

Antibody-mediated rejection after adult living-donor liver transplantation triggered by positive lymphocyte cross-match combination

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Abstract

A 46-year-old female suffering from liver cirrhosis was referred to us for living-donor liver transplantation (LDLT). Pre-transplant lymphocyte cross-match tests were positive. The recipient showed immunoreactivity against donor human leukocyte antigen (HLA) Class I antigens, a finding confirmed by flow cytometry. Additional tests confirmed donor-specific lymphocyte immunoreactivity against HLA B 55. As no other suitable donor was available, we performed LDLT coupled with splenectomy, despite the positive cross-match. Tacrolimus, methylprednisolone and mycophenolate mofetil were used postoperatively for immunosuppression. The postoperative course was uneventful until Day 3 when blood tests showed disorders in liver function and the patient's condition suddenly worsened. Although intensive care (including plasma exchange) was given, her condition continued to deteriorate. Flow cytometry initially showed that immunoreactivity against Class I antigens was down-regulated immediately after LDLT, but further testing showed that it had increased again. We diagnosed humoral rejection based on clinical, immunological and histopathological findings and suggest that this was mediated by an immune response to donor-specific antigens. The patient experienced multi-organ failure and died on post-operative Day 9.

Keywords antibody-mediated rejection, cross-match, human leukocyte antigen, humoral rejection, liver transplantation

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Introduction

Classically, allograft rejection in organ transplantation is considered to be mediated by alloantigen recognition by T cells. Immunosuppressants such as cyclosporine and tacrolimus have shown good results in controlling the rejection process, and therapies for acute cellular rejection mediated by T cells (such as steroid pulse) are also well-established. However, though positive lymphocyte cross-match combinations of

donor and recipient are rare, humoral rejection (HR) or antibody-mediated rejection (AMR) is still a serious problem after organ transplantation because treatment is difficult and in some cases, grafts are lost.

The importance of lymphocyte cross-matching and human leukocyte antigen (HLA) histocompatibility have been reported for kidney transplantation and combined kidney-liver transplantation [1-4]. The role of anti-donor HLA antibodies in graft loss is also well-known [5,6]. However, the impact of lymphocyte cross-matching and HLA compatibility upon HR or AMR after liver transplantation (LT) is still unclear.

We report the case of a patient referred to us for a living-donor liver transplantation (LDLT) with a positive cross-match that had a poor post-operative outcome, and discuss strategies to further improve the prognosis in such cases.

Case report

A 46-year-old female was admitted suffering from well-developed liver cirrhosis. Hepatitis C virus infection was diagnosed at 39 years of age and she had been treated at an-

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Conflict of Interest: None

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other hospital for the last seven years. Although the number of different medications used to treat the condition (furosemide, spironolactone, ursodeoxycholic acid, lactulose, and branched-chain amino acids) and their dosages had slowly increased over the last year, her condition was not well-controlled. She had frequent episodes of esophageal variceal rupture over the last year and had suffered from intractable ascites and a right pleural effusion. Because of her deteriorating condition, she was referred to our division for LDLT. On admission, she had a low-grade fever and cell counts in the ascites and pleural effusion were 2270 /mm³ and 2580 /mm³, respectively. We diagnosed spontaneous bacterial peritonitis and pleuritis which were managed pre-operatively by drainage, hydration and cefotaxime i.v. The low-grade fever disappeared after treatment. Her status according to the United Network for Organ Sharing was IIB. Her scores for Child-Pugh and the model for end-stage liver disease were 14 and 25, respectively.

Pre-transplant lymphocyte cross-match tests were performed using direct complement-dependent cytotoxicity (CDC) and anti-human globulin assays (anti-human immunoglobulin lymphocytotoxicity test, AHG-LCT) [7,8]. The results of these tests were positive. Moreover, the patient showed strong reactions against donor HLA Class I antigens (Fig. 1). Also, flow cytometry (FCM) showed that the lymphocytes of the recipient were reactive against HLA Class I antigens (Fig. 2). The HLA typing of both the recipient and the donor is shown (Fig. 3). We also performed additional tests to assess the patient's immunoreactivity to specific HLA Class I antigens. The lymphocytes of the recipient showed strong immunoreactivity against HLA Class I loci including HLA B 55. Tests showed that the donor had this HLA B

locus (Fig. 3), which meant that the patient could potentially mount a donor-specific anti-HLA antibody response after transplantation.

Although the results of the cross-matching tests were positive for this particular donor and recipient, the ABO blood group was compatible and the patient had no history of receiving blood transfusions from the donor. As we were unable to find a more suitable donor, the ethics committee of our institution granted approval for the procedure and written informed consent was obtained from both the recipient and the donor. During surgery we found that the patient had splenomegaly and developed collateral vessels (umbilical vein and coronary vein) and so we performed a splenectomy and ligation of collateral vessels to obtain improved intra-operative control of portal venous pressure. We reported that portal venous pressure <15 mmHg is a key for successful LDLT [9,10], and the final pressure was 13 mmHg in this case. The surgery lasted 822 minutes and intra-operative blood loss was 7700 mL. The graft was a left-lobe graft and the graft weight was 450 g. The graft:recipient weight ratio was 0.91. The patient received 24 units of red cells, 16 units of plasma and 30 units of platelets during the procedure. We used tacrolimus, methylprednisolone and mycophenolate mofetil as immunosuppressants and the trough level of tacrolimus was kept at 8–10 ng/mL during the early post-operative period. Methylprednisolone was given intravenously (1 mg/kg) once daily from post-operative Day (POD) 1 to POD 3 followed by 0.5 mg/kg once daily for the next 3 days. The dosage of mycophenolate mofetil was 10 mg/kg/d from POD 1.

Post-operative splanchnic in-flow and out-flow were excellent as assessed by Doppler ultrasound studies. The

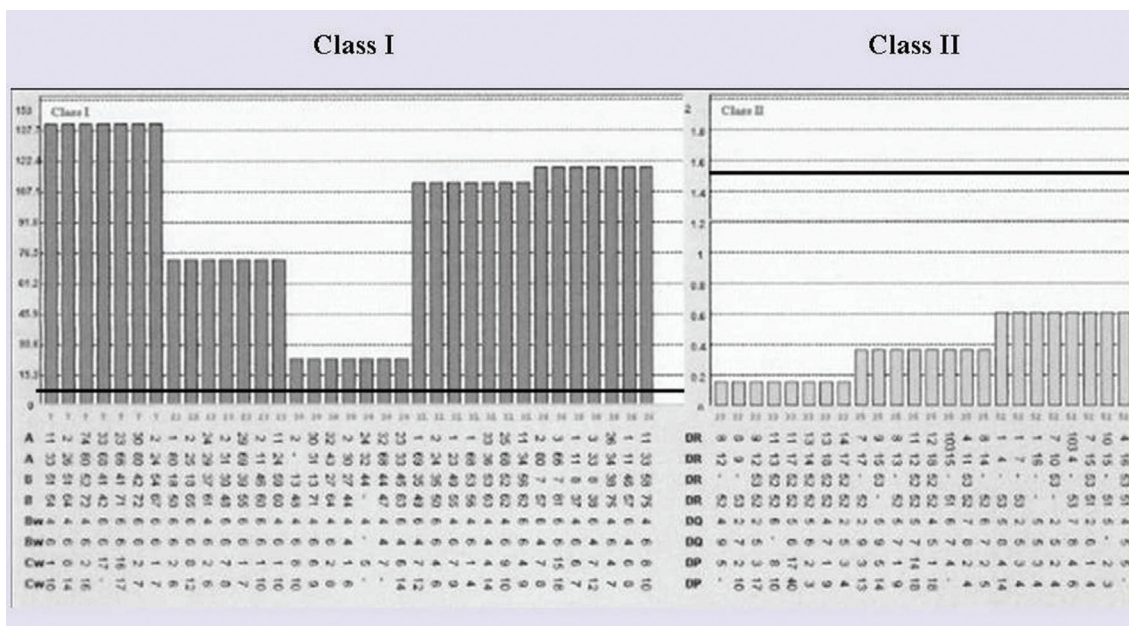


Figure 1 Recipient's lymphocyte reactivity against HLA class I and II antigens. Recipient lymphocytes had obvious immunoreactivity against donor HLA class I antigens, though reactivity against donor HLA class II antigens was below the threshold level. The threshold level was 1.53 (horizontal lines)

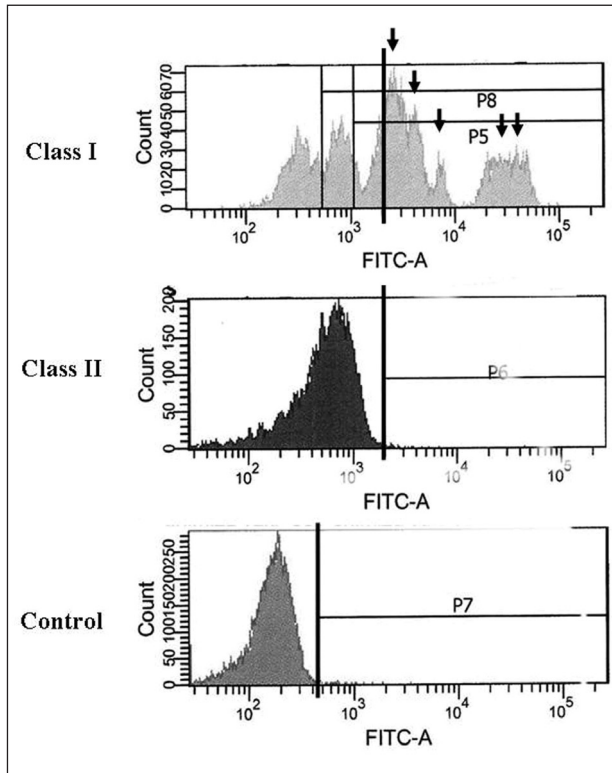


Figure 2 Recipient pre-transplant immunoreactivity against donor antigens, as assessed by FCM. The recipient's lymphocytes clearly show reactivity against donor HLA class I antigens (arrows). The vertical lines represent reactivity against the same antigen in a third party (other recipients).

post-operative course was uneventful until POD 3 when the patient experienced a sudden elevation of serum lactate dehydrogenase (LDH) levels, a decrease in the platelet count and severe fragmentation of red blood cells. Serum total bilirubin (T-Bil) levels were increased after POD 3 leading to a prolonged case of jaundice. On POD 4 a chest X-ray was taken and showed an acute respiratory distress syndrome-like condition. Blood gas analysis revealed significant respiratory insufficiency. The patient's respiratory function worsened to a point where she required mechanical ventilation. Plasma exchange (PE) (80 mL/kg/d) was performed daily after POD 4 (Fig. 4) and she received steroid pulse therapy (methylprednisolone at 10 mg/kg, i.v.) from POD 5. The gated area represented immunoreactivity against Class I antigens, and the percentages were calculated as the counts in the gated area/the whole counts. The percentages at pre-LDLT, PODs 2, 5, 6, 8 and 9 were 71.7, 1.7, 1.9, 11.2, 7.3 and 25.8 %, respectively. Although immunoreactivity against HLA Class I antigen was down-regulated during the early period after LDLT it increased again from POD 6. Note that this immunoreactivity was down-regulated on POD 5 even though graft dysfunction began on POD 3 and that this immunoreactivity remained from POD 6 even after repeated PE. On POD 8, peripheral blood examination showed evidence of hemolysis and that haptoglobin levels had fallen (<5.0 mg/dL). Percutaneous microecchymosis was noted and coagulation profiles were consistent with disseminated intravascular coagulation (DIC). The patient's condition worsened and she did not respond to further treatment, including daily PE. On POD 9 we performed a liver needle biopsy under US guidance. Histopathological examination clearly showed severe graft damage

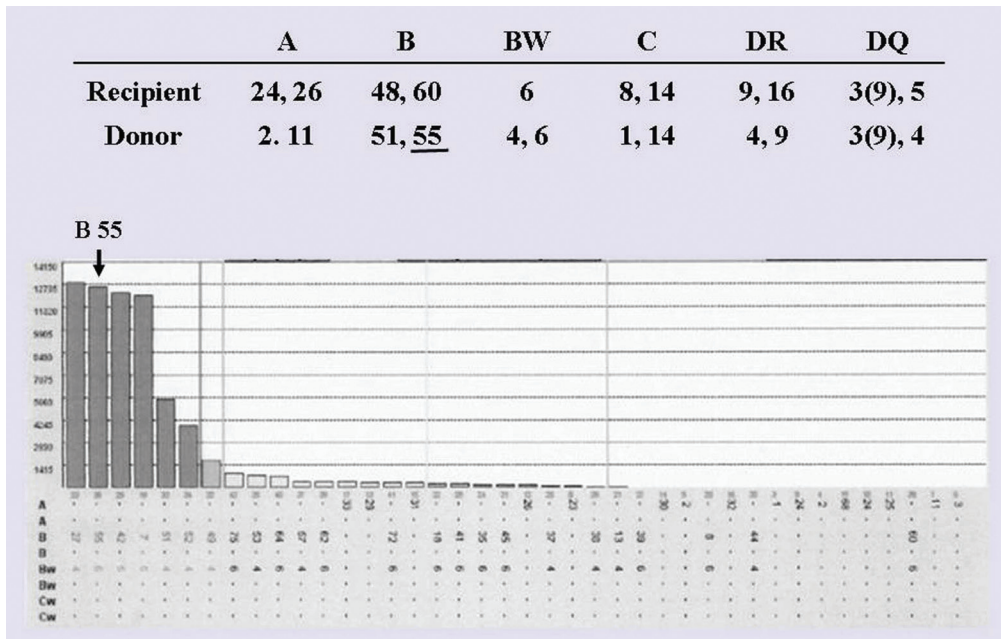


Figure 3 Serological HLA typing of both the recipient and donor and the recipient's lymphocyte immunoreactivity against specific HLA class I antigens. The recipient was not homozygous for HLA loci. The donor has the HLA-B 55 locus (underlined). The recipient's lymphocytes show specific activity against HLA-B locus 55 (black arrow).

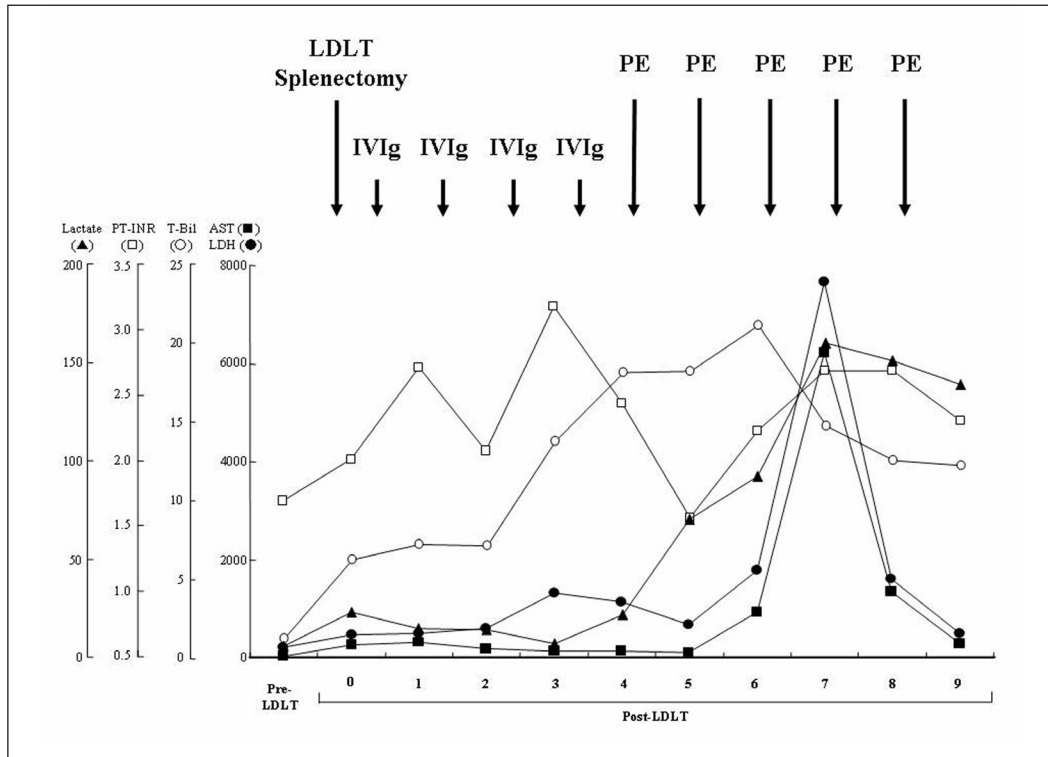


Figure 4 Changes in the patient's blood biochemistry after LDLT. Temporal changes in each of the variables are represented as follows: closed square, AST; closed circle, LDH; open circle, T-Bil; open square, PT-INR; closed triangle, lactate.

(Fig. 5). We diagnosed HR mediated by an antigen-specific immune response to the donor tissue based on the clinical, immunological and histopathological findings. The patient experienced multi-organ failure accompanied by DIC and died at POD 9 despite intensive treatment.

Discussion

In HCV recipients, we need to consider HCV recurrence after LDLT, although our results in HCV recipients are currently excellent [11]. Previously, post-operative recovery of the platelet counts was limited when severe thrombocytopenia existed [12]. Recipients with HCV may require combination therapy with ribavirin and interferon after LDLT [11]. Therefore, in our institution, concurrent splenectomy was performed in HCV recipients to treat thrombocytopenia regardless of the PVP level. In this case, we performed splenectomy based on HCV, though a well-controlled portal venous pressure was confirmed as a result. Previous studies have reported that many other factors are crucial for LT outcomes [13-20], and intra-operative factors in this case, such as operative time, blood loss and massive blood transfusion, seemed to affect the post-operative course and outcome.

There have been many contradictory reports regarding the importance of cross-matching and HLA compatibility

in LT [26-29]. Some studies have reported the importance of appropriate cross-matching while others have concluded that a positive cross-match has no bearing on the outcome of LT [21-29]. Therefore, the significance of a positive cross-match combination between donor and recipient still remains a matter of debate within the field. Some investigators have suggested that HLA histocompatibility for Class I is crucial for graft survival after LT while others have indicated there may be a dualistic effect of HLA histocompatibility in liver allogeneic grafts. They suggest that although HLA histocompatibility reduced the incidence of allograft rejection it may also enhance other immunological mechanisms which can lead to allograft dysfunction [23,25-29]. Thus, there is still no consensus on the importance of cross-matching and HLA compatibility in the LT field.

Previous reports have shown that a cross-match can change from a positive one to a negative one after organ transplantation [2-4]. Strict real-time evaluation based on the results of immunological assays is important for adequate treatment after LDLT. Peri-operative monitoring of allogeneic antigens by FCM is a method suitable for clinical use because it can be performed repeatedly, non-invasively and in real-time. Based on our FCM results it appears that in this case lymphocytes reactive against HLA Class I antigens can be controlled during the early post-operative period but proliferate again after this initial period of down-regulation. It is worth noting that immunoreactivity against HLA Class I antigens was down-

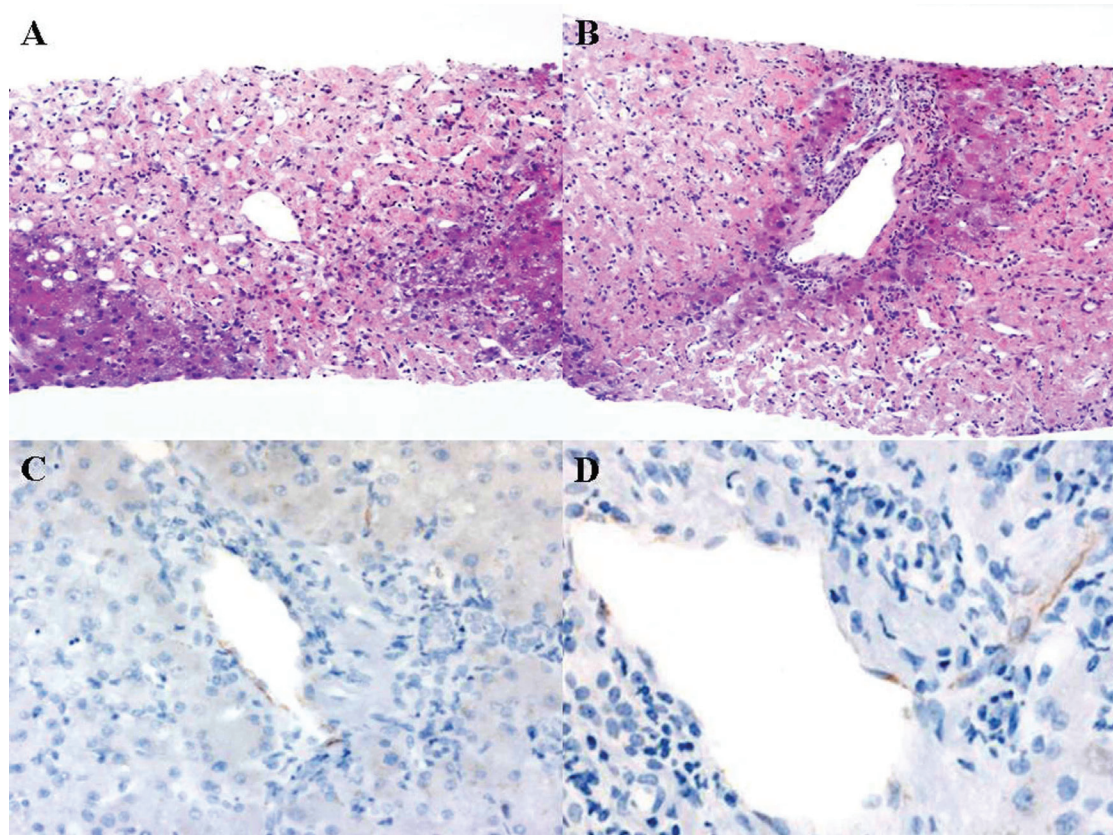


Figure 5 Histopathological findings from liver needle biopsy. The hematoxylin-eosin stained specimens show massive necrosis of hepatocytes and disappearance of bile ducts (A and B). C4d immunostaining shows endothelial-positive (C) and stromal-positive (D) staining in portal areas. These findings indicate humoral rejection.

regulated on POD 5 even though graft dysfunction was evident from POD 3, and that this immunoreactivity remained from POD 6 even after repeated PE. A possible explanation for the phenomenon seen on POD 5 is the immunoabsorption of anti-graft antibodies by PE [30]. This case suggests that PE can have positive effects on the anti-graft immune response in the initial period after LDLT, but repeated PE has limited use as a treatment for HR or AMR. Some investigators have suggested that more aggressive immunosuppression is probably needed in immunologically high-risk patients, including those with a positive cross-match [31,32]. In our case, the target trough level of tacrolimus was slightly low due to the consideration of the patient's pre-operative infectious condition, and the intravenous administration of immunoglobulins (IVIg) was also low-dose just as a complement. This case suggests that strong immunosuppression may be needed in positive cross-match cases in order to maintain a negative cross-match after LDLT.

PE and high-dose IVIg are considered to be the standard therapies for HR or AMR after organ transplantation [33-35]. However, splenectomy is considered as a suitable intra-operative strategy to prevent post-operative AMR [36]. In our case, splenectomy and intensive post-operative treatment were not successful. Therefore, we hypothesize

that pre-operative induction therapy to prevent HR or AMR after LDLT is crucial in positive cross-match LDLT recipients. The usefulness of the anti-CD20 antibody (rituximab) is well reported in this respect. Rituximab is key in order to prevent HR or AMR after organ transplantation, including LDLT [33,34,37]. The use of a living related donor may leave more time for immunological testing and the induction of suitable preconditions for LT than is the case for a cadaver donor LT. Therefore, having studied the literature around pre-operative conditioning for positive cross-match LDLT recipients, rituximab treatment alongside PE prior to LDLT is now under consideration in our institution.

Although the use of living related donors maybe leave more time to select a suitable donor, donor compatibility is still a serious problem and this will continue to be the case. There is an obvious limitation of suitable donors in the case of LDLT. There were no ideal candidates in the case we present here and so we performed LDLT regardless of the positive cross-match. Because of the shortage of compatible donors and the difficulties in treating HR or AMR successfully, peri-operative strategies for cross-match positive LDLT recipients are sorely needed.

Currently, by using an advantage of flexible timing in LDLT, a pretransplant preconditioning already overcame

the ABO incompatibility in LDLT [37]. As described above, LDLT has an advantage for preoperative immunological testing and the induction of suitable preconditions for LT than is the case for a cadaver donor LT. Because the influence of lymphocyte cross-matching is considered to be debatable in LT, including deceased donor LT, many transplant centers in the United States and Europe do not perform cross-match tests before LDLT or only investigate lymphocyte cross-matching retrospectively for cost-saving reasons. We have demonstrated convincingly that a positive lymphocyte cross-matching has a negative impact on LDLT. Because not all of our lymphocyte cross-matching positive cases died, we suggest that positive lymphocyte cross-matching itself does not contraindicate LDLT, but advanced immunologic strategies should be established for lymphocyte cross-matching-positive LDLT as well as for ABO-incompatible LDLT.

In conclusion, we suggest that our case will be thought-provoking for organ transplant surgeons and may provide important information about the use of novel immunological strategies for the management of positive cross-match LDLT recipients. Further improvements in peri-operative immunological strategies and further case studies will be indispensable in achieving improved results for positive cross-match combinations in LDLT.

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