Indian J Gastroenterol (January–February 2014) 33(1):72–76
DOI 10.1007/s12664-013-0424-0

CASE SERIES

The use of ABO-incompatible grafts in living donor liver transplantation—First report from India

A. S. Soin · V. Raut · R. Mohanka · A. Rastogi · S. Goja · M. Balachandran · S. Saigal · N. Saraf · P. Bhangui · K. R. Sumana · P. Singla · T. Srinivasan · N. Choudhary · A. Tiwari · V. Raina · D. Govil · N. Mohan · V. Vohra

Received: 22 August 2013 / Accepted: 13 October 2013 / Published online: 27 December 2013

Abstract ABO incompatibility is the commonest reason for rejection of donors in living donor liver transplantation (LDLT). The donor pool could be expanded by 25 % to 35 % if the ABO barrier is overcome. In the absence of pre-conditioning, transplantation across the blood groups is fraught with the almost universal risk of antibody-mediated rejection (AMR) that rapidly leads to graft loss. However, AMR can be prevented by removal of preformed antibodies and reducing their production by B cells. We describe our initial experience of three cases of ABO-incompatible (ABO-i) LDLT: a 42-year-old male, an 8-month-old male and a 28-month-old female, all of blood group O+ who received blood group B + right lobe, B + left lateral segment, and A + left lateral segment liver grafts, respectively. Pre-LDLT conditioning included administration of anti-CD20 antibody (Rituximab®) to the adult 4 weeks prior, and four to seven sessions of double-filtration plasmapheresis to all, to remove preformed antibodies and achieve anti-donor blood group antibody (ADA) titers of ≤1:16 IgG and ≤1:8 IgM, respectively. In addition, cases 1 and 3 received mycophenolate mofetil for 7 days prior to LDLT. After LDLT, all three patients achieved normal graft function over 8–17 days with no evidence of AMR and without the need for further plasmapheresis. Postoperative complications included portal vein thrombosis (one successfully re-explored), CMV (one), *Pseudomonas* and *Klebsiella* sepsis (one each), and abdominal collection (one treated with percutaneous drainage). All are currently well with normal graft function and low ADA titers at 8, 16, and 19 months after ABO-i LDLT.

Keywords Antibody-mediated rejection · Plasmapheresis · Rituximab

Introduction

While living donor liver transplantation (LDLT) is an established alternative to deceased donor grafts, there are inherent limitations to its universal applicability to all potential recipients. Most centers including ours require donors to be close relatives under 55 years [1]. The commonest reason for screened potential donor rejection is ABO-incompatibility (ABO-i) [2]. It is estimated that nearly 25 % to 35 % additional transplants would be possible if the ABO barrier could be overcome [3]. Swap transplantation and ABO-incompatible liver transplantation (ABO-i LT) are two ways this can be done.

ABO-i LT was first described by Starzl et al. in 1969 [4]. Antibody-mediated rejection—the bane of these transplants—can be avoided by removal of preformed antibodies and reducing their production by B cells. Depending on the center and era, this has been done with various combinations of plasma exchange [5], anti-CD 20 monoclonal antibody [6], intravenous immunoglobulin before and after the transplant, splenectomy [7], portal venous and/or hepatic arterial infusion of prostaglandins, gammaxylae mescelte (a protease inhibitor which inhibits platelet aggregation) [8], high dose post-transplant immunosuppression, and post-transplant plasma exchange. Most of the current protocols have been simplified with plasma exchange (always) and preoperative anti-CD 20 antibody (usually) being a part of the protocol.

We launched our swap and ABO-i LT programs 4 and 1.5 years ago, respectively. Majority of those with group A/

See editorial on doi:10.1007/s12664-013-0435-x.

A. S. Soin (✉) · V. Raut · R. Mohanka · A. Rastogi · S. Goja · M. Balachandran · S. Saigal · N. Saraf · P. Bhangui · K. R. Sumana · P. Singla · T. Srinivasan · N. Choudhary · A. Tiwari · V. Raina · D. Govil · N. Mohan · V. Vohra
Medanta Institute of Liver Diseases and Transplantation, Medanta-The Medicity, Gurgaon, Haryana 122 001, India
e-mail: absoin@gmail.com

B mismatch was enrolled into our swap program whereas those with non-O to O mismatch were offered ABO-i LT. We describe our initial experience of the first three cases of ABO-i LT.

Methods

During January 2012 to December 2012, 478 patients were evaluated for liver transplantation. Of these, 213 were found to have suitable donors and underwent LDLT with their own blood group-matched donors. Fifty-six had willing but ABO-incompatible donors. Based on experience of other centers [9–13], an age-based protocol was evolved for ABO-i LT, which is outlined in Table 1.

In addition to the standard counseling prior to LDLT [1], all patients, donors, and their families were counseled in detail about the nature and potential adverse of the pre- and post-transplant treatments detailed in Table 1, as well as the higher risk of antibody-mediated rejection, hepatic arterial thrombosis, biliary complications and graft failure after transplant. An informed consent was then obtained. Standard piggyback LDLTs were performed [14]. The three patients underwent a left lateral sector, a reduced left lateral sector, and a modified right lobe graft, respectively, as per previously described techniques [1].

Immunosuppression

Our standard immunosuppression included a triple drug regimen with tacrolimus, mycophenolate mofetil (MMF), and steroids. The latter were withdrawn at 3 months. Peri-transplant treatment protocol for ABO-i LDLT included pre-transplant rituximab, MMF and plasmapheresis (PP), and post-transplant intravenous immunoglobulin (IVIG) (see Table 1). The adult patient received 100 mg rituximab 28 days prior to the provisional date of LDLT. We confirmed elimination of B cells from circulation by demonstrating the reduction of absolute CD-19+ lymphocyte count to 0–5 cells/mm^3 in blood. Oral MMF 500 mg/twice a day was started 7 days prior to LDLT. PP was performed with blood group AB plasma depending upon anti-ABO antibody titers before transplantation to reduce anti-donor blood group antibody (ADA) IgG and IgM titers to 1:16 or 1:8 or less, respectively. Tacrolimus trough level was maintained higher than usual, between 10 and 15 ng/mL in the first 2 weeks, 7–10 ng/mL between 2 and 12 weeks, and 5–7 ng/mL until 6 months. Prednisolone was given at a dose of 2 mg/kg for 1 week, then at 1 mg/kg for 2 weeks, then gradually tapered to 0.5 mg/kg by the fourth week and stopped after 3 months (Table 2).

Summary of cases

Case-1: This patient was a 42-year-old male with blood group O+. He was diagnosed with ethanol-related decompensated chronic liver disease with ascites, subacute bacterial peritonitis, hepatic encephalopathy, and upper gastrointestinal bleeding. He was abstinent for 1 year. His only suitable donor was his brother-in-law whose blood group was B+. Patient received 100 mg rituximab IV under close monitoring. His CD-19 cell counts (in cubic millimeters) on days −30 (baseline), −15, −7, 7, and 14 were 500, 100, 2, 1, and 1, respectively. His ADA titer and infective parameters were regularly monitored for 3 weeks. PP was done with AB blood group fresh frozen plasma; his ADA IgG and IgM titers gradually fell from 1:512 and 1:256 to 1:8 and 1:4, respectively, immediately prior to transplant, as shown in Fig. 1. During the preparation phase, he developed *Klebsiella* infection which responded to culture-based antibiotics. He underwent a modified right lobe LDLT with a graft to recipient weight ratio (GRWR) of 1.05, 28 days after administration of rituximab. His postoperative ADA titer remained within the desired range as shown in Fig. 1, and his liver functions normalized within the first week. He did not show any evidence of rejection. Postoperatively, he had urinary tract infection due to *Klebsiella* on day 9 and cytomegalovirus (CMV) infection on day 11, which responded to culture-based antibiotic and ganciclovir, respectively. He was discharged home on postoperative day (POD) 15. He is now living normal life 8 months after ABO-i LDLT.

Table 1 Protocol of ABO-incompatible living donor liver transplantation

		Pre-transplant	Post-transplant
Monitoring targets	CD-19 cell count	Day −7, 0–5	Day 7, 0–5
	ADA IgG titer	Day −1, ≤1:16	≤1:32 for 12 weeks
	ADA IgM titer	Day −1, ≤1:8	≤1:16 for 12 weeks
Age-based treatment	<1 year	PP if needed	IVIG
	1–7 years	PP, MMF	Extra steroid and tacrolimus, IVIG
	>7 years	Rituximab, PP, and MMF	Extra steroid and tacrolimus, IVIG, and PP if needed

ADA anti-donor blood group antibody, *MMF* mycophenolate mofetil, *IVIG* intravenous immunoglobulin, *PP* plasmapheresis

Table 2 Summary of patient characteristics

			Pre-transplant			Post-transplant									
Case	Age/ sex	Donor–recipient blood group	Rituximab	PP	ADA titer at LT IgG; IgM	AMR	PP	Peak ADA titer IgG; IgM	Bacterial infection	Vascular complication	CMV infection	IVIG	ICU Stay (days)	HOSP Stay (days)	F/U (mt)
1	42/M	B to O	100 mg	7	1:8; 1:8	No	No	1:4; 1:2	Yes	No	Yes	No	5	15	8
2	0.8/M	B to O	No	4	1:8; 1:4	No	No	1:32; 1:16	Yes	No	No	POD 7–8	8	16	16
3	2.4/F	A to O	No	4	1:16 1:8	No	No	1:32; 1:16	Yes	PVT POD 7	No	POD 1–7	12	22	19

ADA anti-donor blood group antibody, *CMV* cytomegalovirus, *PP* plasmapheresis, *POD* postoperative day, *PVT* portal vein thrombosis, *F/U* (*m*) follow up in months

Case-2: This 8-month-old male child had a history of jaundice and clay-colored stools since day 5 of life. He developed ascites since the age of 2 months. His blood group was O, and both of his parents were B positive. He received his mother's left lateral section with a GRWR 2.01. His preoperative ADA titer was 1:128 which was reduced to 1:16 at time of surgery after four sessions of PP (Fig. 2). He received IVIG postoperatively for 4 days. His liver function tests normalized within the first week after the transplant. His anti-B titer on POD 7 rose to 1:32 for which he received IVIG on POD 7 and 8. He developed abdominal sepsis with high drain cell count and *Pseudomonas aeruginosa* on culture, sensitive to colistin, which was treated accordingly. Postoperatively, his ADA titers remained at 1:32 or less between POD 1 and 14 (Fig. 2). He was discharged 21 days after surgery and he is doing well now.

Case-3: This 28-month-old female child underwent a Kasai portoenterostomy for extrahepatic biliary atresia at the age of 38 days. After a year of uneventful course, she developed recurrent cholangitis, hematemesis, ascites, and hepatic encephalopathy. Her blood group was O, and her grandmother of blood group A was the only otherwise suitable donor in the family. The child received four PPs on alternate days starting 7 days prior to transplant. MMF was started as per protocol (see above). The transplant was performed with the donor's left lateral section with a GRWR of 1.75. She received IVIG intraoperatively and postoperatively for 5 days at dose of 10 g/day. Her preoperative ADA titer was 1:256, which was gradually reduced to 1:8 after four sessions of PP, as shown in Fig. 2. She was re-explored on POD 6 for portal vein thrombosis, which was treated with thrombectomy and thrombolysis. She developed localized abdominal collection which was treated with drainage and antibiotics as per culture sensitivity. Postoperatively, her ADA titers remained at 1:32 or less between POD 1 and 14 (Fig. 2). She is now well with normal liver function and has achieved normal milestones of development 21 months after ABO-i LDLT.

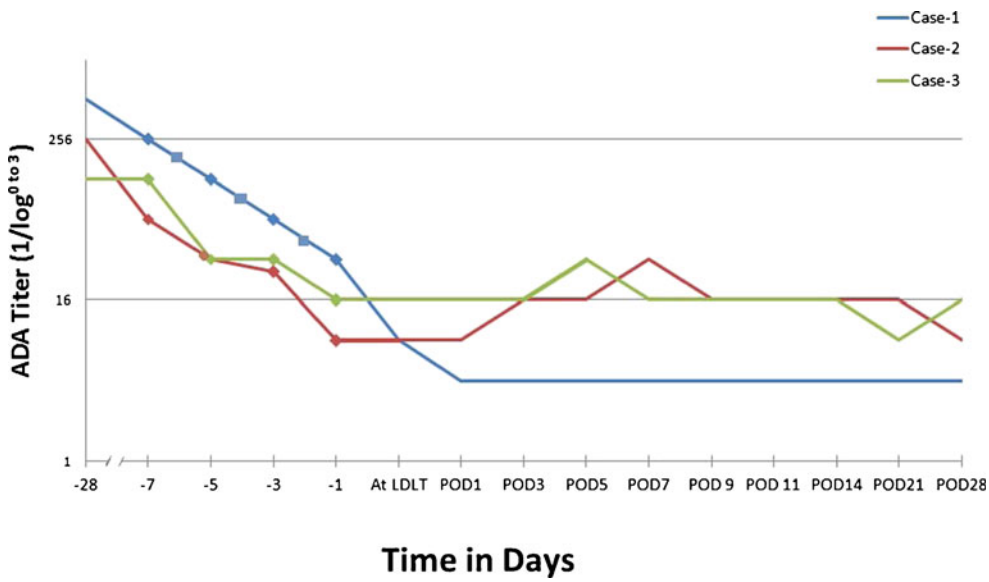

Fig. 1 Perioperative anti-blood group antibody titers. Preoperative rituximab and plasmapheresis reduced the titers to 1:16 level, postoperatively rising titers in cases 2 and 3 were treated with IVIG.(*diamond* plasmapheresis; *POD* postoperative day)

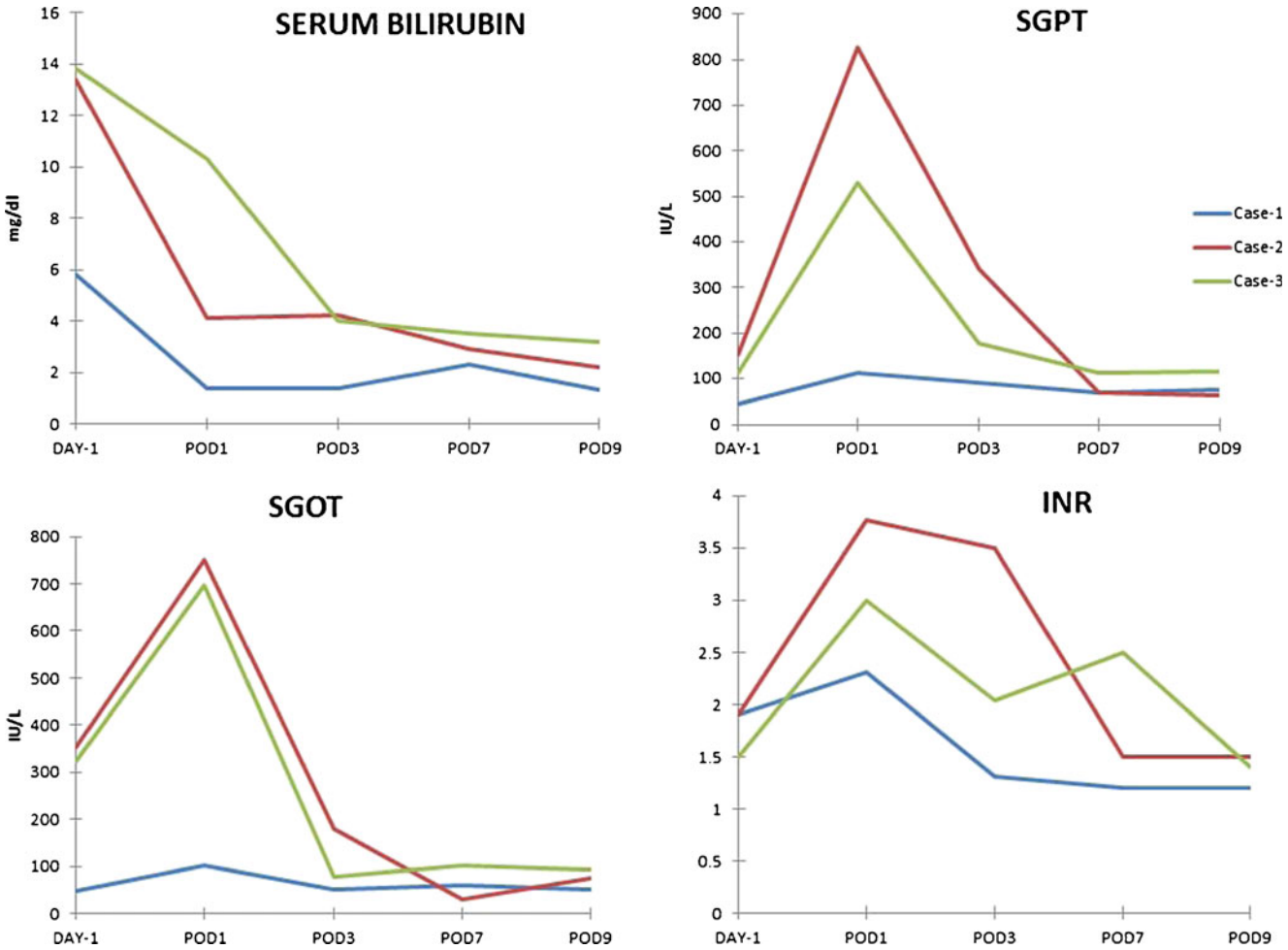

Fig. 2 Perioperative liver function tests. In all three patients, serum bilirubin, SGOT, SGPT, and INR were stabilized by POD 9

Discussion

Initial animal experiments by Professor Starzl [4] demonstrated that the liver is "a privileged organ" with much greater resistance to acute rejection than the kidney or heart. However, in 1987, Rego et al. [15] reported hyperacute rejection after ABO-i LT despite the "privileged" status of the liver. Later, Gugenheim et al. [16, 17] also confirmed hyperacute rejection and lower graft survival of ABO-i LT. In his series of 17 ABO-i transplants, Gugenheim postulated immunological damage as the cause of low graft survival and reported antibody-mediated rejection as a cause of graft failure in six patients. He also acknowledged an increased incidence of arterial thrombosis and progressive cholangitis in ABO-i grafts. Similarly, Sanchez-Urdazpal [18] confirmed an increased incidence of cholangitis, bile leak, cellular rejection, and hepatic artery thrombosis in ABO-i LT.

In Asia, LDLT is predominant due to a scarcity of deceased donors. In India, as in many other countries, the living donor pool is restricted to the family members as emotionally unrelated donation is prohibited by law, and friends are rarely approved as donors. Thus, it is sometimes difficult to find blood group-matched donors within the family. To overcome the ABO barrier, we started swap liver transplantation between two families with A to B and B to A type ABO mismatch. However, for blood group O recipients, swap LT is not an option. We therefore, selected such recipients to launch our ABO-incompatible LDLT program.

Our immunosuppression protocol for ABO-i LDLT included rituximab, plasmapheresis, MMF without portal vein infusion, and splenectomy. Rituximab is a monoclonal antibody against the CD-20 antigen, which is expressed on the B cells of all developmental stage except plasma cells. These cells also express the CD-19 antigen, which is widely used as surrogate serum marker of extent of CD-20 positive cell depletion. Although action of rituximab starts within 3 days; effective depletion of B cells is seen only after 3 weeks. Therefore, we administered rituximab 3 weeks prior to LDLT and monitored B cell depletion using CD-19 estimation. As the plasma cells escape from rituximab action [19], MMF was started 7 days prior to LDLT to remove plasma cells. Preformed ADA were removed with double-filtration

plasmapheresis involving the separation of plasma from whole blood followed by further processing through a plasma fractionator, where substances with molecular weight more than 67,000 were filtered out and rest of plasma returned to the body. Splenectomy was not included in the protocol as other series [20] have shown similar postoperative ADA titers after rituximab irrespective of splenectomy. We avoided the local infusion therapy due to the risks of bleeding and portal vein infusion, and its doubtful role in other studies [21] in preventing antibody-mediated rejection when rituximab and efficient perioperative PP are employed.

There were two pediatric patients in this series. Egawa et al. showed that patient's age is an important factor in acceptance of ABO-i grafts [22]. Infants have the almost similar outcomes after compatible and ABO-i LDLT due to immaturity of the immune system and failure to mount a significant ADA response. The ADA response increases with age. Preoperatively, in both children, four sessions of PP were sufficient to bring down the ADA titer to <1:16. Rituximab was therefore, omitted. Postoperatively, they were given IVIG. In both patients, liver graft function gradually recovered, with no rejection episode. Both had an episode of bacterial infection which responded to appropriate antibiotics. A more aggressive preoperative immunosuppression protocol was adopted for the adult patient including rituximab, PP, and MMF, which resulted in effective deletion of B cell population and preformed ADA prior to transplant. In the post-transplant period, he did not require any PP, and his peak ADA titer was 1:4. He developed the bacterial and CMV infections which responded to appropriate antimicrobials. All three patients had low ADA titers at 8, 16, and 19 months after transplant.

This series describes our initial experience with ABO-i LT, a procedure that helps to increase the donor pool in predominantly live donor-based liver transplant programs. While splenectomy and intravascular infusions may not be necessary as part of the ABO-i protocol, these procedures overcome the ABO barrier. This predisposes patients to a higher incidence of perioperative infections, but in our experience, they can be successfully managed. Once the patient escapes antibody-mediated rejection for the first 4 weeks, further problems are uncommon.

References

1. Soin A. Live donor liver transplantation: Donor selection, safety and techniques of donor hepatectomy. *In*: Sahni P, (ed). IASG Textbook of Surgical Gastroenterology. Delhi. Byword Books. 2014 (In press).
2. Hwang S, Lee SG, Moon DB, et al. Exchange living donor liver transplantation to overcome ABO incompatibility in adult patients. Liver Transpl. 2010;16:482–90.
3. Warner PR, Nester TA. ABO-incompatible solid-organ transplantation. Am J Clin Pathol. 2006;125:S87–94.
4. Starzl TE. Experience in Hepatic Transplantation. Philadelphia W.B Saunders Co; 1969.
5. Hanto DW, Fecteau AH, Alonso MH, Valente JF, Whiting JF. ABO-incompatible liver transplantation with no immunological graft losses using total plasma exchange, splenectomy, and quadruple immunosuppression: evidence for accommodation. Liver Transpl. 2003;9:22–30.
6. Kawagishi N, Satoh K, Enomoto Y, et al. New strategy for ABO-incompatible living donor liver transplantation with anti-CD20 antibody (rituximab) and plasma exchange. Transplantat Proc. 2005;37:1205–6.
7. Renard TH, Andrews WS. An approach to ABO-incompatible liver transplantation in children. Transplantation. 1992;53:116–21.
8. Yoshizawa A, Sakamoto S, Ogawa K, et al. New protocol of immunosuppression for liver transplantation across ABO barrier: the use of rituximab, hepatic arterial infusion, and preservation of spleen. Transplant Proc. 2005;37:1718–9.
9. Egawa H, Inomata Y, Uemoto S, et al. Biliary anastomotic complications in 400 living related liver transplantations. World J Surg. 2001;25:1300–7.
10. Song GW, Lee SG, Hwang S, et al. Successful experiences of ABO-incompatible adult living donor liver transplantation in a single institute: no immunological failure in 10 consecutive cases. Transplant Proc. 2013;45:272–5.
11. Stewart ZA, Locke JE, Montgomery RA, Singer AL, Cameron AM, Segev DL. ABO-incompatible deceased donor liver transplantation in the United States: a national registry analysis. Liver Transplant. 2009;15:883–93.
12. Egawa H, Ohdan H, Haga H, et al. Current status of liver transplantation across ABO blood-type barrier. J Hepatobiliary Pancreat Surg. 2008;15:131–8.
13. Raut V, Uemoto S. Management of ABO-incompatible living-donor liver transplantation: past and present trends. Surg Today. 2011;41:317–22.
14. Soin AS, Mohanka R, Singla P, et al. Segment IV preserving middle hepatic vein retrieval in right lobe living donor liver transplantation. J Am Coll Surg. 2011;213:e5–e16.
15. Rego J, Prevost F, Rumeau JL, et al. Hyperacute rejection after ABO-incompatible orthotopic liver transplantation. Transplant Proc. 1987;19:4589–90.
16. Gugenheim J, Samuel D, Fabiani B, et al. Rejection of ABO incompatible liver allografts in man. Transplantat Proc. 1989;21(1 Pt 2):2223–4.
17. Gugenheim J, Samuel D, Reynes M, Bismuth H. Liver transplantation across ABO blood group barriers. Lancet. 1990;336:519–23.
18. Sanchez-Urdazpal L, Sterioff S, Janes C, Schwerman L, Rosen C, Krom RA. Increased bile duct complications in ABO incompatible liver transplant recipients. Transplantat Proc. 1991;23(1 Pt 2):1440–1.
19. Pescovitz MD. Rituximab, an anti-cd20 monoclonal antibody: history and mechanism of action. Am J Transplant. 2006;6(5 Pt 1):859–66.
20. Raut V, Mori A, Kaido T, et al. Splenectomy does not offer immunological benefits in ABO-incompatible liver transplantation with a preoperative rituximab. Transplantation. 2012;93:99–105.
21. Ikegami T, Taketomi A, Soejima Y, et al. Rituximab, IVIG, and plasma exchange without graft local infusion treatment: a new protocol in ABO incompatible living donor liver transplantation. Transplantation. 2009;88:303–7.
22. Egawa H, Oike F, Buhler L, et al. Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. Transplantation. 2004;77:403–11.