Title

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Author(s)

Hori, Tomohide; Uemoto, Shinji; Takada, Yasutsugu; Oike, Fumitaka; Ogura, Yasuhiro; Ogawa, Kohei; Miyagawa-Hayashino, Aya; Yurugi, Kimiko; Nguyen, Justin H; Hori, Yukinobu; Chen, Feng; Egawa, Hiroto

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Kyoto University
Does a positive lymphocyte cross-match contraindicate living-donor liver transplantation?

Tomohide Hori, MD, PhD,a,b* Shinji Uemoto, MD, PhD,a Yasutsugu Takada, MD, PhD,a Fumitaka Oike, MD, PhD,a Yasuhiro Ogura, MD, PhD,a Kohei Ogawa, MD, PhD,a Aya Miyagawa-Hayashino, MD, PhD,c Kimiko Yurugi, PhD,d Justin H. Nguyen, MD, PhD,b Feng Chen, PhD,b Yukinobu Hori, LLM,c Hiroto Egawa, MD, PhDa

aDepartment of Transplant Surgery, cDepartment of Diagnostic Pathology and dDepartment of Transfusion Medicine and Cell Therapy, Kyoto University Hospital, Kyoto, 606-8507, Japan.
bDepartment of Transplant Surgery, Mayo Clinic, FL 32224, USA.
cNagoya Economic University Graduate School of Law, Aichi Prefecture, 484-8504, Japan

*Corresponding author: Tomohide Hori, Ph.D., M.D.

Department of Transplant Surgery, Kyoto University Hospital, 54 Shogoinkawara-cho, Sakyo-ku, Kyoto 606-8507, Japan

Phone: +81-75-751-3111; FAX: +81-75-751-3106; E-mail: horit@kuhp.kyoto-u.ac.jp
Abstract

**Background.** There is still no consensus on the importance of lymphocyte cross-matching (LCM) in the field of living-donor liver transplantation (LDLT).

**Methods.** LCM examinations are routinely performed before LDLT, and the results of complement-dependent cytotoxicity were used in this study. A total of 1157 LDLT cases were evaluated. The recipients were divided into four groups based on the LCM and ABO compatibilities: (i) negative LCM and identical/compatible ABO; (ii) negative LCM and incompatible ABO; (iii) positive LCM and identical/compatible ABO; and (iv) positive LCM and incompatible ABO. The diagnosis of antibody-mediated rejection (AMR) was made based on the clinical course, immunological assays and histopathological findings. C4d immunostaining was added if AMR was suspected.

**Results.** The LCM-positive LDLT recipients showed significantly poorer outcomes than the LCM-negative recipients. Among the LCM-positive recipients, 44.1% of recipients eventually died and 85.2% of recipients revealed positive C4d findings. The survival rate of LCM-positive and ABO-incompatible group was 0.50. The survival days were compared with the LCM-negative and ABO-identical/compatible group, and the LCM-positive and ABO-identical/compatible group clearly showed early death after LDLT, although the ABO-incompatible groups did not show significant. The factors of age, disease, pre-transplant scores, LCM, ABO compatibility and graft-recipient weight ratio showed statistical significance in multivariate analysis for important
factors of LDLT outcomes. However, the LCM and ABO compatibilities had no synergetic effects on the LDLT survival.

**Conclusion.** HLA antigens are more widely expressed than ABO antigens, and advanced immunological strategies must be established for LCM-positive LDLT as well as for ABO-incompatible LDLT.
Introduction

The impacts of lymphocyte cross-matching (LCM) and human leukocyte antigen (HLA) histocompatibility have been reported for organ transplantation.\textsuperscript{1-4} Antibody-mediated rejection (AMR) is still a serious problem after organ transplantation,\textsuperscript{5,6} although liver transplantation (LT) involving a positive LCM combination between donor and recipient is rare, even under donor shortage. Within the LT field, it is generally believed that positive LCM does not contraindicate LT, because many contradictory reports have been published.\textsuperscript{3,4,7-9} Some investigators have suggested the importance of appropriate LCM while others have concluded that positive LCM has no impact on LT outcomes.\textsuperscript{3,4,7-9} In the transplant immunity field, several investigators have indicated that HLA histocompatibility has dualistic effects (reduction of rejection incidence and enhancement of allograft dysfunction) in liver allografts.\textsuperscript{8} Therefore, there is still no consensus on the importance of LCM in the LT field. This study focused the effects of positive LCM on living donor LT (LDLT) outcomes.

Patients and Methods

LCM examinations (complement-dependent cytotoxicity (CDC) and anti-human globulin assays) are routinely performed before LDLT in Kyoto University Hospital. The results of direct CDC assays, which were performed to detect anti-donor antibodies\textsuperscript{10}, were used in this study.
Briefly, 1 µL of donor lymphocyte suspension and 5 µL of recipient serum, which was obtained before LDLT, were incubated in a Terasaki plate (Nunc, Roskilde, Denmark) at room temperature for 30 min. Next, 5 µL of rabbit complement was added to each well, and the mixtures were incubated at room temperature for 60 min. After the addition of 2 µL of 5% eosin solution, the cells were fixed with formalin. The plate was examined by phase-contrast microscopy (IMT-2; Olympus, Tokyo, Japan). The results were considered positive when more than 20% of the donor lymphocytes were killed by the recipient's serum.10

A total of 1157 LDLT cases during 13.5 years (485 pediatric and 672 adult recipients from January 1996 to June 2009) were evaluated. The clinical courses of all the recipients were followed for 2075.8±1531.9 days (range, 2 (dead case) to 5041 days) after LDLT. The immunosuppressive regimens including those for ABO incompatibility were described in detail elsewhere.11,12 The recipients were divided into four groups based on the LCM and ABO compatibilities: (i) negative LCM and identical/compatible ABO (n=952; 399 pediatric and 553 adult recipients); (ii) negative LCM and incompatible ABO (n=171; 74 pediatric and 97 adult recipients); (iii) positive LCM and identical/compatible ABO (n=30; 11 pediatric and 19 adult recipients); and (iv) positive LCM and incompatible ABO (n=4; 1 pediatric and 3 adult recipients). All positive LCM cases showed the immunoreactivity against HLA Class I antigens in this study.

The diagnosis of AMR was made based on the clinical course, immunological assays and histopathological findings. C4d immunostaining was added if AMR was suspected.4 Regarding the methodology for C4d immunostaining, our procedures including the evaluation and control
materials were described in detail elsewhere. Briefly, portal stromal or endothelial staining was considered to be positive to minimize false-positives.

Univariate and multivariate analyses were used for between-group comparisons of LDLT outcomes as follows: Mann-Whitney U test for early death; Kaplan-Meier method (log-rank) for the survival rates; logistic regression analysis for important factors predicting outcomes; and multiple analysis of covariance for the effects and synergetic effects of the factors on the survival rates. All the calculations were performed using SSPS-II (SPSS Japan, Tokyo, Japan). Values of $p<0.05$ were considered to indicate statistical significance.

Results

The LCM-positive LDLT recipients ($n=34$) showed significantly poorer outcomes than the LCM-negative recipients ($n=1123$) ($p<0.0001$). Among the LCM-positive recipients, four received re-LDLT owing to graft loss. Fifteen recipients eventually died (44.1%) because of AMR or chronic rejection (9 cases), portal or hepatic arterial thrombosis (3 cases) and sepsis (3 cases). C4d staining was performed in 27 cases and revealed positive findings in 23 cases (85.2%). Portal endothelial immunostaining was the most common staining pattern, and this staining was observed in all 23 cases. Portal stromal immunostaining was positive in 12 cases.

The survival curves of each group are shown in Fig. 1. Four patients were LCM-positive and ABO-incompatible. Three of these four patients received histopathological examinations and
were positive for C4d staining (one endothelial and two stromal immunostaining patterns). Two of the four patients finally died, while one patient recovered after re-transplantation. Therefore, the survival rate of this group was 0.50.

The LCM-positive recipients seemed to die earlier than the LCM-negative recipients (Fig. 1). Therefore, the survival days for the dead cases in each group were compared with the LCM-negative and ABO-identical/compatible group (Table 1). Although the ABO-incompatible groups did not show significant differences, the LCM-positive and ABO-identical/compatible group clearly showed early death after LDLT.

Previous studies have documented the important factors for LT outcomes, such as recipient age, disease, donor age, scores of model for end-stage liver disease (MELD) or pediatric end-stage liver disease (PELD), ABO incompatibility, cold ischemic time, operative time, blood loss and graft-recipient weight ratio (GRWR).\textsuperscript{11,15-22} To investigate which factors are important for LDLT outcomes, multivariate analyses were performed (Table 1). The factors of age, disease, MELD/PELD scores, LCM, ABO compatibility and GRWR showed statistical significance.

To investigate the effects of LCM and ABO compatibilities and the synergetic effects of these factors on LDLT survival, multivariate analyses were performed in 1121 recipients who were followed for at least 1 year and dead cases (Table 1). The LCM and ABO compatibilities each had significant effects on the LDLT survival. However, they had no synergetic effects on the LDLT survival.
Discussion

The LDLT recipients with positive LCM showed the poorest outcomes in their survival curve and the most drastic postoperative courses, even compared with the ABO-incompatible cases. Possible explanations for these findings are as follows: (i) HLA antigens are more widely expressed than ABO antigens; (ii) ABO-incompatible recipients have recently achieved excellent results similar to ABO-identical/compatible cases using established strategies, although the overall survival of the ABO-incompatible cases still showed a significant difference compared with that of the ABO-identical/compatible cases; and (iii) LCM-positive recipients still do not receive any treatments to prevent AMR occurrence (i.e., they receive usual immunosuppression after LDLT and no specialized preconditioning before LDLT).

There have been many contradictory reports regarding importance of HLA compatibility in LT. Some investigators have suggested that HLA histocompatibility for Class I is crucial for graft survival after LT while others have documented that there may be a dualistic effect of HLA histocompatibility in liver allografts. They suggested that although HLA histocompatibility reduced the incidence of rejection, it may also enhance other immunologic mechanisms which can lead to allograft dysfunction. Our results advocated the previous opinion that HLA histocompatibility for Class I is important for LT outcomes.

Previous studies have reported that several factors are crucial for LT outcomes, and that LDLT outcomes are excellent especially in pediatric recipients. The overall outcomes in pediatric LDLT are also excellent compared with adult LDLT in our institution. The results of our
multivariate analyses, which revealed the importance of age, disease and GRWR, are easy to understand from the viewpoints of previous opinions and pediatric outcomes. The importance of the MELD/PELD scores and ABO incompatibility is also consistent with previous reports. However, LCM also had a large impact on the LDLT outcomes in this study.

We previously reported that standard therapies during and after LDLT (i.e., plasma exchange, high-dose immunoglobulins and splenectomy) have limited use as AMR treatments and that strong immunosuppression is needed to maintain a negative LCM after LDLT. Moreover, we hypothesized that preoperative induction therapy to prevent AMR may confer a large advantage similar to the case for ABO incompatibility, because LDLT leaves more time for immunological examinations and the induction of suitable preconditions compared with deceased donor LT. Furthermore, we suggested that stronger immunosuppression is required for positive LCM than for ABO-incompatible cases, because HLA antigens are more widely expressed than ABO antigens. Hence, modified regimens based on our regimen for conquering ABO incompatibility are considered to be suitable strategies for LCM-positive LDLT, and we have already initiated a prospective study including monitoring methods.

Since LCM influences are considered to be debatable in LT, including deceased donor LT, many transplant centers in the United States and Europe skip cross-mach tests before LDLT or only investigate LCM retrospectively, for cost-saving reasons. We have clearly demonstrated that positive LCM has a fatal impact on LDLT. Since not all of our positive LCM cases died, we suggest that positive LCM itself does not contraindicate LDLT. However, we conclude that advanced
immunological strategies must be established for LCM-positive LDLT as well as for ABO-incompatible LDLT.
References


Significance of C4d staining in ABO-identical/compatible liver transplantation. Mod Pathol 2007; 20: 676-84.


Figure legend

Fig.1 The survival curves of each group after LDLT