Case Report

Approach to a case of multiple irregular red cell antibodies in a liver transplant recipient: Need for developing competence

Ravi C. Dara, Aseem K. Tiwari, Prashant Pandey[1], Dinesh Arora

Departments of Transfusion Medicine, Medanta-The Medicity, Gurgaon, Haryana, [1]Jaypee Hospital, Noida, Uttar Pradesh, India

Abstract:

Liver transplant procedure acts as a challenge for transfusion services in terms of specialized blood components, serologic problems, and immunologic effects of transfusion. Red cell alloimmunization in patients awaiting a liver transplant complicate the process by undue delay or unavailability of compatible red blood cell units. Compatible blood units can be provided by well-equipped immunohematology laboratory, which has expertise in resolving these serological problems. This report illustrates resolution of a case with multiple alloantibodies using standard techniques, particularly rare antisera. Our case re-emphasizes the need for universal antibody screening in all patients as part of pretransfusion testing, which helps to identify atypical antibodies and plan for appropriate transfusion support well in time. We recommend that the centers, especially the ones that perform complex procedures like solid organ transplants and hematological transplants should have the necessary immunohematological reagents including rare antisera to resolve complex cases of multiple antibodies as illustrated in this case.

Key words:

Irregular antibody, liver transplant, allo-immunization

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Correspondence to:
Dr. Ravi C. Dara,
Department of Transfusion Medicine, Medanta-The Medicity, Sector - 38, Gurgaon - 122 001, Haryana, India.
E-mail: rcdara@gmail.com

Introduction

Liver transplantation is a treatment of choice for patients with acute or chronic end-stage liver disease (ESLD). Liver transplants earlier required a large amount of blood transfusions, but the transfusion requirement in liver transplant has declined during the last few years.[1] Liver transplant procedures act as a challenge for transfusion services in terms of specialized blood components, serologic problems, and immunologic effects of transfusion. Blood transfusion itself is recognized poor prognostic factor in liver transplant recipients because of its adverse effects such as transfusion reactions, viral and bacterial contamination of blood products, and transfusion related immune modulation.[2] Red cell alloimmunization adds to this as the presence of red cell alloantibodies in patients awaiting a liver transplant may cause delay or unavailability of compatible red blood cell (RBC) units creating pressure over caregivers. Compatible blood units can be provided by well-equipped immunohematology laboratory with expertise in resolving serological problems. Such competence is essential for a successful liver transplantation program. In this report, we present our approach in a patient awaiting a liver transplant with clinically significant multiple red cell alloantibodies.

We present a case of 49-year-old male diagnosed with ESLD and scheduled for liver transplant. Immunohematology laboratory received a sample for alloantibody identification and providing compatible red cell units required for liver transplant. All procedures were performed as per the departmental standard operating procedure and manufacturer's instructions were followed.

Blood Grouping

On blood grouping the patient was A Rh (D) positive.

Direct Antiglobulin Test and Autocontrol

The patient sample was direct antiglobulin test (DAT) positive (4+; polyspecific) and autocontrol was also positive (2+). In monospecific DAT cassette, the anti-IgG was 3+ and anti-C3d was negative. Cold acid elution (Elutions-System, BAG Amtsgerichtsstra Health Care, Germany) was performed on the patient's red cells. The eluate was tested for antibody specificity, but remained inconclusive.

Autoadsorption was performed using two sets of papain-treated (Liquipap, Tulip Diagnostics, Goa,

India) autologous red cells. The antibody screen and identification was performed using adsorbed plasma.

Irregular antibody screening

Using column agglutination technology, the patient's adsorbed plasma was screened for irregular antibodies using commercially available three cell reagent panel (Surgiscreen, Ortho Clinical Diagnostics, Johnson and Johnson, USA), which showed varying strength of agglutination in SC I (2+), SC II (3+), and SC III (3+), respectively [Table 1], suggesting multiple antibodies.

Antibody identification

Eleven-cell identification panel resolve panel A (Ortho Clinical Diagnostics, Johnson and Johnson, USA) showed positive reactions with cells 3, 4, 5, 6, 7, 8, 9, and 10 [Table 2] which was suggestive of antibody against E, c, Le^a, and s antigens.

Select cells

Four select cells [Table 3] from resolve panel B (Ortho Clinical Diagnostics, Johnson and Johnson, USA) were used confirming the presence of Anti-c and anti-E alloantibody and ruled out the presence of antibody against Le^a and s antigens. Patient's red cell antigen phenotyping for E and c was also negative confirming the presence of anti-c and anti-E alloantibody.

Antigen negative compatible units

11 units of red cells were required; 45 RBCs were initially typed to find out c and E antigen negative units and 23 c antigen negative and E antigen negative units (c–, E–) O Rh (D) positive RBC units were identified. Out of these 23 (c–, E–), only seven units were AHG cross-match compatible suggesting the presence of another alloantibody.

Extended antigen phenotyping

Rare antisera (Ortho Clinical Diagnostics, Johnson and Johnson, USA) were used in conventional test-tube technique. Typing of all compatible and incompatible units was done for Fy^b, Jk^a and s antigen (since Fy^b and Jk^a antigens were ruled out only once in cell 11 of the panel; and s was not ruled out). All compatible units were Fy^b– (c–, E– and Fy^b–), while incompatible units were Fy^b+ (c–, E– and Fy^b+) suggesting the third antibody as "anti-Fy^b." This finding was further confirmed by treating the red cells of incompatible units (c–, E– and Fy^b+) with enzyme (Liquipap, Tulip Diagnostics) and cross-matched with patient's serum and these incompatible units became compatible and antigen phenotyping of patient's red cell for Fy^b antigen was negative confirming the presence of

Table 1: Antibody screen (Surgiscreen)

Cell no.	Rh-hr	Donor number	Rh-hr							Kell						Duffy		Kidd		Sex linked	Lewis		MNS				P	Lutheran		Cell no.	Test result
			D	C	E	c	E	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		AHG phase
1	R1R1	312646	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	1	2+
2	R2R2	312578	+	0	+	+	0	0	0	0	+	+	0	+	/	+	+	+	+	0	+	0	+	+	+	+	0	+	0	+	2	3+
3	Rr	112723	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	+	+	+	0	+	+	0	0	+	0	+	3	3+
	Patient																														Auto	2+

AHG: Anti-human globulin

Table 2: Antibody identification resolve A panel

Cell no.	Rh-hr	Donor number	Rh-hr							Kell						Duffy		Kidd		Sex linked	Lewis		MNS				P	Lutheran		Cell no.	Test result
			D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		AHG phase
1	R1wR1	310042	+	+	0	0	+	0	+	0	0	+	0	+	/	+	+	0	+	+	0	0	0	+	+	+	+	+	+	+	1	0
2	R1R1	305760	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	0	+	0	+	+	0	+	0	+	0	+	2	0
3	R2R2	308595	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	0	+	3	3+
4	R_0r	312318	+	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	0	+	0	0	+	+	+	+	+	0	+	4	3+
5	r'r	310289	0	+	0	+	+	+	0	0	0	+	0	+	/	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	5	2+
6	r''r	312316	0	0	+	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	0	+	0	+	0	+	+	0	+	6	3+
7	Rr	308645	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	0	+	0	+	+	0	+	+	0	+	7	3+
8	Rr	312308	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	+	+	+	0	0	+	0	+	0	0	+	8	3+
9	Rr	311303	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	9	3+
10	Rr	311877	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	0	+	0	+	0	+	0	+	+	0	+	10	3+
11	R1R1	312320	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	0	0	+	+	0	+	11	0
	Patient																														Auto	2+

AHG: Anti-human globulin

Table 3: Select cells from - resolve B panel

Cell no.	Rh-hr	Donor number	Rh-hr							Kell						Duffy		Kidd		Sex linked	Lewis		MNS				P	Lutheran		Cell no.	Test result (IAT)
			D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		
13	Rr	311762	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	0	+	0	+	+	0	+	+	+	0	+	13	3+
18	R1R1	302528	+	+	0	0	+	0	0	0	0	+	+	+	0	+	0	0	+	+	+	+	0	+	0	+	0	+	0	+	18	0
19	R1R1	308648	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	+	0	0	+	0	+	+	0	+	19	0
20	RzR1	311720	+	+	+	0	+	0	0	0	0	+	0	+	/	+	+	+	+	0	+	0	+	0	+	0	+	+	0	+	20	2+

IAT: Indirect antiglobulin test

anti Fy^b antibody [Table 4]. These red cell alloantibodies were also re-confirmed on a fresh sample of the patient. Considering the antigen negative frequency in our donor population for c, E and Fy^b (50.52%, 81.1% and 43.85%) 12 O Rh (D) positive units were typed for finding four more compatible units.[3] Thus, total of 11 (c–, E– and Fy^b–) RBC cross-match compatible units were issued and transfused to the patient during liver transplant. Intra-operatively, he received five red cells, six fresh frozen plasmas, one cryoprecipitate, and two single donor platelets without any adverse effects like hemolysis. In the postoperative period, one red cell unit was transfused which remained uneventful. Titers of anti-c, anti-E and anti-Fy^b alloantibody were 32, 2, and 2, respectively. The patient had a previous history of transfusion of four units of A Rh (D) positive red cell units in some other hospital 3 months ago. This previous transfusion could be the source of alloimmunization.

The patient was followed-up after the liver transplant during the hospital stay and telephonically after his discharge from the hospital. The patient continues to be alright with normal liver function tests and normal hemoglobin (10.2 g%).

Discussion

Considering the alloimmunization due to transfusion, overall 1% of patients in the general population and 18.6% in multi-transfused patients develop RBC alloantibodies.[4] The incidence of red cell alloantibodies at our center in general patient population is 1%[5] while in multi-transfused patient's in India incidence is 7.7% as reported by Patel *et al.*[6] In the first UK published survey of red cell alloimmunization in adults undergoing liver transplant, 8% of adults were reported to have red cell antibodies, out of which 6.8% were clinically significant. This was in contrast with their general patient population in which the prevalence of red cell alloimmunization was 2-3%.[7] Luzo *et al.* have reported 23% incidence of red cell alloantibodies among liver transplantation patients in their center.[8] In our patient awaiting a liver transplant, three red cell alloantibodies were identified of which two were againstRh (anti-c, anti-E) and one against Fy (anti-Fy^b) blood group system. The most common red cell alloantibody in liver transplants recipients reported by Mushkbar *et al.* from UK was anti Rh and anti Kell (K).[7] Ramsey *et al.* also reported anti K, Rh and Jk^a as the most common red cell antibodies in 496 adults and 286 children undergoing 1000 consecutive transplants in Pittsburgh.[9]

For finding out compatible units for this patient with multiple antibodies large number of units are to be typed. In our case, 57 units were typed to get 11 antigen negative compatible blood units, which shows that 19% of units were antigen-negative and this corroborates with expected antigen-negative units. Other approaches could also be of benefit in providing transfusion with difficult RBC antibodies like intra-operative blood salvage[10] and use of rare donor registries[11] for respective antigen negative blood units, while if blood requirement is quite high, ABO-compatible units that have not been typed for all antigens may be transfused initially and once the patient stabilizes, antigen-negative RBCs are given again at the end of the procedure, to minimize the risk of serious delayed hemolytic transfusion reactions.[12] This strategy takes advantage of hemodilution and antibody washout in initial stages of transfusion. However, the timing of switching back to antigen negative compatible blood can't be clearly defined, potentially putting the patient at risk for delayed hemolytic transfusion reaction and increasing postoperative morbidity. Thompson *et al.*[13] suggested to avoid at least complement fixing antibodies when sufficient antigen-negative blood cannot be obtained as they can potentially cause severe intravascular hemolysis. Another approach would be to do plasma exchange to remove or reduce the titers of clinically significant alloantibodies.

This case highlights the importance of antibody screening and identification as a part of pretransfusion testing in identifying irregular red cell antibodies and proper planning for transfusion support as failure to recognize all the irregular antibodies in a patient may lead to hemolytic transfusion reaction. In our case, anti Rh (anti-c and anti-E) was identified in time while anti-Fy^b was initially missed as cell 11 in our identification panel was negative which was later identified, while cross-matching antigen (c and E) negative units. This might be due to loss of blood group antigens (labile antigens) from reagent cells, as they were towards the end of their recommended storage period. Fy^a and Fy^b tend to elute from red cells stored in low pH low-ionic strength medium, and even after a prolonged storage, or mixing, in saline at pH-7.[14] Recent guidelines by British blood transfusion society for pretransfusion compatibility procedures state that reagent cells should be preserved in the temperature-controlled environment in diluents shown to minimize loss of blood group antigens during storage and stability of screening cells should be validated locally for routine use in laboratory. BCSH also recommends the use of controls, containing weak examples of antibodies (weak anti-D <0.1 IU/mL) and weak anti-Fy^a to assure the sensitivity of the test procedure and integrity of antigen expression of reagent red cells during storage.[15] This case also highlights the importance of extended phenotyping as anti-Duffy antibody was identified by extended phenotyping of all cross-match compatible and incompatible units. Extended phenotyping plays a major role, especially when multiple antibodies are suspected, but reagent control or AB serum control with the same technique should be incorporated.[15]

Table 4: Phenotyping of incompatible and compatible units

Unit no.	c	E	Fy^a	Fy^b	S	s	Jk^a	Jk^b	Test results (IAT)	Test results (enzyme)
28299	0	0	+	0	+	+	+	0	Compatible	—
28314	0	0	+	0	+	+	+	+	Compatible	—
28319	0	0	+	0	+	+	+	0	Compatible	—
28253	0	0	+	0	+	+	+	0	Compatible	—
28271	0	0	+	0	+	+	+	+	Compatible	—
28143	0	0	+	0	+	+	+	+	Compatible	—
28204	0	0	0	+	+	+	+	0	Incompatible	Compatible
28278	0	0	+	+	+	+	+	+	Incompatible	Compatible
28300	0	0	0	+	+	+	+	0	Incompatible	Compatible
28760	0	0	+	+	+	+	+	0	Incompatible	Compatible

IAT: Indirect antiglobulin test

Transfusion service provides vital support of the liver transplant program and preparation of transfusion support for organ transplant starts early in the preoperative period with antibody screening and identification to identify irregular antibodies and appropriate transfusion support in time. Our case re-emphasizes the need for antibody screening as part of pretransfusion testing. We also recommend that all centers, especially those which perform solid organ transplants and hematological transplants should develop necessary competence to resolve complex cases of multiple antibodies. Since developing such competences could be challenging, we can identify an existing laboratory with requisite competence as a reference laboratory to solve such complex cases.

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