

Short Paper

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Safety assessment of RhD-positive red cell transfusion in RhD-negative liver-transplant recipients: Single-centre report from India

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Background & objectives: The number of blood components required during a liver-transplant surgery is significant. It is challenging for blood transfusion services to provide the required RhD-negative red blood cells (RBCs) for recipients during the peri-operative period. This retrospective study presents safety data of transfusing RhD-positive RBCs in RhD-negative living donor liver-transplant (LDLT) recipients during the peri-operative period with six-month follow up for risk of developing alloantibodies.

Methods: All RhD-negative patients who underwent LDLT and were transfused ABO-compatible but RhD-positive RBC units between January 2012 and May 2018 were included in the study. Twenty one RhD-negative patients who received a total of 167 RhD-positive RBCs peri-operatively were chosen for alloantibody screening. All the patients were started on triple immunosuppression drugs as per the standard hospital protocol. Blood grouping, cross-match and antibody screening were done by column agglutination technique.

Results: Post-transplant antibody screen (weekly for 12 wk) was negative, and none of the patients developed anti-D alloantibodies till their last follow up (mean 21 months).

Interpretation & conclusions: Our observations suggest that it may be safe to use RhD-positive RBCs peri-operatively in RhD-negative LDLT recipients with low risk of alloimmunization.

Key words Alloantibody - living donor liver transplants - red blood cell - RhD-negative - transfusion

Introduction

Liver transplant has become an established treatment modality for end-stage liver disease patients¹. While deceased donor liver transplant constitutes more than 90 per cent of liver transplantation in the

western world, in India and other Asian countries, most transplants are living donor liver transplants (LDLTs)^{1,2}. Blood transfusion services (BTSs) are a vital part of LDLT programme that provides various blood components. Although the number of required blood components during surgery has gone down over

the last few years due to better donor selection, careful patient monitoring, improved surgical techniques and use of intra-operative blood salvage, it still continues to be significant^{3,4}.

India is currently witnessing a surge in liver transplantation programmes. There are about 30 functioning liver-transplant centres in India, and new centres are being added every year. India has emerged as regional hub for liver transplants for South-East Asia⁵. An established liver transplant programme is in place at the Institute of Liver Transplantation and Regenerative Medicine, Medanta- the Medicity, Gurugram, India, with close to one transplant a day. The BTSs at the centre maintains adequate blood component inventory including red blood cells (RBCs), fresh frozen plasma, platelet concentrate and cryoprecipitate to support the transplant programme. However, in cases where the recipient is RhD negative, it is sometimes challenging to provide the required RhD-negative RBCs during the peri-operative period. This is crucial considering that India has lower RhD-negative population as compared to western countries⁶⁻⁸.

In this retrospective study, safety data of transfusing RhD-positive RBCs in RhD-negative LDLT recipients during peri-operative period are presented with six-month follow up for risk of developing alloantibodies.

Material & Methods

Setting: This retrospective study was conducted at the department of Transfusion Medicine in collaboration with the department of Liver Transplantation and Regenerative Medicine at Medanta- The Medicity, Gurugram, India, a tertiary care hospital from January 2012 to May 2018. The study was approved by the Institutional Ethics Committee and written informed consent was obtained from all the patients.

Patient selection: All RhD-negative patients who underwent LDLT and were transfused ABO-compatible but RhD-positive RBC units and underwent antibody screening at least 28 days after the last RhD-positive RBC transfusion were included in the study. The decision to transfuse RhD-positive RBC units to RhD-negative patients was based on the inventory levels in the hospital BTS. Female patients of childbearing age, children below 18 yr of age and patients with pre-formed alloantibody(s) were excluded from the study. Immunoprophylaxis with RhIg (Rho (D) immune globulin) was not given

to any patients. All the patients were started on triple immunosuppression drugs [mycophenolate mofetil (MMF), tacrolimus and glucocorticoids] before the surgery as per the standard hospital protocol.

Data collection: Patient and donor demographics (age, gender, indication for liver transplant and blood group of recipient and donor), relevant clinical history (immunoprophylaxis), transfusion records (intra and post-operative RBC transfusion) and follow up with the results of antibody screening were collated from hospital information system and manual data forms.

Demographics: A total of 1946 patients underwent LDLT during the study period; of these 1585 were males and 361 were females. The mean age of the male recipients was 48.9 yr and of the female recipients was 49.3 yr. The peri-operative RBC consumption was 0-18 RBC units with a mean of 5 units. During the study period, a total of 100 RhD-negative patients underwent liver transplant. Of these 100 patients, 77 patients were excluded from the study population because they received RhD-negative compatible units (17 were children below 18 yr of age, 57 were females in childbearing age group and three patients had pre-formed anti-D antibodies). A total of 23 RhD-negative patients received RhD-positive RBCs. Two patients died within two months of transplant due to transplant-related complications and were excluded from the study population.

Laboratory protocol: All patient samples were subjected to ABO and RhD grouping and antibody screening at the time of admission to the hospital. ABO and RhD grouping was done by column agglutination technology (CAT) using ABD cards and reverse diluents cards (Ortho Clinical Diagnostics, Rochester, USA). Antibody screening was performed by indirect antiglobulin test (IAT), using low ionic strength solution-anti-human globulin (AHG)-based CAT (Ortho Clinical Diagnostics, Rochester, USA) and commercially available three-cell reagent panel (R1R1, R2R2 and rr phenotype; 0.8 per cent Surgiscreen, Ortho Clinical Diagnostics, Rochester, USA). Screening for antibody was also performed using two-step enzyme (papain) method in neutral cards (Ortho Clinical Diagnostics, Rochester, USA). Post-operatively, screening for unexpected antibody by both AHG and enzyme methods was done on weekly intervals for 12 weeks and then every three months till the last follow up.

Results and Discussion

RhD-negative recipients receiving RhD-positive red blood cell (RBC): The mean age of recipients, gender ratio and mean number of RBC units transfused intra-operatively were similar to the previous study published from our centre³. The mean age of the 21 patients included in the study cohort was 45 yr, with a male-to-female ratio of 4.3:1. Three of the four females, included in the study, were post-menopausal, and the decision to infuse RhD-positive RBC in the fourth woman was taken because she had completed her family and RhD-negative inventories were critical. These 21 RhD-negative patients received a mean of eight RhD positive RBC units intra-operatively (range 2-20 RBCs). In addition, three of these patients also received upto two RhD-positive

RBC units in the immediate post-operative period (within 24 h).

Follow up for unexpected antibody development: Post-transplant, protocol antibody screen (weekly for 12 wk) was negative and none of the patients developed anti-D alloantibody, thereafter, until their last follow up. Minimum period of follow up was six months while the maximum was 90 months with a mean of 21 months. In our study, none of the 21 patients who were given RhD-positive RBC either intra (n=18) or both intra- and post-operatively (n=3) produced IAT detectable anti-D antibodies (Table).

The Rh system is one of the most polymorphic and immunogenic blood group systems with antigen-D

Table. Summary of RhD-negative liver-transplant recipients

Case number	Gender/age	Diagnosis	Blood group		Red blood cell transfused		Follow up with antibody screen (months)
			Recipient	Organ donor	Intra-operative RhD+	Post-operative RhD+	
1	Male/58	HCC	B negative	B positive	13	0	90
2	Female/39	Crypto	O negative	O positive	20	0	32
3	Female/48	NASH	B negative	O positive	18	0	66
4	Male/47	HCV	A negative	A negative	6	1	26
5	Male/45	HCV	O negative	O positive	6	1	6
6	Male/39	HBV	O negative	O positive	8	2	8
7	Male/22	HBV	AB negative	B positive	4	0	27
8	Male/46	ALD	O negative	O positive	7	0	26
9	Male/49	Crypto	B negative	B positive	4	0	25
10	Female/48	ATT	O negative	O positive	5	0	20
11	Male/48	ACLD	A negative	O positive	10	0	23
12	Male/51	HBV	O negative	O negative	8	0	8
13	Male/35	HBV	AB negative	A positive	4	0	6
14	Male/41	HCV	AB negative	O negative	10	0	16
15	Male/46	NAFLD	O negative	O positive	8	0	11
16	Male/53	ACLD	O negative	O positive	2	0	7
17	Male/52	ACLD	O negative	O negative	4	0	6
18	Male/44	ACLD	O negative	O positive	8	0	6
19	Male/42	ACLD	O negative	O positive	10	0	6
20	Female/45	AI-CLD	O negative	O positive	4	0	7
21	Male/49	HCC	B negative	B negative	4	0	6

HCC, hepatocellular carcinoma; Crypto, cryptogenic; NASH, non-alcoholic steatohepatitis; HCV, hepatitis C virus; HBV, hepatitis B virus; ALD, alcoholic liver disease; ATT, anti-tubercular therapy; ACLD, acute-on chronic liver disease; NAFLD, non-alcoholic fatty liver disease; AI-CLD, autoimmune chronic liver disease

having the highest immunogenicity among Rh antigens⁹. Approximately 80 per cent RhD-negative immunocompetent individuals develop Rh antibodies within 2-5 months after receiving a single unit (200 ml) of RBC¹⁰. The risk of alloimmunization, or formation of new irregular antibody, depends on multiple factors including antigenic differences between donor and recipient RBC, dose of RBC transfused, frequency of transfusion, recipient immune status and immunogenicity of the donor antigens. In the present study cohort, LDLT patients received multiple units of highly immunogenic RhD-positive RBC. Lack of alloimmunization can possibly be explained by the fact that all the patients undergoing LDLT are maintained under high degree of immunosuppression, which is consistent with other published data¹¹⁻¹⁴. For example, Casanueva *et al*¹² found no evidence of alloimmunization in Rh-negative orthotopic liver-transplant recipients (n=17) transfused with RhD-positive RBCs. This was attributed to cyclosporine-A, an immunosuppressive drug which affects the humoral response, inhibiting lymphocyte activation and primary immune response. Yuan *et al*¹³ reported similar findings in liver transplant recipients (n=15) with a more recent immunosuppressive drug, mycophenolate mofetil (MMF) that exerts inhibitory effects on B- and T-cell proliferation. In the present study, the risk of alloimmunization was possibly even lower since the immunosuppressive drug protocol included, both, tacrolimus (calcineurin inhibitor) as well as MMF, besides steroid.

Burin des Roziers *et al*¹⁵ reported development of transient and early anti-D antibody in two out of 20 RhD-negative liver-transplant recipients receiving RhD-positive RBC. This weak anti-D reacted only with papain-treated RBCs at 10 and 11 days without any sign of immune haemolysis and became undetectable quickly. However, no such transient anti-D antibody was found in the present study cohort.

By transfusing RhD-positive RBC in RhD-negative patients, authors did not allow any postponement or cancellation of LDLT, a life-saving surgery. This practice also allowed for management of the inventory of limited RhD-negative RBC units and allowed other patients (in other medical and surgical disciplines) to undergo surgeries using RhD-negative RBC instead of RhD positive, since the risk of alloimmunization was higher,

e.g. cardiac surgery. Further studies are warranted to prove the relative safety of transfusing RhD-positive RBC in solid organ-transplant cases. It is quite possible that we may have similar results (no or minimal alloimmunization) are observed in kidney transplant recipients as well, since this category of patients, too, receive substantial immunosuppressive drugs. In conclusion, it is suggested that it is safe to use RhD-positive RBCs peri-operatively in RhD-negative LDLT recipients with low risk of alloimmunization.

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Conflicts of Interest: None

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