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Case Report:

Minor ABO-Incompatible Bone Marrow Transplantation in β-Thalassemia Major

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Abstract

Background: Allogeneic bone marrow transplantation (BMT) primarily requires human leukocyte antigen (HLA) compatibility, whereas ABO blood group compatibility is not mandatory. However, transplantation across major or minor ABO incompatibility may result in clinically significant complications, including hemolysis, delayed engraftment, and Passenger Lymphocyte Syndrome (PLS). Elevated anti-ABO antibody titers in either donor or recipient can compromise transplant outcomes. Careful pre-transplant immunohematological evaluation is therefore essential to minimize these risks. **Aim:** This study aimed to prevent hemolytic complications, optimize engraftment, and improve clinical outcomes in a case of major ABO-incompatible allogeneic BMT. **Methods:** A retrospective descriptive case study was conducted on a nine-year-old female patient diagnosed with major β-thalassemia, blood group B, scheduled for allogeneic BMT from her HLA-identical (100% match) brother with blood group O. Donor anti-ABO antibody titers were measured using the standard tube agglutination method with serial serum dilutions. Testing was performed using immediate spin (IS) and anti-human globulin (AHG) phases against B-group red blood cells. Based on antibody titration results, the harvested bone marrow graft underwent appropriate cell manipulation prior to infusion. Post-transplant monitoring included clinical assessment and laboratory evaluation for evidence of PLS and engraftment kinetics. **Results:** Donor anti-B antibody titers were 1:32 at the IS phase and 1:256 at the AHG phase, indicating significant IgG-mediated reactivity. Following graft manipulation, total nucleated cell recovery was 98.5%, while $CD34^+$ stem cell recovery was 88.3%, remaining within an acceptable therapeutic range. The patient received the manipulated graft without acute hemolytic complications. Serial clinical and laboratory evaluations revealed no evidence of PLS. Successful hematopoietic engraftment was subsequently achieved. **Conclusion:** Pre-transplant quantification of anti-ABO antibodies and appropriate graft manipulation can effectively mitigate hemolytic risk in major ABO-incompatible BMT. This individualized approach supports safe engraftment and favorable clinical outcomes, emphasizing the importance of immunohematological assessment in ABO-mismatched transplantation settings.

1. Introduction

While human leukocyte antigen (HLA) matching is necessary for bone marrow transplantation, ABO incompatibility does not carry problems for effective hematopoietic stem cell transplant (HSCT) (1). This is because antigens of ABO blood group do not present on pluripotent or early committed hematopoietic stem cells (HSCs). Therefore, unlike solid organ transplantation, the use of ABO mismatched donors is adequate for HSCT (2).

Mohammad Vaezi and his colleaques suggested that in clinical practice, for the patient who has many susceptible donors, the one with compatible ABO blood groups has to be chosen to enhance the transplanation outcomes, as improving the overall survival and the transfusion requirements (3). Aaron and his colleaques also proposed that when the opportunity exists, an ABO matched donor is preferred than an ABO mismatched donor (4).
The first research work about allogenic transplant with ABO-incompatible donor was studied by Buckner et al in 1978 (5). This current study aims to define the clinical workup for dealing with minor ABO incompatible HSCT from bone marrow source, with determining stem cell recovery. This work was done on the first ABO-incompatible stem cell transplant in our center. We aim to improve patient outcome, reduce morbidity and mortality post-transplant, reduce transfusion needs, and promote engraftment. A careful teamwork is needed, involving

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hematopathologist, bone marrow transplant physician, and blood bank
specialist to manage effective transfusion (6). The transfusion therapy must be carried out according to the scheme in **Table 1** below, which has been modified based on Gajewski work (7):

Table 1. Guidelines of Selection of ABO Blood Group for RBCs and Blood Components for Patients Subjecting to HSCT with Myeloablative Conditioning:

Incompatibility type	ABO of donor	ABO of recipient	Choice of RBC		Choice of platelet		Choice of plasma
			First choice	Second choice	First choice	Second choice	
Major	A	O	O		A	AB	A-AB
Major	B	O	O		B	AB	B-AB
Major	AB	O	O		AB	-	AB
Major	AB	A	A	O	AB	-	AB
Bidirectional	B	A	O		AB	B	AB
Bidirectional	A	B	O		AB	A	AB
Major	AB	B	B	O	AB	-	AB
Minor	O	A	O		A-AB	B-O	AB-A
Minor	O	B	O		B-AB	A-O	AB-B
Minor	O	AB	O		AB		AB
Minor	A	AB	A	O	A-AB	-	A-AB
Minor	B	AB	B	O	B-AB	-	B-AB

Note. Adapted from Gajewski work (7).

All platelets and RBC concentrates must be leuko-depleted and irradiated. Platelet concentrates should preferably be produced by apheresis donation and if there is no possibility of obtaining platelet concentrates between the first and second choice, then the products must be delivered plasma depleted.

2. Materials and Methods

This is a retrospective descriptive study. Data was collected from the clinical registry. Nine years old female with thalassemia major, she had received many blood transfusions since she was diagnosed. Her weight was 31 kilograms. The donor was her brother who was fully HLA matched, 12 years old, weighing 35 kilograms. The blood group of the patient was B; that of the donor was O. Descriptive case report was adopted.
The antibodies' titration was done in the hematology laboratory. Serial dilutions of the donor's serum in saline were made; it was up to 1/1024. Serial antibody titration was done on donor's serum with 3% recipient's group B RBC suspensions. The reactions were then read after IS at room temperature, then after incubation at 37° C for 30 min with bovine albumin, and lastly, adding anti-human globulin (AHG) and read. Test result was the reciprocal of the highest dilution. In the scheduled date, donor underwent bone marrow harvest. Two harvest bags were identified, samples were taken to the lab for sterility tests and complete blood count together with CD34+ and CD3+ cell count (markers for stem cells, T-lymphocytes respectively).Depending on the antibody's titer, cell manipulation was planned, the strategy was eventually aimed at removal of harmful anti ABO antibodies from the donor's serum. Stem cell's bags are then taken to the Stem Cells' Processing Lab. Under sterile conditions, inside the biological safety cabinet class II, workers started processing; the bone marrow bags were connected to empty bags for collection of the plasma which will be extracted; then centrifugation followed by plasma extraction.
The aim of centrifugation is to separate plasma, then plasma removal, which is in turn aim to eliminate any antibodies that could lead to future transfusion reactions in the recipient. Knowing that the researcher supposed inevitable loss of stem cells during manipulation. The stem cells right now are ready to be infused through central venous line in the patient circulation.In the following thirty days post infusion of the stem cells, the patient is monitored for clinical manifestation and laboratory tests for Passenger Lymphocyte Syndrome (PLS). This was done by daily monitoring of all the following tests: hemoglobin concentration, reticulocyte count, haptoglobin level, indirect bilirubin level, platelet and white blood cell (WBC) count. Also alternate-day testing of direct anti globulin test (DAT) and lactate dehydrogenase (LDH) level.
For ethical issues, study followed the ethical principles summarized in the Declaration of World Medical Association of Helsinki about medical research concerning human subjects. This study is also approved by the Ethical Committee of Research at the Nineveh Directorate of Health on May 8th, 2025 (number: 19774). Written informed consent was obtained from the patient's legal guardian for publication of case report.

3. Results:

Antibodies' titration after IS at room temperature, the reading revealed titer of 32. Then after AHG phase, the reading revealed 256. The parameters before and after manipulation were demonstrated in **Table 2**.

Table 2. Stem Cell Product Parameters, Pre and Post Manipulation:

Parameters	Bag 1 pre	Bag 2 pre	Total	Bag 1 post	Bag 2 post	Total	Recovery %
Net product weight gr	411.00	452.00		183.00	179.00	362.00	
Hematocrit %	27.60	25.30		62.40	59.60		
Net volume ml	391.43	430.48	821.90	174.29	170.48	344.76	41.90
RBC volume ml	108.03	108.91	216.94	108.75	101.60	210.36	97.00
Plasma volume ml	283.00	321.00	604.00	65.00	68.00	133.00	22.40
TNC * 10^8 *	128.19	57.04	185.23	124.86	57.54	182.39	98.50
Total $CD34^+$ * 10^6	261.00	98.00	359.00	224.00	93.00	317.00	88.30

** TNC is the total nucleated cells*

There were two bone marrow bags, with different hematocrits (mean 26.45%), then after the product had been concentrated, the hematocrit increased significantly (mean 61.00%).
The total net volume of the two bags was 821.90 ml pre-manipulation, then the net volume decreased significantly post-manipulation (344.76 ml).
Plasma volume is calculated by subtracting the RBC volume from the total volume, the total amount of plasma removed is 469.00 ml, so a reduction of 77.60% (recovery of 22.40%).
While the TNC was not reduced, the total $CD34^+$ * 10^6 count also did. There was a very good recovery of TNC (98.50%) with minimal loss was observed, but a lower recovery, although high recovery of $CD34^+$ (88.30%). The critical point was achieved, since good recovery of the $CD34^+$ cells during manipulation.
This case report contained clinical assestment of both patient and donor, laboratory mesurment of anti ABO titers, and quantitaive analysis of the parameters of the stem cells before and after manipulation, including product weight, volme, hematocrit, TNC count and CD34+ cells counting. The decision of plasma reduction was done based on increased donor anti B antibody titers.
The patient then was infused with the stem cells. Then she monitored for any clinical manifestation and laboratory tests to predict PLS. This was done by daily and alternating-day tests. The results on day +25 post-transplant were summarized in **Table 3**.

Table 3. Parameters Post Stem Cell Transplant:

Parameter	Result
Hb	10.1 mg/dL
Reticulocyte count	1 % of total RBC count
Haptoglobin	130 mg/dL
Indirect bilirubin level	0.6 mg/dL
Platelet count	150 x 10^9/L
Neutrophil count	2.7 x 10^9/L
DAT	negative
LDH	280 U/L

Only descriptive statistics were used, and no hypothesis testing was conducted.

4. Discussion

Although the peripheral blood HSCs in thalassemia was associated with faster engraftment and is easier procedure, particularly in pediatrics, but it is linked with a higher incidence of acute and chronic GVHD. Consequently bone marrow source was chosen (8).

Matthias et al study's results found that mismatch in ABO blood group has a significant effect on the outcome of transplantation (9), and imprtant immunohematological events, thretening the post transplant program (10). Other study found that there was no proof of big effects of incompatability of ABO blood group on the outcome of BMT among patients with leukemia (11). While there is no significant association between Rh matching with any of transplanation endpoints (12).
The PLS is defined as finding donor anti-ABO antibodies within the period of one month of transplantation (13). Donors' lymphocytes inside the minor ABO mismatched graft produce isohemagglutinins (IHAs) which can destroy recipient's RBCs, leading to delayed onset hemolysis, mediated by donor's lymphocytes. A condition known as PLS. Its onset is between one and three weeks after transplanation and is self-limiting as these donor-derived antibodies will disappear within a maximum period of three months (14). That is why any hemolysis occurs after ABO incompatible HSCTs, means passive transport of donor's lymphocytes, causing PLS, thus measurement of anti ABO antibodies are indicated and reasonable (15).
Patient was monitored for PLS, which could start from the day +5 up to +30 (16), while other studies showed that it may start in day 7-14 post infusion (17).Diagnosis of PLS is usually suspected if there is a sudden low hemoglobin, high reticulocyte, low haptoglobin, high indirect bilirubin, positive direct anti-globulin test (DAT) (16, 18).
In our case, in the thirty days post infusion of stem cells, there was no decrease in hemoglobin, normal reticulocyte count, normal haptoglobin level, normal indirect bilirubin, negative DAT indicating that there was neither IgG nor IgM. Also, there was an increase in the platelet and WBC counts. Indicating that there was good engraftment and no PLS.Early recognition of hemolysis, supporting with antigen-negative or matched blood transfusion and proper management are critical to improve clinical outcomes (10). Adopting forceful donor compatible RBC transfusions must be tried in all cases (19).
Anju Dubey and his colleagues highlighted the significance of close monitoring of PLS in patients underwent ABO mismatched renal transplant, especially when finding hemolysis, positive DAT, and identification of relevent antibodies in patient's serum and RBC eluate (20).
It is difficult to study a consistent effects of ABO-incompatible transplantation in the letreture, due to the little patient number, different graft resources, different conditioning and GvHD prophlaxis regimens (21).
Whereas HSCs that were collected through apheresis cell separators have already been plasma and RBCs depleted (7). The IS test reveals cold IgM antibodies, while after AHG phase, warm IgG antibodies were detected.
Such stem cell transplantation will be going on across minor ABO incompatibility. i.e. the IHAs in the donor's serum might attack antigens on the recipient's erythrocytes, which may lead to mild delayed immune

hemolytic reaction in the recipient. Plasma reduction on the graft was done to decrease the harmful IHAs antibodies in donor's plasma to avoid serious complications (22).

Our work is in accordance with Tekgündüz et al, 2016, who proposed that the plasma reduction from the graft is commonly used to prevent harmful reaction from donor derived IHAs toward recipients' erythrocytes. Tekgündüz also touched that plasma reducion procedure is useful when the antibodies titer exceeds 1/256 in donor's graft. They also recommended that plasma reduction is not indicated when IHAs titer is less than 1/256, in order not to loose the stem cells (6).

Another study conducted by Yan-Ru and colleaques on outcome of transplanation in sever aplastic anemia, the researchers found that even in haploidentical donors, ABO compatible donors were prefereed than ABO minor incompatible ones, if other haploidentical donors are accessible (23). The workup regrding donor-recipient pre transplantation including ABO phenotype of both to be prepared for any transfusion needs, monitor clinical laboratory results, and plan for graft manipulation (10).

Post transplant monitoring for success of transplanataion especially regarding PLS avoidance done over six weeks post transplant, via monitoring blood tests and finding rising level of white blood cells, RBCs, and platelets. In addition to frequent screening tests for viral, bacterial and fungal infections to assess immunity status. Monitoring for acute GvHD via examining skin rash, diarhoea, liver function tests. Renal function tests, serum electrolytes. Indicating that the new marrow is working.

The patient also asked to adhere to infection control as hand washing, crowds avoiding, weight and blood pressure monitoring at home. Awareness of the expected complications and follow up of patients will help to detect early trouble and manage efficiently, thus decreasing the life threatening and fatal events attributed to ABO mismatched HSC (21).Teamwork working between transfusion and transplantation expertises will assist in early detection of clinically significant antibodies and choosing right management and treatment (10).

5. Conclusion

The ABO discrepancies between donors and recipients need careful evaluation of the transplant protocol and specific systematized approach. Followed by establishing transfusion policy for such recipient, conditioning regimen, cellular processing protocol. The clinical implications of the study strategies includes prevention of immune-mediated hemolysis, preventing delayed RBCs engraftment and pure RBC aplasia. In addition to the importance of active communication between the blood bank and the clinical team, which is mandatory to resolve and manage any immune-hematological event, especially acute sever hemolysis. There is also a need for improving local and regional practices relating to transplants, which will eventually facilitate access to transplants, wait-listed patients, specialist availability and donors' procurements.

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زراعة نخاع عظمي غير متوافق جزئيًا مع فصيلة الدم ABO في حالة الثلاسيميا الكبرى بيتا / تقرير حالة

الخلاصة

الخلفية: تتطلب زراعة نخاع العظم الخيفي في المقام الأول توافق مستضدات الكريات البيضاء البشرية (HLA)، بينما لا يُعد توافق فصيلة الدم ABO شرطًا أساسيًا. مع ذلك، قد تؤدي الزراعة في ظل عدم توافق كبير أو صغير مع فصيلة الدم ABO إلى مضاعفات ذات أهمية سريرية، بما في ذلك انحلال الدم، وتأخر انغراس النخاع، ومتلازمة الخلايا اللمفاوية العابرة (PLS). يمكن أن يؤدي ارتفاع مستويات الأجسام المضادة لفصيلة الدم ABO لدى المتبرع أو المتلقي إلى الإضرار بنتائج الزراعة. لذا، يُعد التقييم المناعي الدموي الدقيق قبل الزراعة ضروريًا لتقليل هذه المخاطر. **الهدف:** هدفت هذه الدراسة إلى منع مضاعفات انحلال الدم، وتحسين انغراس النخاع، وتحسين النتائج السريرية في حالة زراعة نخاع عظمي خيفي غير متوافق جزئيًا مع فصيلة الدم ABO. **الطرق:** أُجريت دراسة حالة وصفية استرجاعية على مريضة تبلغ من العمر تسع سنوات، شُخِّصت بمرض الثلاسيميا بيتا الكبرى، فصيلة دمها B، ومُرشَّحة لعملية زرع نخاع عظمي من متبرع مطابق لها نسيجيًا (تطابق 100%) من فصيلة دمه O. قُيست عيارات الأجسام المضادة لـ ABO لدى المتبرع باستخدام طريقة التراص الأنبوبي القياسية مع تخفيفات متسلسلة للمصل. أُجري الاختبار باستخدام مرحلتي الطرد المركزي الفوري (IS) والغلوبولين المضاد للإنسان (AHG) ضد خلايا الدم الحمراء من فصيلة B. بناءً على نتائج معايرة الأجسام المضادة، خضع طعم نخاع العظم المُستأصل لمعالجة خلوية مناسبة قبل الحقن. شملت المتابعة بعد الزرع التقييم السريري والتقييم المختبري للكشف عن أي دليل على متلازمة ما بعد الزرع (PLS) وحركية الانغراس. **النتائج:** بلغت عيارات الأجسام المضادة لـ B لدى المتبرع 1:32 في مرحلة الطرد المركزي الفوري و1:256 في مرحلة الغلوبولين المضاد للإنسان، مما يشير إلى تفاعل كبير بوساطة الغلوبولين المناعي IgG. بعد إجراء معالجة الطعم، بلغت نسبة استعادة الخلايا النووية الكلية 98.5%، بينما بلغت نسبة استعادة الخلايا الجذعية 88.3% $CD34^+$، وهي نسبة تقع ضمن النطاق العلاجي المقبول. تلقى المريض الطعم المُعالج دون حدوث مضاعفات انحلالية حادة. ولم تُظهر التقييمات السريرية والمخبرية المتكررة أي دليل على متلازمة الخلايا اللمفاوية العابرة. وقد تحققت عملية انغراس الدم بنجاح لاحقًا. **الخلاصة:** يُمكن للقياس الكمي للأجسام المضادة لـ ABO قبل الزرع، وإجراء معالجة مناسبة للطعم، أن يُخفف بشكل فعال من خطر انحلال الدم في عمليات زرع نخاع العظم غير المتوافقة مع فصيلة الدم ABO. يدعم هذا النهج الفردي انغراسًا آمنًا ونتائج سريرية مُرضية، مما يُؤكد أهمية التقييم المناعي الدموي في حالات زرع نخاع العظم غير المتوافقة مع فصيلة الدم ABO.

الكلمات المفتاحية: عدم توافق فصيلة الدم ABO؛ زرع نخاع العظم؛ انغراس؛ طعم؛ معالجة؛ متلازمة الخلايا اللمفاوية العابرة.