Original Article

Utility of Donor Plasma with Soluble A and B Secretory Substances (SAS) to Neutralize A, B Isoagglutinin in ABO-Incompatible Hematopoietic Transplant Recipients

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Abstract

Introduction In case of a major ABO-incompatible transplant team is typically concerned with the potential of increasing hemolysis when infusing blood that is incompatible with ABO and the risk of pure red cell aplasia in the recipient due to increased antidonor isoagglutinin level.

Objectives This study was designed to assess changes in the levels of anti-A and anti-B antibody titers in ABO-incompatible hematopoietic transplant recipients after transfusion of donor plasma having A and B secretory substances.

Materials and Methods Blood donors of AB type were screened for their secretor status, and fresh frozen plasma (FFP) was prepared as per standard operating procedure in the department. Plasma was tested for the presence of soluble ABO substances. This plasma was infused at a predetermined dosage of 5 to 10 mL/kg based on their in vitro neutralization concentrations in patients undergoing ABO-incompatible hematopoietic stem cell transplants and monitored for changes in their AB isoagglutinin levels.

Results A total of three patients, two with a diagnosis of chronic myeloid leukemia and one with acute myeloid leukemia, received the plasma infusions with individualized cumulative doses starting at 5 up to 25 mL/kg. Group AB FFP containing soluble ABO substances decreased antibody titer levels variedly (64→8, 64→16, and 32→16). No adverse events were reported that could be attributable to the transfusion.

Conclusion AB donor's FFP with soluble ABO substances given to ABO-incompatible stem cell transplant recipients as desensitization therapy is effective in reducing the ABO isoagglutinin titer, and no clinical and laboratory evidence of hemolysis in patients was found.

Keywords
- soluble ABO substances
- ABO-incompatible HSCT
- ABO isoagglutinin
- anti-A
- B neutralization

Introduction

A hematopoietic stem cell (HSC) transplant can be done between donors and recipients with red blood cell (RBC) antigen disparity.[1] Stem cell transplants are frequently ABO incompatible, unlike solid organ transplantation.[2] ABO incompatibility can cause hemolysis, PRCA, graft versus host disease, and increased transfusion requirement.[3]

ABO-incompatible hematopoietic stem cell transplantation (HSCT), occurring in 30 to 50% of allogeneic transplants,

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can impose a significant clinical burden due to hemolysis and PRCA.[4] Clinically significant hemolysis occurs in ~10 to 15% of ABO-mismatched HSCTs and is more common in peripheral blood stem cell grafts due to higher lymphocyte content.[5] ABO incompatibility consistently increases red cell transfusion burden during engraftment.[6]

PRCA is a delayed donor-type erythroid aplasia seen predominantly after major ABO-incompatible HSCT, resulting from persistent host plasma cell–derived isoagglutinins that destroy donor erythroid precursors. The reported incidence ranges from 10 to 30% of ABO-incompatible transplants, depending on conditioning intensity and immune modulation.[7] It manifests as a prolonged transfusion-dependent anemia with reticulocytopenia and absent red precursors in marrow beyond day 60 to 100 posttransplant. High pretransplant anti-A/B immunoglobulin G (IgG) titers are a major risk factor for it.[8] A titer of 1:32 is often used as the threshold to initiate interventions, such as plasmapheresis or plasma exchange, to lower the antibody levels.[9]

Secretors are those in whom the ABO antigens are present on epithelial cells and in body secretions, which include saliva, gastric fluids, tears, breast milk, semen, and vaginal and cervical secretions.[10] A majority (up to 80%) of individuals have A, B, and H antigens found in the soluble form in body secretions such as saliva, plasma, tears, and other body fluids as soluble ABO group substance (SAS).[11] These substances are antigenically similar to ABO blood group antigens present on RBCs and do react/combine with antibodies in the same fashion.[12] Hence, donor plasma can neutralize ABO antibodies and reduce ABO antibody levels in the recipient, and reduce humoral graft rejection.

To our knowledge, till now, no other studies have been published from India regarding the donor plasma with soluble ABO substances infusion to reduce anti-A and anti-B antibody titer in ABO-incompatible transplant recipients, so it will help in knowing the effect of neutralization by soluble A and B antigens from the secretor donor.

Objectives

This single-center study aims to assess changes in the levels of anti-A and anti-B antibody titers in ABO-incompatible hematopoietic transplant recipients after transfusion of donor plasma having A and B secretory substances.

Materials and Methods

Study Setting and Design

This clinical cohort study was done in the Departments of Transfusion Medicine and Medical Oncology in a tertiary care teaching hospital in Southeastern India from September 2022 to June 2024.

Study Procedures

For the Blood Donors

Only AB blood group donors were chosen. Saliva and blood samples were collected. Saliva and plasma secretor status were ensured by the hemagglutination inhibition method. Once the presence of SAS was confirmed, the donors were bled, and fresh frozen plasma (FFP) was prepared as per the standard operating procedure followed in our department for preparing components from whole blood. These were stored in −40 degrees deep freezers as per regular practice.

For the ABO-Incompatible Transplant Recipients

Pretransplant immunohematological work included grouping, Coomb's test, and antibody screen. A pre-transplant anti-A, B antibody titer was performed. In vitro neutralization was done (plasma with SAS was incubated with the serial dilutions of the patient's plasma) in the laboratory to see the proportions at which the neutralization is completed.

In vitro neutralization assay: 100 µL of AB donor plasma and ABO-incompatible hematopoietic transplant recipient plasma were taken in a test tube and mixed. This mixture was incubated at room temperature for a minimum of 20 minutes. One drop of 5% suspension of AB donor cells was added to this mixture, centrifuged, and looked for agglutination. The presence of agglutination suggests that the neutralization is incomplete. The donor plasma proportion was increased in single multiples (1:1,1:2, and so on) till the neutralization was complete. All the FFPs selected neutralized the patient plasma in a titer range of 32 to 64.

Plasma Transfusion

FFP transfusion was given to the patient 24 hours before HSC infusion at incremental doses starting at 5 mL/kg at intervals of 12 hours before and continued after HSC infusion. Blood samples were collected from the patients to determine the extent of in vivo neutralization by performing anti-A and anti-B titers. Titers were performed before every subsequent transfusion and the last one within 24 hours of posttransfusion. Further transfusion of FFP was decided based on the neutralization.

The study methodology is summarized as a flow diagram in ►**Fig. 1**. As generally a titer of 1:32 is considered critical for clinically initiating interventions such as plasmapheresis, we considered a titer below 1:32, which is 1:16, as the target for the clinically comfortable titer levels. Once the titer level is ≤ 16, the patient is followed up as per regular clinical transplant protocol.

Primary and Secondary Outcomes

This study was expected to evaluate the reduction of anti-A and/or anti-B antibody titers after the transfusion of secretor AB donor plasma.

Safety and Efficacy End Points: The safety end points were adverse events related to plasma transfusion. The efficacy end points included prevention of incompatible stem cell transplant-related events such as hemolysis due to HPSCs infusion and engraftment-related issues.

Inclusion and Exclusion Criteria

Patients who are undergoing ABO-incompatible HSC transplant were included in the study. The patients who could not

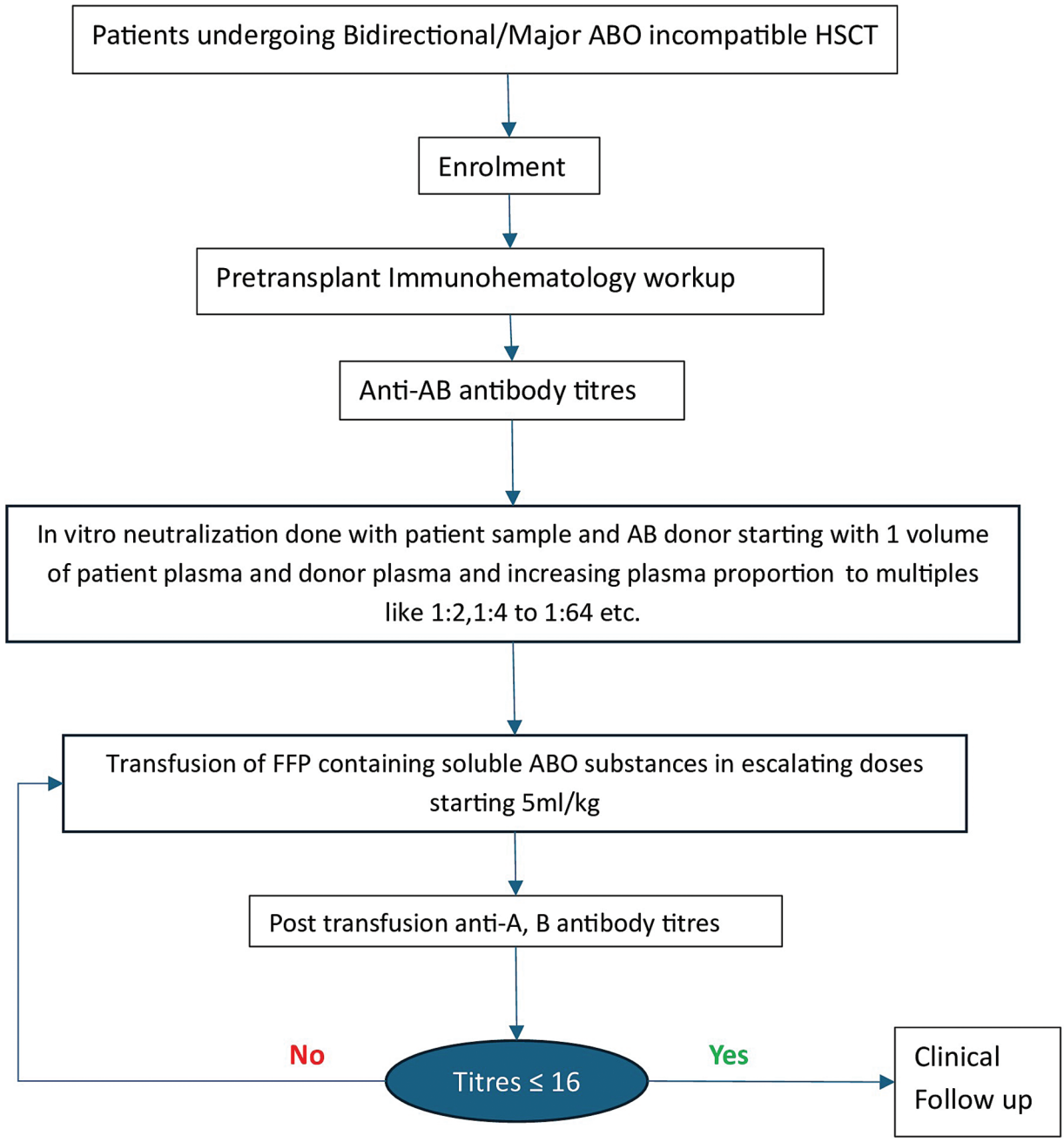

Fig. 1 Flow diagram depicting the study methodology. FFP, fresh frozen plasma; HSCT, hematopoietic stem cell transplantation.

receive the FFP transfusion or could not complete the transfusion were excluded.

Statistical Analysis

Given the small number of participants, the data are presented descriptively without statistical analysis.

Ethical Approval

This study was conducted per the ethical standards in the 1964 Declaration of Helsinki and its later amendments. Ethical approval was obtained from the Institutional Review Board, with approval number JIP/IEC/2022/081. Informed consent was obtained from all individual participants included in the study. Participants were provided with detailed information about the study's purpose, procedures, potential risks, and benefits, and their participation was entirely voluntary.

CTRI Registration: The study was registered in the Clinical Trial Registry India with registration number CTRI/2022/10/046554.

Minor Deviations: Though the intention was to give plasma infusions at incremental doses of 5 ml/kg, in patient 2, during the first transfusion patient had to undergo a procedure, and plasma infusion had to be interrupted. It could not resume immediately due to the slot adjustment for diagnostic scheduling. However, this is unlikely to affect the study outcome, as the outcome is based on the cumulative dose of plasma the patient has received.

In the case of patient 3 the she weighed only ~34 kg and was in distress periinfusion of stem cells. The second infusion had to be abandoned as her distressed continued. As she had received more than the minimum required dose of 5ml/kg/episode, we included her in the study.

Results

Participants and Treatment

Three participants aged 21, 27, and 31 years were included. They received a cumulative dosage of 10 to 20 mL of FFP/kg of the recipient's weight. The target dosage of HPSCs for each patient was 6×10^6 cells/kg. The quantity of blood product ranged from 210 to 290 mL. We did not use volume reduction due to the risk of losing the stem cells.

Safety and Efficacy: None of the patients had any adverse reactions to FFP transfusion (immediate or delayed up to 14 days). None of the patients had clinically identifiable hemolysis or delayed engraftment.

Patient 1

A 27-year-old woman was incidentally diagnosed with chronic myeloid leukemia (CML) in the chronic phase in 2018. After multiple lines of treatment with imatinib and later dasatinib, she was planned for an HSC transplant.

Her blood group was O positive. The donor was a 10/10 HLA-matched sister with blood group B positive. Pretransplant anti-B titer by tube method was 64 for immunoglobulin M (IgM) in the saline phase and 64 for IgG in the AHG phase (►**Table 1**).

During admission, the patient had a central line infection for which antibiotics were given, and on day 5 of the transplant, developed febrile neutropenia with thrombocytopenia, which led to hematemesis and was managed with transfusion support. No clinical sign of hemolysis or deranged laboratory parameters was found. Neutrophil engraftment happened on day 10 and platelet engraftment on day 11. The patient was discharged on the 19th day of the transplant. The patient is in the remission phase and on follow-up at our center.

Patient 2

A 21-year-old man, case of CML with no comorbidities, was incidentally diagnosed in February 2020; bone marrow biopsy showed 3% blast and was started on imatinib; in view of multiple lines of tyrosinase kinase inhibitor failure, the patient was started on asciminib; after 6 weeks of therapy, bone marrow biopsy showed marrow in remission and was planned for ABO-incompatible transplant.

His blood group was O positive; immunohematological work-up showed negative direct antiglobulin test and indirect antiglobulin test. The donor was the brother with a 10/10 HLA match, with blood group A positive. The pretransplant anti-A titer by tube method was 64 for IgM in the saline phase and 64 for IgG in the AHG phase (►**Table 2**).

Neutrophil was engrafted on day 10 of the transplant, and platelet was engrafted on day 11 of the transplant. The patient was discharged on the 23rd day of the transplant.

Patient 3

A 31-year-old woman diagnosed with acute myeloid leukemia was planned for a peripheral blood stem cell transplant after a second relapse following induction therapy. The donor was the brother, who was a 12/12 HLA match. The patient's blood group was B positive, and the donor was AB positive and was thus a major ABO-incompatible transplant. Pretransplant anti-A titer by tube method was 32 for both IgM in the saline phase and IgG in the AHG phase (►**Table 3**).

A stem cell dose of 6×10^6 cells/kg was infused into the patient. Neutrophil engraftment was documented on D10,

Table 1 Patient 1

Episode	Dose (mL/kg)	Timing of HSC infusion	Volume transfused (mL)	Pretransfusion titer		Posttransfusion titer	
				IgM	IgG	IgM	IgG
1	5	−24 h	300	64	64	32	16
2	10	−12 h	552	32	16	16	16
3	5	+12 h	304	16	16	8	8

Abbreviations: HSC, hematopoietic stem cell; IgG, immunoglobulin G; IgM, immunoglobulin M.

Table 2 Patient 2

Episode	Dose (mL/kg)	Timing of HSC infusion	Volume transfused (mL)	Pretransfusion titer		Posttransfusion titer	
				IgM	IgG	IgM	IgG
1	3	−24 h	200	64	64	32	64
2	5	−12 h	392	32	64	32	64
3	10	+12 h	593	32	64	32	32
4	5	+24 h	362	32	32	16	16

Abbreviations: HSC, hematopoietic stem cell; IgG, immunoglobulin G; IgM, immunoglobulin M.

Table 3 Patient 3

Episode	Dose (mL/kg)	Timing of HSC infusion	Volume transfused (mL)	Pretransfusion titer		Posttransfusion titer	
				IgM	IgG	IgM	IgG
1	5	−24 h	166	32	32	32	32
2	7.5	−12 h	264	32	32	16	16

Abbreviations: HSC, hematopoietic stem cell; IgG, immunoglobulin G; IgM, immunoglobulin M.

Table 4 Baseline characteristics of the participants with the outcome in terms of reduction in antibody titers

Pt	Age (y)	Gender	Weight (kg)	Diagnosis	Patient's blood group	Donor's blood group	Pretransplant titer	The volume of FFP transfused (mL)	Posttransplant titer
1	27	Female	55	CML	O positive	B positive	64	1,149	8
2	21	Male	70	CML	O positive	A positive	64	1,548	16
3	31	Female	42	AML	B positive	AB positive	32	430	16

Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; FFP, fresh frozen plasma.

and platelet engraftment on D11. No signs or symptoms of engraftment syndrome were noticed. The patient required only one packed red blood cell and two single-donor platelet transfusions during this period. No signs or laboratory features suggestive of gross hemolysis were noted.

The summary of all three patients is shown in ►**Table 4**.

Discussion

This study was done to assess the changes in the levels of anti-A and anti-B antibody titers in ABO-incompatible hematopoietic transplant recipients after transfusion of donor plasma having A and B secretory substances. All the donors from whom FFP was included in the study were males to avoid the chances of transfusion-related acute lung injury. All were of the AB blood group, as this plasma is considered universal due to the absence of anti-A and anti-B in donor plasma.

It has been suggested that, to neutralize anti-A in group B recipients and anti-B in group A recipients, groups A and B FFP, respectively, might be more effective than group AB FFP. However, no such requirement has been shown for O group recipients. Groups A and B individuals are also usually shown to have low titers of ABO IgG antibody, unlike group O individuals. In our study, we used AB plasma uniformly for better inventory management and logistics.[13] Also, two of our patients were O-group individuals. The comparison with various studies is summarized in ►**Table 5**.[14–16]

Table 5 Summary of various studies on the usage of SAS for isoagglutinin neutralization

Study	Participants	Intervention	Outcomes
Won et al (2015)[13]	ABO-incompatible kidney transplant patients	FFP containing soluble ABO substance as replacement fluid for plasma exchange	Group AB FFP containing soluble ABO substances decreases ABO antibody levels more efficiently than albumin does
Stussi et al (2009)[3]	ABO-incompatible stem cell transplant patients	Donor type ABO-incompatible PRBC transfusion or plasma exchange	Pretransplant elimination of these isoagglutinins effectively prevents pure red cell aplasia and delayed red blood cell engraftment, which is dependent on the quantities of antidonor isoagglutinin
Curley et al (2012)[8]	All major or bidirectional ABO-mismatched allogeneic hematopoietic stem cell transplant recipients	Plasma infusions using donor-type secretors with or without plasma exchange	Donor-type secretor plasma infusions have been related to low rates of posttransplant pure red cell aplasia and are safe and effective in reducing progenitor cell infusion-associated hemolysis
Benjamin et al (1998)[15]	ABO-incompatible bone marrow transplant in a chronic myeloid leukemia patient	Plasma pheresis with donor-type plasma	Anti-A titer was found to be 256 before transplant, and later on, the patient developed graft vs. host disease; the titer was found to be 16,000, so 19 cycles of plasmapheresis were done with donor-type plasma, and the titer was reduced to 8

(*Continued*)

Table 5 (*Continued*)

Study	Participants	Intervention	Outcomes
Basu et al (2015)[16]	ABO-incompatible stem cell transplant	Donor-type plasma	In the first case, the IgM titer was reduced from 32 (pretransplant) to 8 (5 weeks after transplant), and IgG 16 (pretransplant) to 2 (5 weeks after transplant). In the second case, the IgM titer was reduced from 16 (pretransplant) to 4 (21 weeks after transplant), and IgG from 512 to 2 (21 weeks)

Abbreviations: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; IgG, immunoglobulin G; IgM, immunoglobulin M; FFP, fresh frozen plasma; PRBC, packed red blood cell; SAS, soluble A and B secretory substance.

Table 6 Comparison of various desensitization therapies available

Attribute	SAS-mediated neutralization using plasma	Immunoadsorption column	Plasma pheresis
Mechanism of action	Anti-A/B isoagglutinin depletion by A/B antigens in secretor plasma through antigen–antibody interaction	Selective removal based on hydrophobic adsorption	Nonselective removal by centrifugation or filtration based
Substitution fluid	Plasma (FFP)	No substitution fluid is required	Albumin or plasma
Adverse effects	Mild risk of transfusions	Reactions to the membrane	Reactions specific to the substitution fluid
Cost (in INR)	~ 10,000	~ 2 Lakhs	~ 20,000–30,000 per procedure
Consumables	Only the blood administration set	Membrane efficacy is reduced for subsequent uses	Fresh kit to be used for every procedure
Risk of infection	TTI	Cross-infection risk due to the reuse of the column and survival decrease due to severe infection	TTI if blood products are used
Duration of stay in hospital	No additional stay is required	Prolonged hospitalization is required for multiple procedures	Hospitalization is required for the required number of cycles
Risk of hemolysis	Nil if AB plasma is used	Risk of hemolysis and rupture of the filter fiber	Risk of hemolysis and filter clotting

Abbreviations: FFP, fresh frozen plasma; SAS, soluble A and B secretory substance; TTI, transfusion-transmissible infection.

SAS is present in the plasma of all individuals, regardless of their secretor status, with much larger amounts in the plasma of secretors comparatively.

SAS neutralizes ABO antibodies, but there is also a risk that it stimulates B cells to produce immune ABO antibodies several days after plasma exchange and might evoke humoral rejection of allografts, especially in kidney transplants. However, this is unlikely to be an issue here, as the B cell population is also replaced by donor type in HSCT.

Transfusion reactions are a risk in the transfusion of plasma. Still, these are mostly mild and easily manageable with the current-day quality of plasma production and testing with molecular methods. The transfusion received is also regular in patients undergoing allogeneic transplants, especially single donor platelets peritransplant, which has a sizeable amount of plasma. The various other desensitization strategies available and the comparison with them are given in ►**Table 6**.

Conclusion

AB donor's FFP with soluble ABO substances given to ABO-incompatible stem cell transplant recipients as desensitization therapy is effective in reducing the ABO isoagglutinin titer, and no clinical and laboratory evidence of hemolysis in patients was found. A titer reduction to values of 16 or less seems to be sufficient to prevent immediate hemolysis pending further studies to confirm this hypothesis.

Funding
None.

Conflict of Interest
None declared.

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