduces, however does not eliminate, the risk of developing clinically significant alloantibodies. This risk is higher in light of the low prevalence of alloantibodies; extended phenotype matching is not routinely performed for haematological stem cell donors, although this has been previously recommended.⁶ As a result, rare cases such as this can occur, where a clinically significant antibody may present challenges in transfusion support for patients undergoing allogeneic stem cell transplant.

Pre-transplant strategies to complement transfusion support are generally reported. Plasmapheresis for reduction of antibody titre pre-transplant for ABO incompatible transplants is a long established practice which can be easily extended to non-ABO red cell alloantibodies, although the

utility of this practice is contentious. While the titre of antibody will transiently decrease, whether this has any meaningful effect on subsequent haemolysis is unclear. Alternative methods of pre-transplant alloantibody titre reported in the literature include rituximab administration,⁷ use of immunosuppression such as prednisone,⁸ or even the more daring proposal of *in vivo* immunoabsorption by donor red cell transfusion prior to transplant.⁹ The proteasome inhibitor bortezomib has also been used successfully in a case of refractory post-transplant haemolysis due to ABO incompatibility.¹⁰

A 55-year-old female presenting with pancytopenia was diagnosed with favourable risk acute myeloid leukaemia [nucleophosmin 1 (NPM1) positive, FMS-like tyrosine kinase 3 internal tandem repeat (FLT3-ITD) negative] per European Leukaemia Network criteria in 2017.¹ Her medical history was significant for Type 2 diabetes mellitus, hypertension and hypercholesterolaemia. The patient had a history of four completed uncomplicated pregnancies, with no haemolytic disease of the foetus and newborn or haemolysis. The patient had no prior transfusions. At initial presentation, her ABO group was A positive, with an Rh phenotype of Cde/Cde, Kell negative. Anti-c and anti-E antibodies were detected at presentation via DG Gel Card (Grifols, Spain) column agglutination method. The only immune sensitising event identified was pregnancy, and she was of Italian ethnicity.

She underwent induction chemotherapy with 7:3 (idarubicin and cytarabine) and two high-dose cytarabine consolidation cycles and successfully achieved a minimal residual disease negative remission. During this time, she was transfused with 23 units of packed red cells, all of CDe phenotype. Unfortunately 18 months later she developed progressive pancytopenia, and bone marrow aspirate was conclusive for therapy related myelodysplasia with re-emergence of NPM1 and new cytogenetic abnormalities (der(17)t(1;17)(q21;q25). The patient was planned for a fludarabine and melphalan reduced intensity conditioned matched unrelated allogeneic stem cell transplant.

Donor Rh phenotyping was undertaken due to the patient's anti-c antibody, identifying a clinically significant Rh incompatibility (cde/cde), with scarcity of suitable red cell donors. The patient's anti-c antibody was quantified at 13.8 IU/mL using the Astoria-Pacific analyser (bromelain methyl cellulose method) at the NSW Lifeblood reference laboratory. Three sessions of plasmapheresis were performed on days –4, –3, –2 of the transplant, depleting the anti-c antibody to 3.3 IU/mL (titre 1:32). At transplant there was a non-significant rise to 1:64 (Fig. 1).

Due to scarcity of compatible red cell products, the transplant centre liaised closely with the Australian transfusion reference laboratory, Lifeblood, and a strategy of pre-transplant transfusion and a conservative post-transplant haemoglobin goal (haemoglobin >7 g/dL) using Cde/cde blood was adopted. Close monitoring for haemolysis, with frequent elution and antibody quantification was pursued.

During her inpatient stay, seven units of O negative red cells were infused. Five units of matched Cde red cells were available through scheduled donation, followed by two units

Fig. 1 Anti-c antibody quantification across transplant.

of Cde/cde blood. On day +31, anti-c was eluted from the patient's red cells. During this time, biochemical markers of haemolysis as well as haemoglobin level were consistent with a mild degree of haemolysis. Haptoglobin was 1.9 g/L on day +21, and dropped to <0.1 g/L by day +32, rising again on day +49, and 1.1 g/L on day +61. Other biochemical markers of haemolysis such as lactate dehydrogenase were unreliable in the setting of clearance on dialysis commenced for oliguric acute renal failure secondary to sepsis.

Seven months following transplant, the patient's Rhesus phenotype switch was confirmed as cde (Fig. 2).

Following review of the available literature, we considered several risk reduction and management strategies for our patient.

Option 1: Fully Rhesus phenotype matched blood. This is obviously the desired approach which eliminates risk of the alloantibody causing haemolysis of donor cells. Alloantibodies against common Rhesus antigens present a challenge in the availability of compatible donors. Cde phenotype occurs in <0.2% of Australian donors, hence the difficulty in obtaining compatible blood for our patient. The availability of suitable donors more generally depends on the ethnicity of the recipient.

Option 2: Lowest possible antigen doses. In the event that fully compatible blood cannot be identified, one strategy is to use blood with the lowest antigen dose. In our case this would mean use of Cde/cde blood, and the chance of developing significant haemolysis with this blood type was thought to be low. However, this phenotype occurs in <1% of Australian donors so would not necessarily ensure supply.

Option 3: Ignore the D antigen. It is accepted practice that O positive blood can be transfused to ABO untyped men and women who are not of childbearing potential, in certain clinical situations. One strategy considered for our patient was provision of D positive blood to allow matching of

antigens with known antibodies. In the setting of immunosuppression for transplant, the risk of the patient developing an anti-D antibody *de novo* is theorised to be low. A 30 patient study of patients post-transplant from an RhD incompatible donor showed no antibody developed at 32 weeks follow up.¹¹ Nonetheless, such small cohort studies do not include patients with existing red cell alloantibodies seen such as our patient. In our patient, development of an anti-D antibody, would force the use of D negative blood even in emergency situations, which is predominantly homozygous for c-antigen and thus likely compounding the pre-existing problem.

Option 4: Use of frozen phenotype matched cells. Frozen product is held by reference red cell laboratories for rare blood types, and in some clinical scenarios is an option to overcome shortages of fresh product availability. The process of thawing and deglycerolisation results in loss of red cells, and the lifespan of surviving cells when transfused is thought to be reduced.^{12,13} Furthermore, irradiation is required for product transfused to transplant recipients; however, there is limited evidence for the viability of blood irradiated following thaw. There is some evidence that *in vitro* red cell viability is comparable between irradiated products whether they are thawed or fresh, however this finding has not been shown *in vivo*.¹⁴

Following multidisciplinary input from transfusion scientists and transplanters at our facility, it was decided that the safest approach would be to use low c-antigen dose units and monitor the anti-c titre and haemolysis markers. This would allow the reservation of D positive blood to be used in the event that high volume emergency transfusion was required. In the event of development of a *de novo* D antibody, or a rising anti-c titre with evidence of clinically significant haemolysis, we would request frozen units from the reference red cell laboratory as a salvage option.

Clinically significant red cell alloantibodies rarely present a problem in transplant, due to their low population prevalence, as well as the fact that transplant is skewed towards younger patients reducing the chances of prior immunogenic exposures. As such, it is not of common consideration in the donor selection process. Strategies to manage donor directed alloantibodies during transplant require planning and individualisation, depending on local phenotypic prevalence and donor availability. This case demonstrates the safe and effective use of low dose c-antigen red cells in a patient with known c-antigen alloimmunisation, in the acute post-transplant period.

The limited available evidence suggests a role for both pre- and post-transplant interventions. Booth *et al.* outline the

Fig. 2 Rhesus typing pre- and post-transplant.

approach for ABO incompatible transplants, whereby red cell depletion of stem cell products is considered for major ABO mismatch, and plasma depletion for minor ABO mismatch. The same principle is essentially employed for transfusion support in patients with pre-transplantation-ABO alloantibody. Recipient plasma antibody clearance with therapies such as plasmapheresis or rituximab is followed by transfusion of products negative for the stimulating antigen.¹⁵

This case highlights the role of donor red cell extended phenotyping for intended transplant recipients with known clinically significant alloantibodies, to guide donor selection.

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1. Heim MU, Schleuning M, Eckstein R, *et al.* Rh antibodies against the pretransplant red cells following Rh-incompatible bone marrow transplantation. *Transfusion* 1988; 28: 272–5.
2. Hows J, Beddow K, Gordon-Smith E, *et al.* Donor-derived red blood cell antibodies and immune hemolysis after allogeneic bone marrow transplantation. *Blood* 1986; 67: 177–81.
3. Shaw BE, Logan BR, Spellman SR, *et al.* Development of an unrelated donor selection score predictive of survival after HCT: donor age matters most. *Biol Blood Marrow Transplant* 2018; 24: 1049–56.
4. Transfusion Science Standing Committee. Australian and New Zealand society of blood transfusion. *Guidelines for Transfusion and Immunohaematology Laboratory Practice*. 1st Edition (revised). Sydney: ANZSBT, 2020.
5. Moinuddin I, Fletcher C, Millward P. Prevalence and specificity of clinically significant red cell alloantibodies in pregnant women - a study from a tertiary care hospital in Southeast Michigan. *J Blood Med* 2019; 10: 283–9.
6. Franchini M, Gandini G, Aprili G. Non-ABO red blood cell alloantibodies following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2004; 33: 1169–72.
7. Matsui M, Kajiwaru K, Tsukada M, *et al.* A case of Philadelphia chromosome-positive acute lymphocytic leukaemia with type I CD36 deficiency. *Vox Sang* 2021; Jun 14: 10.1111/vox.13119.
8. Xu Z, Duffett L, Tokessy M, Cote J, Goldman M, Saldenberg E. Anti-AnWj causing acute hemolytic transfusion reactions in a patient with aplastic anemia. *Transfusion* 2012; 52: 1476–81.
9. Scholl S, Klink A, Mücke L-O, Schilling K, Höffken K, Sayer HG. Safety and impact of donor-type red blood cell transfusion before allogeneic peripheral blood progenitor cell transplantation with major ABO mismatch. *Transfusion* 2005; 45: 1676–83.
10. Hosoba S, Jaye DL, Cohen C, Roback JD, Waller EK. Successful treatment of severe immune hemolytic anemia after allogeneic stem cell transplantation with bortezomib: report of a case and review of literature. *Transfusion* 2015; 55: 259–64.
11. Cid J, Lozano M, Fernández-Avilés F, *et al.* Anti-D alloimmunization after D-mismatched allogeneic hematopoietic stem cell transplantation in patients with hematologic diseases. *Transfusion* 2006; 46: 169–73.
12. Eades B. Freezing and recovering rare red blood cells using glycerol. *Immunohematology* 2020; 36: 85–8.
13. Wintch R, James PM. Viability of cryopreserved red blood cells studied over 96 hr post-thaw. *J Surg Res* 1977; 23: 88–95.
14. Winter KM, Johnson L, Webb RG, Marks DC. Gamma-irradiation of deglycerolized red cells does not significantly affect in vitro quality. *Vox Sang* 2015; 109: 231–8.

15. Booth GS, Gehrie EA, Bolan CD, Savani BN. Clinical guide to ABO-incompatible allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2013; 19: 1152–8.

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Monocytoid switch in an adult with B-cell precursor acute lymphoblastic leukaemia characterised by the PAX5 P80R mutation

To the Editor,

Lineage switch leukaemias are a rare entity, thought to be a result of genetic instability in the malignant clone or through unmasking of a dormant subclone following initiation of cytotoxic chemotherapy. In most reported cases, the switch has become evident after completion of induction therapy. Here we report a case of lineage switch after administration of steroids alone, in the context of a PAX5-P80R mutation.

A 60-year-old nurse was referred to hospital by her general practitioner who noted moderate to severe cytopenia, with a neutrophil count of $0.8 \times 10^9/L$, haemoglobin of 85 g/L, platelets of $9 \times 10^9/L$ and 71% circulating blasts. She presented clinically with mild abdominal pain and fatigue in the preceding week. She had no significant past medical history. Bone marrow examination confirmed acute leukaemia with 90% pro-lymphoblasts, having an immunophenotype of CD10, CD19, cytoplasmic CD79a, and partial expression of TDT and CD34. Positron emission tomography scan demonstrated increased FDG uptake in an enlarged spleen, pancreas and numerous lymph nodes above and below the diaphragm. After diagnostic work-up, dexamethasone 4 mg thrice daily was commenced as pre-phase therapy on day 3. Lumbar puncture was deferred due to profound thrombocytopenia refractory to transfusions. She was then transferred to a neighbouring institution which offered blinatumomab and abbreviated chemotherapy for frontline treatment, in the context of a clinical trial. The white cell count was $4.9 \times 10^9/L$ after 4 days of steroids, with the blast percentage falling to less than 1%.

The white cell count rose steadily from this point, however, and reached $108 \times 10^9/L$ on day 6 following commencement of steroids. Flow cytometry (Fig. 1) was repeated and revealed a single population of blasts with a monoblastic immunophenotype, positive for CD4, CD14, CD64, and CD11b, and negative for CD34, not noted at the initial diagnostic analysis. The erstwhile lymphoblast population had disappeared. This finding was confirmed on repeat bone marrow examination (Fig. 2).

Due to the loss of CD19 expression on the prevailing blast cell population, the patient was deemed ineligible for the clinical trial and returned to our institution. With a revised diagnosis of mixed phenotype acute leukaemia (MPAL), induction chemotherapy with 7+3 (idarubicin) was commenced. The induction phase was complicated by