Case Report

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Outcome of a Major ABO-incompatible Haematopoietic Stem Cell Transplantation: A Case Report

Transfusion Medicine Section

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ABSTRACT

The ABO determinants are not present on the cell surface of Haematopoietic Progenitor Cells (HPCs), which allows them to be transplanted into recipients regardless of their ABO type. The difficulties associated with an ABO-incompatible HPC transplant depends on the mismatch, which can be classified as major, minor, or bidirectional. In the present case report, the recipient's (42-year old female) blood group was O Rh (D) positive, while the donor's (47-year-old male) blood group was A Rh (D) positive, resulting in a major ABO-incompatible transplant. Investigation of the donor were within normal limits. Given the high anti-A titres in the recipient {O Rhesus D Rh (D) positive}, Fresh Frozen Plasma (FFP) from an A Rh (D) positive donor (the donor's blood group) was transfused for four days. Once the levels were below the critical value, Peripheral Blood Stem Cell (PBSC) collection from the donor was done and later, the product was subsequently transfused to the recipient. On day 25 and day 29 post-PBSC infusion, the recipient achieved neutrophil and platelet engraftment, respectively. A delay in Red Blood Cell (RBC) engraftment Pure Red Cell Aplasia (PRCA) was noticed, and the recipient has been transfusion-dependent. Given mild Graft-versus-host Disease (GVHD) and molecular relapse, Donor lymphocyte was collected from the same donor and infused (DLI). A switch in the recipient's blood group was later noted (5 months post-DLI infusion), and RBC engraftment was achieved. Here, the authors discussed the difficulties encountered when a significant ABO mismatch occurred after an allogeneic haematopoietic Stem Cell Transplant (SCT).

Keywords: Allogenic haematopoietic stem cells, Donor lymphocyte infusion, Pure red cell aplasia

CASE REPORT

A 42-year-old female was admitted to the hospital with complaints of breathlessness and tiredness for one week. Bone marrow aspiration and flow cytometry suggested acute myeloblastic leukaemia with 68% blasts. Karyotyping revealed a translocation (8;21), and molecular testing showed FLT3 mutation positive. She had undergone two cycles of induction chemotherapy and, since she was FLT3 positive, she was started on Tablet (T.) midostaurin 50 mg Bis in Die (BD) (protein kinase inhibitor) and was counselled for allogenic peripheral Stem Cell Transplantation (SCT) by the haematologist. Blood grouping and typing were found to be 'O Rh (D) positive'. The PBSC donor was a 47-year-old male (brother of the patient). Human Leucocyte Antigen (HLA) typing done for both recipient (patient) and donor was found to be a 12/12 match. The donor's blood grouping and typing was 'A Rh (D) positive'.

The donor was referred to the Department of Transfusion Medicine for Allogenic peripheral SCT. Baseline laboratory investigations for the donor, including complete blood count and serologic tests {Human Immunodeficiency Virus (HIV), Hepatitis B Surface Antigen (HBsAg) and Hepatitis C Virus (anti-HCV)}, yielded results that were within the normal limits and non-reactive. Preprocedural serum calcium was 9.6 mg/dL (reference range: 8.8-10.0 mg/dL).

Pretransplant phase: Since there was an ABO major mismatch in the Haematopoietic Stem Cell Transplantation (HSCT), anti-A titres were tested for the recipient. High titres of anti-A {as per the present Institution's Standard Operating Procedures (SOP) by serial dilution tube method} were noted, therefore, Institution's of A Rh (D) positive' (donor's blood group) FFP were transfused over four days. Since there was a decrease in titre levels, PBSC was planned and carried out [Table/Fig-1].

Stem cell mobilising regimen: The donor received Injection (Inj.) Granulocyte-colony Stimulating Factor (G-CSF) 600 mcg Subcuatneous (s/c) {Once Daily (OD) dose} for five days. On day 5, after obtaining high-risk informed consent, the PBSC collection was attempted for the donor using the machine COM. TEC (Fresenius Kabi), which was loaded with P1YA (stem cell kit) and primed with normal saline and Acid Citrate Dextrose solution A (ACD-A) (anticoagulant) through a peripheral line access. To prevent hypocalcaemia, T. shelcal 500 mg (2 tablets) was given orally every three hours. The donor's vital signs were monitored throughout the procedure and remained stable. A midway Cluster Differentiation 34+ (CD34+) count was sent and was found to be 1780 cells/microlitre.

IgM	IgG	Products given
1:64	1:1024	4 units of "A Rh (D) positive" FFP given×4 days
1:16	1:64	

[Table/Fig-1]: Anti-A titres before PBSC collection (serial dilution-tube method).
FFP: Fresh frozen plasma; Ig: Immunoglobulin

Peripheral stem cell procedure details:

Stem cell mobilisation regimen: Inj. G-CSF 600 mcg s/c (OD dose) for five days.

Blood volume: 4.82 L

Donor weight: 78 kg

Patient weight: 77.3 kg

Product volume collected: 207 mL

After red cell depletion, product volume: 185 mL

Midway CD 34 count=1780 cells/microlitre

Calculated dose=Product volume (185 mL)×CD34 count (1780)/ Recipient weight (77.3 kg)

$=4.1\times10^{6}$/Kg body weight

As there were some financial concerns, cryopreservation of the product was not attempted.

Peritransplant phase: After conditioning the patient, under aseptic precautions, the infusion of the collected PBSC product was transfused over one hour, with the appropriate Premedications and

vitals were monitored and vitals monitored. The patient tolerated the procedure well. The transfusion support is listed in [Table/Fig-2] below.

Postinfusion	Haemoglobin (g/dL)	Platelet (lakhs/cumm)	Products transfused (irradiated)
Day 9	8.7	11,000	SDP-1unit {O Rh (D) positive}
Day 10	8.0	17,000	No transfusion
Day 11	7.7	10,000	PRBC-1unit {O Rh (D) positive} SDP-2 units {A Rh (D) positive}
Day 12	10	10,000	SDP-2 units {A Rh (D) positive}

[Table/Fig-2]: Transfusion support postinfusion.
SDP: Single donor platelets; PRBC: Packed red blood cells

Postinfusion, the patient achieved neutrophil and platelet engraftment on day 25 and day 29, respectively. Red blood cell engraftment was delayed, and hence became transfusion-dependent.

Post-transplant phase: She was discharged and treated on an Outpatient (OP) basis with serial monitoring of her anti-A titre levels [Table/Fig-3]. Blood grouping and typing was done on day 126 and found to still be the recipient's blood group: "O Rh (D) positive" [Table/Fig-4].

Post HSCT	IgM	IgG	Haemoglobin (g/dL)	Products transfused (irradiated)
Day 52	1:32	1:32	5.5	1 unit PRBC {O Rh (D) positive}
Day 58	1:16	1:64	6.1	No transfusion
Day 60	Not seen	Not seen	5.5	2 units PRBC {O Rh (D) positive}
Day 90	1:8	1:64	4.5	2 units PRBC {O Rh (D) positive}
Day 112	1:16	1:128	4.9	2 units PRBC {O Rh (D) positive}
Day 126	1:16	1:64	4.6	2 units PRBC {O Rh (D) positive}

[Table/Fig-3]: Anti-A levels with transfused (serial dilution-tube method).
PRBC: Packed red blood cells

Day	A	B	D	Control	A cells	B cells	Final report
Day 126	0	0	4+	0	4+	4+	O Rh (D) positive

[Table/Fig-4]: Blood grouping and typing of transplant recipient (Day 126) by Column Agglutination Technology (CAT) method.

As her anti-A levels were rising, she was transfused with four units of "Rh (D) positive" FFP and was started on T. Ibrutinib 140 mg (a Bruton kinase inhibitor) and steroids on day 127 Because of, mild GVHD and molecular relapse. The patient and family members were counselled for Donor Lymphocyte Infusion (DLI) on day 134 postinfusion of the PBSC product.

Donor Lymphocyte Infusion (DLI) procedure details: DLI was attempted for the same donor (brother) using a P1YA kit. Machine used was COM.TEC with product volume collected of 22 mL. Lymphocyte subset enumeration (T lymphocyte CD3+absolute count): 48,938.22. Infusion of 2 mL was transfused to the patient immediately postprocedure.

Cryopreservation of the remaining product was done using Dimethyl Sulfoxide (DMSO), 20% human albumin, plasmalyte and heparin, and it was preserved at -80°C. The product (20 mL) was thawed and infused on a later date, increasing the dose regimen. Three months post-DLI, her anti-A titre levels started to decline [Table/Fig-5], and the blood group switched over to the donor's blood group [Table/Fig-6]. Patient is currently lost to follow-up.

Post-DLI	IgM	IgG	Haemoglobin/Products transfused (Irradiated)
Day 60	1:4	1:4	Hb-4.1 g/dL- 2 units PRBC {O Rh (D) positive}
Day 69	1:1	1:1	Hb-6.9 g/dL

[Table/Fig-5]: Anti-A titres by serial dilution-tube method (post-DLI).
Hb: Haemoglobin

Post-DLI	A	B	D	Control	A cells	B cells	Final report
Day 60	0	0	4+	0	3+	3+	O Rh (D) positive
Day 69 [Table/Fig-3]	0	0	4+	0	0.5+	3+	O Rh (D) Positive
Day 80 [Table/Fig-4]	4+	0	4+	0	0	3+	A Rh (D) Positive

[Table/Fig-6]: Blood group switching (post-DLI) by CAT method.

DISCUSSION

The current article presents an intriguing case study of the outcome of an allogeneic SCT that resulted from a major ABO mismatch. Haematopoietic Progenitor Cells (HPCs) are pluripotent stem cells dedicated to becoming lineage-restricted progenitor cells, capable of self-renewal and differentiation. When infused into conditioned recipients, these cells can restore bone marrow function [1]. Allogeneic HPC transplantation restores bone marrow function following high-dose chemotherapy and/or radiation therapy, just like autologous HPC transplantation does [2]. Mismatching of the ABO blood group system does not prevent HPC Transplantation (HCT), in contrast to HLA [3].

When a donor's unique immunodominant sugar moiety (A: N-acetylgalactosamine; B: galactose) is transfused into a recipient who has antibodies against the matching blood group (anti-A, anti-B, and/or anti-A, B), a significant major ABO incompatibility results. Major incompatibility is the existence of antibodies in the recipient against the donor's red blood cell [4].

Here, a cross-reactivity occurs between recipient antibodies {Isohaemagglutinins (IHA)} and donor blood group antigens. PRCA, delayed red cell engraftment, and haemolysis of red cells during graft infusion are the main side-effects of major ABO-incompatible haematopoietic stem cell transplantation (HCT). In the present case report, the patient developed PRCA; despite having repeated packed red cell transfusions (post PBSC product infusion, her haemoglobin levels did not rise. Studies show that the ABO-mismatched patients had a considerably greater rate of red blood cell transfusions [5,6].

Also, due to the separate inheritance of ABO antigens and HLAs, ABO incompatibility can arise in as many as 20-40% of HLA-matched allogeneic HCTs [7,8]. Most guidelines recommend a reduction of a high IHA titres in recipients with a major ABO mismatch [8,9].

In the majority of major ABO-mismatched patients following transplantation, the incompatible anti-A or anti-B titres decline, and reticulocytosis develops when the IgG titre drops to approximately 1:8-1:16 [10]. As per the present study Institution's SOP, the authors managed to monitor the recipient's anti-A levels by serial dilution method (semiquantitative method) by tube technique, hence transfusing the donor's blood group plasma brought the IHA levels below a critical value, and then proceeded with the transplant. The dosage required for an allogenic transplant should be $>3\times10^6$ cells/kg of the recipient's weight; we had given a dosage of 4.1×10^6 cells/kg [11].

Haematopoietic stem cell transplant is a common therapy for many individuals with haematological abnormalities, whether they are inborn or malignant. The classification of red cell incompatibility is based on whether the recipient's isoantigens, IHA, or both are incompatible with the donor's [12]. The PRCA is a rare disorder characterised by a reduction or absence of red blood cell precursors in the bone marrow, resulting in severe anaemia [13]. Zhu P et al., suggested that the persistence of anti-donor isoagglutinins, particularly IgG anti-donor isoagglutinins, ove the first four months after HSCT may be crucial for the development of post-HSCT PRCA [14]. It has been reported that a matched related one is associated with a higher risk for PRCA and prolonged persistence of anti-donor IHA compared to an unrelated donor or a haploidentical donor. Other complications that can lead to PRCA are sibling donors and reduced intensity of conditioning [14].

Regarding transfusion support, while the donor group must be respected for platelet concentrates and FFP, the recipient's RBC concentrates must belong to the same group until the IHAs disappear [15]. Compared to recipients of ABO-compatible transplants, the great majority of patients having major incompatible transplantation have a small increase in the need for red blood cell transfusions due to the quick clearance of anti-donor isoagglutinins [16,17].

Stussi G et al., reported that pre-HCT Plasma Exchange (PLEX), whether used alone or in combined with red blood cell transfusion according to the donor group, may be a reliable procedure to reduce the occurrence of PRCA [18]. However, the present case patient had some financial constraints and preferred to avoid a prolonged hospital stay; hence, PLEX was not done.

For certain individuals with recurring malignant illness, DLI has shown to be an effective therapy choice. Initially, DLI was given as a Bulk Dose Regimen (BDR), which involved a single, sizable dosage of lymphocytes. Since then, evidence has emerged to support the idea that employing an increasing dosage regimen (EDR) may be just as successful in treating leukaemia while causing a lower incidence of GVHD [18-20].

In summary, after identifying the type of major ABO mismatch HSCT, in the pretransplant phase, the authors proceeded to reduce the recipient's anti-A titres by appropriate transfusion support with close monitoring the levels. The authors collected the PBSC product and transfused it to the recipient. Neutrophil and platelet engraftment was achieved; but a delay in RBC engraftment was noticed (peritransplant phase).

In the post-transplant phase, the authors continued to monitored anti-A titres, the switch in blood group (to the donor's group), and her dependency on Packed Red Blood Cell (PRBC) transfusions due to PRCA. DLI was done and gradual switch in blood group to the donor's group was appreciated. During this phase, the need for blood transfusion is also reduced.

CONCLUSION(S)

Recipients of majorly mismatched Haematopoietic Progenitor Cell (HPC) transplants can be at risk for delayed red blood cell production because the recipient's antibodies can lyse newly produced ABO-incompatible RBCs (e.g., the recipient is typing O and the donor is typing A). Despite the efforts to prevent major ABO mismatch HSCT complications, the recipient developed PRCA and required more transfusion support. This should not deter us from proceeding with the transplant in such cases; but need to bear in mind the other factors that contribute to complications also.

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