Original Article

# Clinico-serologic co-relation in bi-directional ABO incompatible hemopoietic stem cell transplantation

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**Abstract:**

**Background:** The ABO blood group system is of prime significance in red cell transfusion and organ transplantation. However, ABO compatibility is not critical in allogenic hemopoietic stem cell transplantation (HSCT) and approximately 40-50% of hemopoietic stem cell transplants are ABO incompatible. This incompatibility may be major, minor or bi-directional. Though there are descriptions of transfusion practice and protocols in ABO incompatible HSCT, there are considerable variations and transfusion support in these patients can be very challenging. **Aims:** The immunohematologic observations in two cases of bi-directional ABO incompatible HSCT have been described, and clinico-serologic correlation has been attempted. **Materials and Methods:** In both cases, peripheral blood stem cell harvests were obtained using the Cobe spectra cell separator. Immunohematologic assessments in the donor and recipient were done as a part of pre HSCT evaluation. Both the standard tube technique and column agglutination method (Ortho Biovue Micro Bead System) was used. Antibody screen was done by column agglutination method using three cell panel (Surgiscreen cells). Isoagglutinin titration was done by the master dilution method and standard validated techniques were used. **Results:** The pattern of laboratory findings in the two cases was different and so were the clinical outcomes. Although there was early engraftment in the first case, the second case developed pure red cell aplasia and this was well-reflected in the immunohematologic assessments. **Conclusion:** Immunohematologic assessment correlated well with the clinical picture and could be used to predict clinical outcome and onset of complications in ABO incompatible HSCT.

**Key words:**

ABO incompatible hemopoietic stem cell transplantation, clinico-serologic correlation, ABO mismatch stem cell transplant

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## Introduction

The ABO blood group system is of prime significance in red cell transfusion and organ transplantation. However, ABO compatibility is not critical for allogenic hemopoietic stem cell transplantation (HSCT) since ABO antigens are not expressed on primitive hemopoietic stem cells. Approximately 40-50% of HSC transplants are ABO incompatible.[1] Thus, there may be major ABO incompatibility when the recipient has isoagglutinins against the donor or minor ABO incompatibility when the donor has isoagglutinins against the recipient. In bi-directional ABO incompatibility, there is a two way mismatch. In ABO incompatible HSCT, the immunohematologic evaluation of the recipient and the transfusion support to be provided can be very challenging. The transfusion support provided can influence the clinical outcome. Benjamin and Antin, in their study, have attributed increased risk of death due to incompatible plasma in ABO incompatible platelet components.[2] In the literature, studies show considerable variation in the immunohematologic assessment undertaken and the transfusion support provided in such patients. In this report, we describe the immunohematologic observations in two cases of bi-directional ABO incompatible HSCT. The pattern of laboratory findings in the two cases was different, and so are the clinical outcomes. However, the laboratory observations correlate well with the clinical profile. In this communication, we wish to highlight that immunohematologic findings could be used to predict the clinical outcome.

Ours is a 160 bedded oncology center in eastern India dealing exclusively with cancer patients. The clinical hematology department performs six stem cell transplants per month. The laboratory evaluations and transfusion support are provided by the transfusion services.

## Case Reports

### Case 1

A 58-year-old male diagnosed as AML-M6 underwent haploidentical HSCT using modified John Hopkins protocol and fludarabine busalfan preparative regimen. In the blood bank, as a part of pretransplant evaluation; blood group, direct antiglobulin test (DAT), antibody screening and isoagglutinin titration and pretransplant donor patient cross match was done. The blood group and DAT were done by both tube method and column agglutination method (Ortho Biovue Micro Bead System). A1 lectin was not used to

subtype group A. Antibody screen was done by column agglutination method using three cell panel (Surgiscreen cells). Standard validated techniques were used. Isoagglutinin titration was done by the master dilution method;[3] the saline method was used for IgM isoagglutinin titration and anti-human globulin (AHG) method for IgG isoagglutinin titration. The end point for the titration was the highest dilution which gave 1+ agglutination. Titers were classified as anti-A, anti-B; IgM, IgG types using A and B reagent red cells prepared in-house. Dithiothrietol was not used while testing for antibody titer. There was no fixed frequency for performing recipient posttransplant isoagglutinin titer; it was, usually, done when the patient sample was received for red blood cell (RBC) cross match or when the patient came for follow-up. The peripheral blood stem cells (PBSC) were harvested using the Cobe spectra cell separator and infused without any product manipulation.

### *Observations*

The blood group of the patient was B positive, and that of the donor (brother of the patient) was A positive. Pretransplant the donor-patient crossmatch showed major and minor incompatibility. The anti-B isoagglutinin titer in the donor was 32 (saline) and 32 (AHG) and anti-A isoagglutinin titer in the recipient was 32 (saline) and 16 (AHG). The patient was given with the consent, four units donor type (A group) plasma 2 days prior to the transplant. The anti-A titer done on the next day, showed a decline from the initial titer of 32 to16 (saline) and from 16 to 4 (AHG).

Posttransplant, there was no immediate hemolysis on infusion of the stem cells. At our center, when the graft is bone marrow, red cell depletion is done using hydroxyl ethyl starch. No manipulation of the product is done for a PBSC harvest as the red cell contamination is <20 ml. Until 2 weeks post transplant, the patient was still group B. By the 7$^{th}$ week, recipient red cells had started declining and donor type red cells had started appearing. By 10 weeks, recipient red cells were no longer detectable, and the front type was A. Posttransplant, the recipient derived isoagglutinins against donor type red cells gradually declined and disappeared. This coincided with the time when the donor type red cells were first detected. At 11 weeks post transplant, the front type was A and the back type was AB. The DAT and antibody screen were both negative. Posttransplant the recipient anti-A titer showed a steady decline from 32 (saline) and 16 (AHG) to undetectable levels by the 7$^{th}$ week.

Posttransplant day 5, 5 mcg/kg granulocyte-colony stimulating factor (G-CSF) was started and an absolute neutrophil count >500/cumm was achieved on day +17. Growth factor support was stopped on day +19. An unsupported platelet count >20,000/cumm was achieved on day +19. Prophylaxis for graft versus host disease (GVHD) was given using a combination of cyclosporine, tacrolimus and mycofenolate. The patient had a spike of fever (day 1-day 4) and was started on piperacillin, tazobactum and amikacin. He also developed mucositis on day +8. The patient received eight RBC units and 10 platelet units (including four single donor platelets) during the initial 5 weeks posttransplant. The results of forward grouping, reverse grouping and the serial posttransplant anti-A titer are shown in Tables 1 and 2 respectively.

## Case 2

A 62-year-old male, case of myelofibrosis, JAK2V617F mutation negative underwent allogenic stem cell transplantation at our center. He was transfusion dependent and had received ruloxitinib and fludarbine-busulphan myeloablative conditioning regimen. The laboratory assessments, evaluation methods and PBSC harvests performed were similar to that described for the previous case.

### *Observations*

The blood group of the patient was A positive, and that of the donor (brother of the patient) was B positive. Pretransplant the donor-patient crossmatch showed major and minor incompatibility. The anti-A isoagglutinin titer in the donor was 16 (saline) and anti-B isoagglutinin titer in the recipient was 16 (saline). There were no infusional complications at the time of transplant other than mild hemoglobinuria, which spontaneously subsided with hydration. Posttransplant the recipient front type was A. Over the subsequent 15 weeks, the reaction with anti-A weakened (both tube and card). At 17 weeks posttransplant, the recipient typed as group O. This implied that recipient red cells had declined and were not detectable, and the donor type red cells were yet to appear; and the red cells detected were the transfused O red cells. The reverse group still continued to be A, though the strength of agglutination had decreased. Thus, implying that the recipient isoagglutinin (anti-B) though had declined over 21 weeks, but had not totally disappeared, and the donor type red cells were yet to appear.

There was a sharp rise in the anti-B isoagglutinin titer from 16 (pretransplant) to 2048 at 3 weeks posttransplant. Thereafter the titer declined gradually and remained at 512 till 13 weeks, though the AHG titer was 1024. Around the same time (6 weeks) posttransplant, the patient developed progressive drop in hemoglobin, though his platelet count and leucocyte counts had started rising. Pure red cell aplasia (PRCA) was suspected and then confirmed by bone marrow biopsy which showed absent erythroid precursors with normal myeloid and megakaryocyte maturation. G-CSF support was started on day +10 and an absolute neutrophil count >500/cumm was achieved on day +15. An unsupported platelet count >20,000/cumm was achieved from day +17. The patient developed a localized erythematous rash surrounding the Hickman's catheter insertion site on day 4 and was empirically started on piperacillin and tazobactum till day +14. Cyclosporine and short course methotrexate were used for GVHD prophylaxis. However, when PRCA was detected, cyclosporine was discontinued, and rituximab was added. He received a total of 42 RBC units in the initial 3 months posttransplant. He also received 26 platelet units (including 15 single donor platelets). Fresh frozen plasma (FFP) units (12) were required

**Table 1: Blood grouping results of case 1**

| Time | Forward grouping | | | | Reverse grouping | | | |
|---|---|---|---|---|---|---|---|---|
| | Tube | | Card | | Tube | | Card | |
| | Anti-A | Anti-B | Anti-A | Anti-B | A cells | B cells | A cells | B cells |
| Pretransplant | Negative | 4+ | Negative | 4 | 4+ | Negative | 4 | Negative |
| Posttransplant 2 weeks | Negative | 3 | Negative | 3 | 2 | 1 | 3+ | Negative |
| 7 weeks | 4+ | 1 | 3+ | Negative | Negative | Negative | Negative | Negative |
| 10 weeks | 4+ | Negative | 3+ | Negative | Negative | Negative | 1 | 1 |
| 11 weeks | 4+ | Negative | 4+ | Negative | Negative | Negative | Negative | Negative |

when he developed sepsis preterminally. The results of forward grouping, reverse grouping and the serial posttransplant anti-B titer are shown in Tables 3 and 4, respectively.

## Discussion

Hemopoietic stem cell transplantation is a treatment option in many hematologic malignancies. A human leukocyte antigens (HLA) matched stem cell donor may not be ABO identical with the recipient since the genes for HLA antigens and the ABO blood group antigens are inherited independently.[4] Due to the limited availability of donors, about 40-50% of HSCT are ABO incompatible, with 5% being bi-directionally incompatible.[1,5] The transfusion support in ABO incompatible HSCT depends on the immunohematologic parameters. The two cases of bi-directional ABO incompatible HSCT described here have contrasting serologic profiles. However, the immunohematologic observations in both correlate well with the clinical outcomes.

In the first case, the patient was initially blood group B, but posttransplant over 3 weeks, the forward type changed to A, and the reverse type continued to be AB. The reason why anti-B antibodies did not develop even when the group has changed to A is not clear. The patient's initial pretransplant anti-A isoagglutinin titer was 32 (saline) and 16 (AHG). He was administered 4 units donor type (group A) plasma pretransplant following which the titer declined to 16 (saline) and 4 (AHG). It is our institutional policy that if the recipient antidonor isoagglutinin titer is more than 16, donor group plasma is administered pretransplant. The practice of pretransplant donor group plasma infusion, to reduce recipient isohemagglutinin titers and facilitate red cell engraftment is variable. A group of authors have proposed that infusion of ABO incompatible plasma containing small amounts of isoantigens, reduces recipient isoagglutinin titers directed against donor red cells.[6-8] However other authors have shown that pretransplant donor group plasma infusion does not reduce incidence of PRCA in ABO mismatched transplants.[9] In our first case, posttransplant, the antidonor isoagglutinins were undetectable at 5 weeks. Clinically this coincided with the time when the patient showed signs of red cell engraftment.

**Table 2: Posttransplant recipient anti-A iso-agglutinin titers - case 1**

| Time | Saline titer | AHG titer |
|---|---|---|
| Pretransplant | 32 | 16 |
| Infused donor type plasma | 16 | 4 |
| 1st week | 8 | 2 |
| 2 weeks | 8 | 2 |
| 5 weeks | — | — |

AHG: Antihuman globulin

Red cell engraftment is said to have taken place when the absolute reticulocyte count is more than 30 × $10^{12}$/L (>1%) and there is independence of RBC transfusions. It is evidenced by 100% donor RBC chimerism in the marrow and coincides with the disappearance of recipient isoagglutinins.[1,10,11] This is well illustrated by the first case. He showed 100% donor chimerism and was independent of red cell transfusions after 5 weeks. This coincided with decline in the anti-A isoagglutinin titer to undetectable levels by the 5th week. Five weeks posttransplant the recipient did not need RBC transfusions and the reticulocyte count was within normal limits.

In contrast, case 2 showed a different serologic profile. At 3 weeks posttransplant, the recipient anti-B saline titer peaked to 2048 from the pretransplant titer of 16. The anti-B titer remained high and only at 19 weeks declined to 16 (pretransplant level). The sharp rise in isoagglutinin titer correlated with the drop in recipient hemoglobin, and heralded the onset of PRCA which was confirmed on bone marrow biopsy. Pure red cell aplasia is defined as reticulocytopenia (<1%) lasting more than 60 days after hemopoietic stem cell transplant, with absent erythroid precursors in the marrow in a recipient who has engrafted myeloid precursors, lymphoid precursors and with megakaryocytes present in the marrow.[11] The reticulocyte count done 2 months posttransplant was 0.05% and chimerism analysis showed mixed chimerism.

The reported incidence of PRCA after a major ABO mismatch varies between 3% and 29%.[10] Several authors have identified risk factors for PRCA and these include nonmyeloblative transplants, use of cyclosporine, persistence of antidonor isoagglutinins and transplants in blood group O/A recipient-donor pairs.[1,12,13] It is however noteworthy that in our case, elevated antidonor anti-B isoagglutinins were responsible for the PRCA. Gmür *et al*. have observed that red cell engraftment and reticulocyte recovery coincide with the decrease in isoagglutinin titer to below 16.[14] These observations are well illustrated in our second case. This recipient was initially group A and 17 weeks posttransplant showed forward type as O. No donor type red cells were detected in front type till 17 weeks, as the recipient still continued to show antidonor isoagglutinins. Here, the sharp increase in the isoagglutinin titer 3 weeks posttransplant, indicated the onset of PRCA. Hence, it appears that immunohematologic assessment with isoagglutinin titer monitoring can be carefully used to predict onset of complications.

In both cases, the transfusion support in the posttransplant period included O Rh(D) positive RBCs and AB group platelets where ever possible. In the first case, AB group platelets were given throughout, however for the second case we were compelled to give out of group platelets due to nonavailability of AB group donors. The second case also received 12 FFP units (AB group)

**Table 3: Blood grouping results of case 2**

| Time | Forward grouping | | | | Reverse grouping | | | |
|---|---|---|---|---|---|---|---|---|
| | Tube | | Card | | Tube | | Card | |
| | Anti-A | Anti-B | Anti-A | Anti-B | A-cells | B-cells | A-cells | B-cells |
| Pretransplant | 4 | Negative | 4 | Negative | Negative | 4 | Negative | 4 |
| Posttransplant 1-week | 4 | Negative | 4 | Negative | Negative | 4 | Negative | 4 |
| 9 weeks | 4 | Negative | 4 | Negative | Negative | 4 | Negative | 4 |
| 13 weeks | 2 | Negative | 3 | Negative | Negative | 4 | Negative | 4 |
| 15 weeks | 1 | Negative | 1 | Negative | Negative | 4 | Negative | 4 |
| 17 weeks | Negative | Negative | 1+ | Negative | Negative | 3 | Negative | 3 |
| 19 weeks | Negative | Negative | Negative | Negative | Negative | 2 | Negative | 3 |
| 21 weeks | Negative | Negative | Negative | Negative | Negative | 1 | Negative | 2 |

**Table 4: Posttransplant recipient anti-B iso-agglutinin titers - case 2**

| Time | Saline titer | AHG titer |
|---|---|---|
| Pretransplant | 16 | — |
| 3 weeks | 2048 | — |
| 4 weeks | 1024 | — |
| 6 weeks | 512 | 512 |
| 9 weeks | 512 | 512 |
| 13 weeks | 512 | 1024 |
| 17 weeks | 32 | 128 |
| 19 weeks | 16 | 16 |
| 21 weeks | 4 | 2 |

AHG: Antihuman globulin

when he developed sepsis. All the platelet and RBC products issued were irradiated (25 Gy). The posttransplant transfusion requirement in the first case was only eight RBC units. This was in sharp contrast to the second case who developed PRCA and was transfused 42 RBC units over 5 months. It has been well documented in literature that patients with PRCA require significantly increased RBC transfusion support.[1,15]

The literature reviewed also shows variable protocols followed in ABO incompatible HSCT. These include reduction of red cells/plasma in the product, in major and minor ABO incompatibilities respectively.[5] However, in the two cases described here, we did not manipulate PBSC product since these were collected by apheresis. Additional interventions like therapeutic plasma exchange can also be performed in the recipient to reduce isoagglutinin titers. Therapeutic apheresis is accepted as supportive therapy (category II indication) as per the ASFA (American Society for Apheresis) guidelines in ABO incompatible HSCT.[16]

In the two cases described here, neither was red cell phenotyping nor was A1/A2 subgrouping performed. Few authors have shown that non ABO red cell alloantibodies can complicate transplant outcomes and hence advocate donor recipient phenotyping.[17] This has also been corroborated by Rowley *et al.* who have found that Rh and Kidd system antigens can affect the clinical outcome.[18] The need to determine donor and recipient ABO subgroups (pretransplant) also needs to be addressed as recently Jaben *et al.* have reported significant posttransplant hemolysis due to anti-A1 developing in a A2 recipient.[19]

Transfusion practices and protocols in ABO incompatible HSCT have been described in the literature, but continue to be challenging. Immunohematologic evaluations can be used to predict the clinical outcome and uniform assessment protocols would help standardize transfusion practice in such patients. Nevertheless, appropriate immunohematologic evaluations can have far reaching effects on the final outcome in HSCT.

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