



# Infectious Complications in Adult ABO-Incompatible Liver Transplantation: Our Preliminary Experience

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The number of ABO-incompatible living donor liver transplantations (ABO-I LDLT) has increased owing to the use of preoperative rituximab for immunosuppression. However, controversy remains regarding adequate immunosuppression owing to rejection and infection. Here, we present 5 cases of our ABO-I LDLT experience, emphasizing rejection and infectious complications, retrospectively. The treatment protocol included prophylactic rituximab followed by plasma exchange prior to transplantation, splenectomy, and immunosuppressive and prophylactic antibiotic regimens after transplantation. Four of the 5 patients also received local infusion therapy via the portal vein. Neither hyperacute nor antibody-mediated rejection occurred. All grafts were functioning well at discharge. Rehospitalization was required for 2 patients due to severe infection within 6 months of transplantation. Invasive aspergillosis was successfully treated in 1 patient, but the other patient died from severe sepsis with overwhelming postsplenectomy infection syndrome. Our results confirm that, although improved immunosuppressive therapy markedly reduces rejection in ABO-I LDLT, it is also associated with an increased risk of various life-threatening infections.

**Key words:** ABO-incompatible transplantation – Antibody mediated rejection – Invasive aspergillosis – Rituximab – Splenectomy

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In Japan, living donor liver transplantation (LDLT) has become more common owing to a severe shortage of deceased donors. However, donor candidates for LDLT are usually limited to relatives and spouses, and some patients do not have an acceptable donor with a compatible blood type. Developments in immunosuppressive protocols, such as preoperative rituximab administration, plasma exchange, and postoperative local infusion therapy, have markedly reduced graft rejection due to ABO-incompatibility (ABO-I); therefore, LDLT is becoming an increasingly popular alternative.<sup>1,2</sup> However, controversy remains regarding the provision of adequate immunosuppressive therapy required for this procedure owing to rejection and infection. Our experience with ABO-I LDLT began in 2008, and since then, we have performed the procedure in 5 patients. In this study, we present our experience with ABO-I LDLT, with a focus on graft rejection and infectious complications.

## Patients and Methods

### *Patients and surgical procedure*

Between January 2008 and December 2014, we performed an ABO-I LDLT on 5 patients (2 men and 3 women; mean age, 53.4 years; range, 35–61 years). The transplant procedures for both donors and recipients have previously been reported.<sup>3</sup> Hepatic arterial reconstruction was performed using a surgical microscopic procedure. Biliary reconstruction was conducted in a duct-to-duct fashion. Concomitant splenectomy was performed in all cases after biliary reconstruction.

This study was reviewed by the institutional ethics committee and was performed in accordance with the ethical standards laid down in the 2000 Declaration of Helsinki, as well as the Declaration of Istanbul 2008. In addition, written informed consent was obtained from all patients prior to their inclusion in this study.

### *ABO-I LDLT protocol*

Our protocol was based on those previously reported in the literature for ABO-I LDLT.<sup>2,4</sup> Each patient was treated with rituximab (Rituxan; Roche Pharmaceuticals, Basel, Switzerland) 2 to 4 weeks prior to transplantation: 400 mg/m<sup>2</sup> for case 1, 350 mg/m<sup>2</sup> for cases 2 to 4, and 300 mg/m<sup>2</sup> for case 5. Rituximab was administered in 2 or 3 doses depending on the patient's condition. Plasma

exchange was performed 2 or 3 times to decrease the antidonor blood type antibody titer to <8.

After the transplant, the immunosuppressive regimen consisted of tacrolimus (Prograf; Astellas Pharma, Tokyo, Japan), mycophenolate mofetil (CellCept; Roche Pharmaceuticals, Basel, Switzerland), and corticosteroids. The tacrolimus dose was adjusted to achieve a trough level of 10 to 15 ng/mL for 2 weeks following the transplant. Thereafter, the target trough level was gradually reduced to approximately 7 ng/mL. These trough levels were the same as those used in ABO identical/compatible LDLT at our institute. The corticosteroids were administered as an initial dose of 2 mg/kg/day, which was tapered gradually.

All patients, except for patient 4, underwent intraportal infusion (IPI) therapy, as reported by Tanabe *et al.*<sup>2</sup> IPI therapy was discontinued in patient 4 because of surgical difficulties. The IPI therapy consisted of prostaglandin E1 (0.01 µg/kg/min until approximately 3 weeks after the transplantation), heparin (during prostaglandin E1 administration), and additional corticosteroids (initial dose of 2 mg/kg/day and was tapered and finally discontinued on postoperative day 14).

The prophylactic regimen for infectious complications was the same as that for ABO identical/compatible LDLT and consisted of a 10- to 14-day course of antibiotics (flomoxef and tazobactam/piperacillin) and micafungin. Oral acyclovir, sulfamethoxazole-trimethoprim, and an amphotericin suspension were given until discharge following the transplantation. Cytomegalovirus (CMV) prophylaxis, including intravenous ganciclovir, was not administered. However, CMV antigenemia was monitored at least twice a week until discharge to allow for preemptive therapy for CMV infection. After discharge, the immunosuppressive therapy was the same as that for ABO identical/compatible LDLT at our institute, consisting of tacrolimus, mycophenolate mofetil, and steroids.

## Results

### *Patient characteristics*

The demographic and clinical characteristics of the patients are summarized in Table 1. The graft types included left lobe with middle hepatic vein (n = 3) and right lobe without middle hepatic vein (n = 2). The mean graft weight-to-recipient weight ratio was 0.93% (range, 0.66%–1.22%), and the graft-to-standard liver volume ratio was 45.1% (range, 36.7%–54.0%).

Table 1 Patient characteristics

Factors	Case 1	Case 2	Case 3	Case 4	Case 5
Ethnology	HBV + HCC	LC (HCV)	PBC	PBC	FAP
Age (y)/sex	58/F	53/M	60/F	61/F	35/M
MELD score	18	12	16	19	6
Donor age (y)/sex	58/M	22/M	33/F	62/M	60/F
Graft type	Lt	Lt	Rt	Lt	Rt
Blood type	A to O	A to O	A to B	A to O	B to A
Plasma exchange (pre, post)	(2, 0)	(2, 0)	(3, 0)	(2, 0)	(2, 0)
Rituximab dose (mg)	620	575	500	550	500

F, female; FAP, familial amyloid polyneuropathy; HCC, hepatocellular carcinoma; LC, liver cirrhosis; Lt, left lobe with middle hepatic vein; M, male; MELD, model for end-stage liver disease; PBC, primary biliary cirrhosis; Rt, right lobe without middle hepatic vein.

There were no emergency transplantation performed. The indications for liver transplantation were primary biliary cirrhosis ( $n=2$ ), hepatocellular carcinoma within Milan criteria ( $n=1$ ), liver cirrhosis due to hepatitis C virus ( $n=1$ ), and familial amyloid polyneuropathy ( $n=1$ ). The mean model for end-stage liver disease (MELD) score was 14.2 (range, 6–19). The living donors were the patients' sons ( $n=2$ ), daughter ( $n=1$ ), husband ( $n=1$ ), or mother ( $n=1$ ). The mean follow-up after the ABO-I LDLT was 1978 days (range, 202–2850 days).

#### Antibody titer and operative outcomes

The serial changes in the antidonor blood type antibody titer are shown in Table 2. The median antibody titer before the plasma exchange was 128 (range, 8–256), and the mean number of preoperative plasma exchange sessions was 2.2 (range, 2–3 times). The median antibody titer immediately before transplantation was 4 (range, 4–8). Postoperative plasma exchange was not performed in any of the patients. The median peak antidonor blood type antibody titer after the LDLT was 8 (range, 2–16). The mean operative time and blood loss were 1028.8 minutes (range, 836–1299 minutes) and 3548.0 mL (range, 1120–8070 mL), respectively.

#### Survival and postoperative complications

All patients were discharged with good graft function after transplantation. No episodes of antibody-mediated rejection, which is characterized by periportal edema and necrosis, as well as positive C4d immunostaining, were encountered.<sup>5</sup> In addition, no episodes of hyperacute rejection were observed. Mild acute cellular rejection was detected in patients 4 and 5, and it was successfully treated with tapered steroid augmentation.

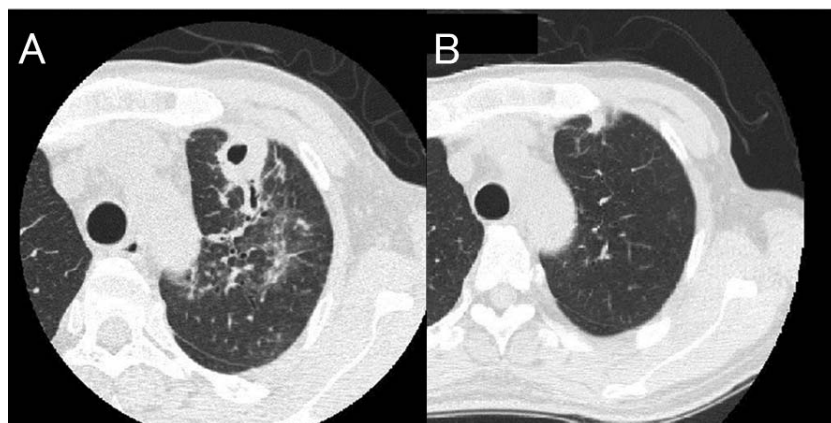
In all cases, CMV antigenemia without symptoms was confirmed between 4 and 8 weeks after transplantation. Generally, preemptive therapy for CMV was ganciclovir administration, which continued at least 2 weeks after antigenemia confirmation. The preemptive therapy was effective, and CMV diseases such as hepatitis, pneumonia, and enteritis were not found in any of the patients. The patients also remained free from infection with other viruses, such as the herpes viruses or Epstein-Barr virus (EBV).

Two patients required hospitalization for serious infectious within 6 months of transplantation. Patient 2 was diagnosed with proven invasive aspergillosis by EORTC criteria, which was diagnosed on chest computed tomography and histo-

Table 2 Antibody titer and operative outcomes

Factors	Case 1	Case 2	Case 3	Case 4	Case 5
Antibody titer (Pre→at Tx→after Tx)	(256→4→16)	(128→4→4)	(256→8→8)	(16→4→8)	(8→8→8)
AMR	–	–	–	–	–
ACR	–	–	–	+	+
Infectious complications	CMV	CMV Inv Asp	CMV Sepsis	CMV	CMV
Other complications	–	–	Bleeding Duo ulcer	Bleeding Biliary stricture	Duo ulcer
Prognosis	93 months, alive	91 months, alive	89 months, alive	7 months, dead	44 months, alive

ACR, acute cellular rejection; AMR, antibody mediated rejection; CMV, cytomegalovirus; Duo ulcer, duodenal ulcer; Inv Asp, invasive aspergillosis; Tx, transplantation.



**Fig. 1** (A) Chest computed tomography (CT) image (lung window settings) showed a 2.5-cm-thick walled cavity in the left upper lobe at the time of diagnosis. (B) After voriconazole administration, the cavity disappeared and only a slight scar was presented on the chest CT scan.

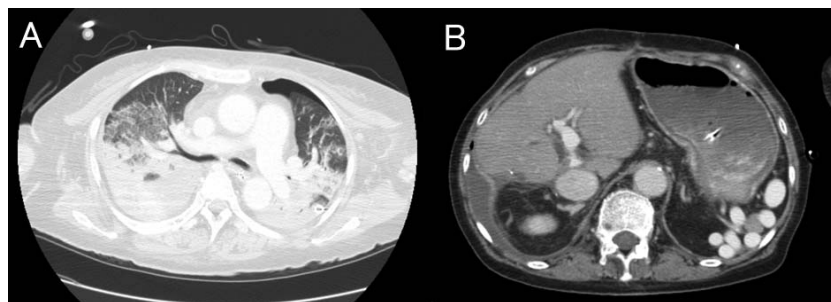
logic specimen obtained by bronchoscopy.<sup>6</sup> Other infections, such as pneumocystis pneumonia or bacterial or viral infections, were not detected at that time. The patient was successfully treated with voriconazole (Fig. 1). Patient 4 was referred to our hospital after being diagnosed with septic shock with multiple organ failure at the regional hospital. The patient had been seen for follow-up in our outpatient department 2 days before her hospitalization; at that time, her graft was functioning well. After admission, the patient received intensive treatment with broad-spectrum antibiotics, antifungal agents, mechanical ventilation, and organ perfusion support. An extensive, detailed examination including whole body computed tomography and serologic examinations of fungal or viral infections were performed in an attempt to locate the infection and treat it definitively (Fig. 2). However, the focus of the infection and causative organism were not found including cholangitis. On the other hand, the infection did not respond to empiric treatment. The patient died from multiple organ failure 202 days after transplantation. Other complications experienced by the patients included intra-abdominal bleeding requiring laparotomy ( $n = 2$ ), upper gastrointesti-

nal ulcer ( $n = 2$ ), and anastomotic stricture of the bile duct ( $n = 1$ ) (Table 2).

## Discussion

In Japan, LDLT is a widely accepted alternative procedure of deceased donor liver transplantation with a favorable prognosis. However, some patients are unable to locate a living donor with a compatible blood type. As a result, ABO-I LDLT has recently become more common, and its prognosis is similar to that of ABO-compatible LDLT.<sup>7</sup> Immunosuppression protocols such as rituximab have markedly reduced the rate of graft rejection; however, they also increase the risk of life-threatening infections. For this reason, it is important to understand the infectious complications.

In our series, the immunosuppression protocol, which included prophylactic rituximab and splenectomy, resulted in no antibody-mediated rejections. Thus, the immunosuppression level provided by the protocol appears to be enough for preventing graft rejection caused by ABO incompatibility. However, the 2 life-threatening infections and high incidence of CMV antigenemia confirm that the immunosuppressive therapy may be associated with serious infectious complications.



**Fig. 2** (A) CT image (lung window settings) showed bilateral infiltrative shadow and pleural effusion at the time of referred to our hospital. (B) Also, abdominal enhanced CT image showed no definite infectious findings.

Although the occurrence of infections is one of the most significant problems associated with solid organ transplantation, there are few previous reports regarding infectious complications of ABO-I LDLT.<sup>8,9</sup> Some studies have reported significantly increased rates of infection with the use of rituximab in ABO-I renal transplantation (ABO-I RT),<sup>10–13</sup> particularly the rates of viral infections. In our series, CMV antigenemia was confirmed, and preemptive therapy was required in all 5 patients. Thus, careful periodic monitoring for viral antigenemia is more important in ABO-I LDLT.

Similarly, fungal infections require careful attention following ABO-I LDLT. Kamar *et al.* reported that rituximab use in ABO-I renal transplant patients increased the frequency of fungal infections.<sup>14</sup> Despite invasive fungal infections having a lower incidence than bacterial or viral infections, the mortality rate is higher in patients with fungal infections.<sup>15,16</sup> In our series, patient 2 developed invasive aspergillosis, which was successfully treated with voriconazole. The outcome of invasive fungal infections generally depends on prompt diagnosis and therapy. Therefore, it is important to recognize that ABO-I LDLT recipients are at a higher risk of invasive fungal infections and require detailed postoperative investigation.

The use of plasma exchange alone does not increase the risk of infection in immunocompromised patients.<sup>17</sup> Although rituximab may induce a pronounced depletion of circulating B cells, it does not affect antibody-producing plasma cells and circulating protective antibodies.<sup>18</sup> Considering these results, we suggest that the combination therapy of standard immunosuppression, rituximab and splenectomy may result in severe and sustained “overimmunosuppression.”

Our experience with patient 4 indicates the risks of infectious complications such as overwhelming post-splenectomy infection (OPSI). Splenectomy for ABO-I LDLT is clinically important to reduce antibody production.<sup>19</sup> Conversely, patients undergoing splenectomy might be at risk of portal vein thrombosis and infection. Notably, OPSI is a life-threatening complication<sup>20</sup> and is a rare condition associated with a high mortality (50%–70%).<sup>21</sup> In patient 4, the acute deterioration despite intensive care suggested a case of OPSI after ABO-I LDLT. Recently, an ABO-I LDLT protocol without splenectomy was reported that showed acceptable outcomes.<sup>22</sup> However, plasma cells left in the spleen may contribute to antibody production.<sup>23</sup> Hence, we do not change to perform splenectomy in our

protocol. Thus, in addition to the usual treatment for LDLT, adequate reduction of immunosuppressants and prophylactic antibiotics or vaccination for pneumococcal, meningococcal, and *Haemophilus influenzae* infection is important in ABO-I LDLT.

Local infusion therapies via the hepatic artery or portal vein are the characteristic therapeutic modalities associated with ABO-I LDLT.<sup>2,24</sup> These therapies were introduced to control single organ disseminated intravascular coagulation and have successfully increased the recipient survival rate; the authors of that study also reported the use of IPI therapy in patients to be safe.<sup>6</sup> The reported effects of IPI also include the benefits of immunologic reactions, as well as suppressed antidonor immune responses.<sup>25</sup> Thus, further investigations are needed to determine the effects of local infusion therapy as well as the associated morbidity.

In conclusion, our protocol involving rituximab and splenectomy resulted in no antibody-mediated rejections. However, life-threatening infectious complications such as sepsis, suspected OPSI, and invasive aspergillosis were noted. Thus, to achieve an optimal immunosuppressive status, immunosuppressive therapies, especially induction therapy by rituximab, should be customized to prevent life-threatening infections in ABO-I LDLT.

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