J Hepatobiliary Pancreat Sci (2013) 20:279–285
DOI 10.1007/s00534-012-0511-0

ORIGINAL ARTICLE

Living donor liver transplantation for patients immunized against human leukocyte antigen

Junichi Shindoh · Yasuhiko Sugawara · Sumihito Tamura · Junichi Kaneko · Noriyo Yamashiki · Taku Aoki · Kiyoshi Hasegawa · Yoshihiro Sakamoto · Norihiro Kokudo

Published online: 10 March 2012

Abstract
Background/purpose The clinical features and perioperative management of liver transplant recipients who are already sensitized against human leukocyte antigen (HLA) prior to transplantation are not yet clear.
Materials and methods Medical records of living donor liver transplant recipients were reviewed and clinical features of the patients possessing anti-HLA antibodies were studied.
Results Among the 470 consecutive living donor liver transplant recipients, 6 patients (1.3%) had preformed anti-HLA antibodies. A review of the postoperative courses of these patients revealed that the problems included platelet transfusion refractoriness (PTR) due to immune-mediated destruction of platelet and thrombotic microangiopathy (TMA). PTR was observed in patients with anti-HLA class I antibodies and only HLA-matched platelet concentrate (HLA-matched PC) relieved thrombocytopenia. Intravenous gammaglobulin had an additive effect to HLA-matched PC in some cases, and platelet transfusion from close relatives might be a substitute for HLA-matched PC in life-threatening situations. Although the etiology of TMA is unremarkable, the incidence was high (67%, 4/6) compared with that in patients who were not sensitized against HLA (5.6%, 26/464; $p < 0.01$). Of the four patients, three were complicated with late-onset TMA.
Conclusions Considering these clinical features, careful preparation and postoperative management are needed for liver transplant candidates with anti-HLA antibodies.

Keywords Human leukocyte antigen · Liver transplantation · Platelet · Thrombocytopenia · Transfusion

Abbreviations

ELISA	Enzyme-linked immunosorbent assay
HLA	Human leukocyte antigen
HLA-PC	HLA-matched PC
HPA	Human platelet antigen
IVIg	Intravenous gammaglobulin
LDLT	Living donor liver transplantation
PC	Platelet concentrate
MPHA	Mixed passive hemagglutination
POD	Postoperative day
PTR	Platelet transfusion refractoriness
TMA	Thrombotic microangiopathy

Introduction

Although previous studies have reported that post-transplant de novo immunization against human leukocyte antigen (HLA) has a negative impact on graft survival [1–3], the presence of anti-HLA antibody or a positive lymphocytotoxic cross-match test result [4] has not always been considered a contraindication for liver transplantation. Actually, alloimmunization against HLA is not very common among liver transplant candidates. A patient who is already sensitized against HLA prior to transplantation,

J. Shindoh · Y. Sugawara (✉) · S. Tamura · J. Kaneko · T. Aoki · K. Hasegawa · Y. Sakamoto · N. Kokudo
Division of Artificial Organ and Transplantation, Department of Surgery, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan
e-mail: yasusugatky@yahoo.co.jp

N. Yamashiki
Department of Surgery, Graduate School of Medicine and Organ Transplantation Service, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

however, has a higher postoperative risk for immune-mediated complications.

Platelet transfusion refractoriness (PTR) is a major problem in patients immunized against HLA [5]. Because most liver transplant candidates usually have thrombocytopenia due to portal hypertension and/or splenomegaly, platelet transfusion is frequently required to avoid hemorrhagic complications. In the presence of the anti-HLA antibody, however, patients would fail to receive the full benefit of platelet transfusions due to immune-mediated platelet destruction. Thus, patients who are preoperatively assessed as positive for anti-HLA antibody require careful preparation and adequate perioperative management.

The clinical features of liver transplant recipients who are already sensitized against HLA, however, are not fully elucidated, and accordingly, no solid strategy has been established for perioperative management of such patients. Therefore, in the present paper, we report our experience with six liver transplant recipients who were already immunized against HLA prior to transplantation, and discuss the optimal perioperative management.

Patients and methods

Patients

A total of 470 patients underwent living donor liver transplantation (LDLT) at our institution between January 1996 and March 2011. Review of the clinical records of the recipients revealed six patients who possessed anti-HLA antibodies prior to and/or just after LDLT. These patients were studied in detail. All six patients had undergone adult-to-adult LDLT from ABO-identical or compatible donors. Our surgical techniques are described in detail elsewhere [6–11]. All six patients received the same immune suppressive treatment with tacrolimus and methylprednisolone, as described previously [12].

Screening of alloantibodies

Screening of alloantibodies is performed as part of the standard preoperative assessment of liver transplant candidates. Screening for anti-nuclear antibody and anti-mitochondrial antibody is routinely performed to assess the background liver disease. After our first experience of postoperative PTR (Case 1 in the following description), however, anti-HLA antibodies and alloantibodies against human platelet antigen (HPA) were added to the routine screening. Detection of anti-HLA antibody was performed by enzyme-linked immunosorbent assay (ELISA) and mixed passive hemagglutination (MPHA) test [13] in the early period (Cases 1 and 2). Recently, a more sensitive fluorescent bead-based assay and MPHA has been used (Cases 3–6). HLA typing for both recipients and donors was routinely performed preoperatively. The presence of antibodies against donor-specific antigen was also screened for by a lymphocytotoxic cross-matching test [14] and ELISA. The patient characteristics and operative data of all six patients are summarized in Tables 1 and 2.

Results

Case 1

A 48-year-old woman underwent LDLT for overlap syndrome of primary biliary cirrhosis and autoimmune hepatitis. Preoperative screening of donor-specific anti-HLA antibodies was negative in lymphocytotoxic cross-matching tests. However, she received multiple platelet transfusions postoperatively due to thrombocytopenia (Fig. 1). Although there were no signs of bleeding or disseminated intravascular coagulation (DIC), the resulting increase in platelet count was less than expected. Her platelet count gradually decreased along with a steep increase in the lactate dehydrogenase–platelet ratio (LDH/Plt ratio), which was suggestive of some kind of platelet destruction. An additional screening test to assess the cause of the PTR revealed the presence of anti-HLA (class I) antibodies. She was negative for anti-HPA antibodies. Therefore, an HLA-matched platelet concentrate (HLA-matched PC) was transfused on postoperative day (POD) 15. After that, both her platelet count and LDH/Plt ratio became stable. The postoperative course of this patient was relatively stable except for mild rejection which was immediately relieved by a steroid pulse therapy. She was discharged from hospital on POD 49. She later died of late-onset thrombotic microangiopathy (TMA) that began on POD 315.

Case 2

A 47-year-old man underwent LDLT for liver cirrhosis associated with hepatitis C. This patient was already immunized against HLA (class I) due to a history of multiple transfusions after a traffic accident. Anti-HPA antibodies were not detected in preoperative screening. A lymphocytotoxic cross-matching test revealed negative results. Because of a shortage of HLA-matched PC, random-donor PC was used postoperatively. Although multiple platelet transfusions were performed, an adequate increase in his platelets was not achieved and the LDH/Plt ratio continued to increase. On POD 3, massive alveolar hemorrhage began spontaneously and the patient went into acute respiratory distress syndrome. Because the situation was critical, platelets from his sister (the LDLT donor) and

Table 1 Patient characteristics

Case	1	2	3	4	5	6
Demographics						
Age (years)/gender	48/F	47/M	37/M	33/F	47/F	44/F
Diagnosis	PBC + AIH	LC (HCV)	LC (HCV), HCC	FHF	PBC	FHF
Blood type (Rh)	O (+)	B (+)	A (+)	A (+)	B (+)	A (+)
Anti-nuclear Ab	+	−	−	−	−	−
Anti-mitochondrial Ab	+	−	−	−	+	−
Anti-HLA Ab (class)	I	I	I	I	II	I
Anti-HPA Ab	−	−	−	−	−	−
Irregular Ab	−	−	−	−	−	−
Major cross-matching test (T/B cell)	−/−	−/−	−/−	−/−	−/±	−/−
Minor cross-matching test (T/B cell)	−/−	−/−	−/−	−/−	−/−	−/−
Graft and surgery						
Donors	Daughter	Sister	Brother	Mother	Daughter	Brother
Blood type combination	Identical	Identical	Identical	Identical	Identical	Identical
Graft type	R	R	R	L (APOLT)	R	R
Graft weight (g)/versus SLV	452/46%	651/48%	588/48%	311/31%	632/53%	872/78%
Operation time (min)	800	1140	863	721	891	712
Blood loss (g)	2903	21125	8490	2940	12030	5550
Transfusions (units)						
RCC	4	22	16	10	34	18
FFP	60	160	24	14	41	30
PC	15	100	80	40	70	40
Splenectomy	No	Yes	Yes	No	No	No
Morbidity and mortality						
Hemorrhage	−	+	−	−	−	+
Acute rejection	+	−	−	−	+	−
Thrombotic microangiopathy	+ (315 POD)	+ (336 POD)	−	+ (55 POD)	−	+ (9 POD)
Mortality	Dead	Dead	Alive	Dead	Alive	Dead

Ab antibody, *PBC* primary biliary cirrhosis, *AIH* autoimmune hepatitis, *APOLT* auxiliary partial orthotopic liver transplantation, *LC* liver cirrhosis, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *FHF* fulminant hepatic failure, *R* right liver graft, *L* left liver graft, *SLV* standard liver volume, *RCC* red cell concentrate, *FFP* fresh frozen plasma, *PC* platelet concentrate, *POD* postoperative day

his mother (see HLA typing results in Table 2) were transfused as an emergency instead of HLA-matched PC. His platelet count increased significantly and the hemorrhagic complications were immediately relieved. Although this patient was discharged on POD 54, he died of late-onset TMA that began on POD 333. Throughout the clinical course, acute/chronic rejection was not proven histopathologically.

Case 3

A 37-year-old man underwent LDLT for hepatocellular carcinoma complicated with hepatitis C. He had a history of multiple transfusions during intensive chemotherapy for a malignant lymphoma at the age of 14. Postoperatively, he was positive for anti-HLA (class I) antibodies in both MPHA and fluorescent bead-based assays, and negative for anti-HPA antibodies. HLA typing revealed a full match in 8 alleles between the recipient and the donor (his elder brother). After an LDLT, he received random-donor PC until an HLA-matched PC was supplied. The decrease in platelet count seemed to accelerate, and more random-donor PCs were used (Fig. 1). Both the platelet count and LDH/Plt ratio were relatively stable after administration of the HLA-matched PC on POD 6. Full recovery of the platelet count was not achieved, however, even after two sessions of HLA-matched PC transfusions. Therefore, intravenous gammaglobulin (IVIg) was administered at a dose of 200 mg/kg for 5 days expecting to block the autoimmune components, and transfusion of HLA-matched PC was also continued. After administration of IVIg, titers of IgG attacking the platelets (PA-IgG) improved from 29.0 to 9.4 ng/10^7 cells, and both platelet count and LDH/Plt recovered dramatically. Apart from this severe PTR

Table 2 HLA typing

	A	B	Cw	DR
Case 1				
Recipient	2, 24	46, 54	1, –	601, 803
Donor	2, –	46, 48	1, 3	803, –
Case 2				
Recipient	24, 33	44, 61	3, –	6, 9
Donor	24, 26	39, 61	3, 7	8, 9
PC donor[a]	26, 33	39, 44	–, –	–, –
Case 3				
Recipient	24, –	35, 52	9, 12	410, 1502
Donor	24, –	35, 52	9, 12	410, 1502
Case 4				
Recipient	2, 33	44, 48	8, 14	1302, 1602
Donor	2, 24	48, 61	8, –	901, 1602
Case 5				
Recipient	24, 26	46, 61	8, 9	803, 1201
Donor	24, 26	61, –	8, –	901, 1201
Case 6				
Recipient	2, 31	39, 61	7, 10	1454, 1501
Donor	2, –	39, 46	1, 7	803, 1501

[a] Patient's mother

episode, his postoperative course has been relatively stable. He was doing well at 20 months after surgery.

Case 4

A 33-year-old woman underwent emergency auxiliary partial orthotopic liver transplantation for fulminant hepatic failure caused by oral antibiotics. Although positive results for anti-HLA antibody (class I) were confirmed preoperatively, it was difficult to prepare the HLA-matched PC because this was an emergency case. Therefore, random-donor PC was used postoperatively. PTR was observed and the LDH/Plt ratio gradually increased (Fig. 1). HLA-matched PC administration significantly improved these parameters. The patient's course was also complicated at POD 52 by late-onset TMA which was successfully treated by intensive therapies including conversion of immunosuppressants and plasma exchange. She died of brainstem hemorrhage, however, on POD 91. Throughout the clinical course, no evidence of graft rejection was confirmed histopathologically.

Case 5

A 47-year-old woman who was diagnosed with end-stage primary biliary cirrhosis underwent LDLT. Preoperative screening by fluorescent bead-based assay revealed that she had anti-HLA (class II) antibodies. She received 20 units of random donor PC twice on POD 3 and 4 due to thrombocytopenia observed early in the postoperative period (Fig. 1). The platelet count then improved without the development of PTR, as in the other five cases with preformed anti-HLA (class I) antibodies. The patient's course was complicated by acute graft rejection which was successfully treated with steroid pulse therapy.

Case 6

A 44-year-old woman underwent emergency LDLT for fulminant hepatic failure due to Wilson's disease. This patient also possessed preformed anti-HLA class I antibodies and the postoperative course was severely complicated with PTR (Fig. 1). On POD 7, sudden massive rectal hemorrhage began at a platelet count of $1.7 \times 10^4/mm^3$ and her general condition deteriorated severely. On POD 9, she met the diagnostic criteria of TMA with severe thrombocytopenia, elevated LDH, and the appearance of fragmented red cells in the blood smear. The patient died of multiple organ failure on POD 20.

Discussion

In the present report, we have reviewed the clinical features of six liver transplant recipients with preformed anti-HLA antibodies. Five cases were positive for anti-HLA class I antibodies and the remaining one was positive for anti-HLA class II antibody. Anti-HPA antibodies or irregular antibodies were not observed. In the preoperative assessment, sensitization against donor-specific antigens was not confirmed except in Case 5.

All six patients received an ABO-identical allograft from a relative and there were no significant signs of postoperative bleeding or disseminated intravascular coagulation. Apparent refractoriness to platelet transfusion was observed in those cases with preformed anti-HLA class I antibodies (i.e., Cases 1–4 and 6) although random-donor PC was used. Case 5 did not develop PTR because platelets usually lack class II antigen.

Typically, platelet count decreased along with a steep increase in the LDH/Plt ratio even after multiple PC transfusions. Furthermore, as observed in Cases 3 and 4, destruction of platelets sometimes seemed to accelerate the more random-donor PC we used. Once HLA-matched PC was administered, however, both platelet count and LDH/Plt ratio immediately stabilized. These results suggest that immune-mediated platelet destruction contributes to PTR in the presence of anti-HLA antibodies.

The efficacy of HLA-matched PC for treating PTR is well known and it is the first choice for immune-mediated PTR [5, 15]. In actual clinical settings, however, is it

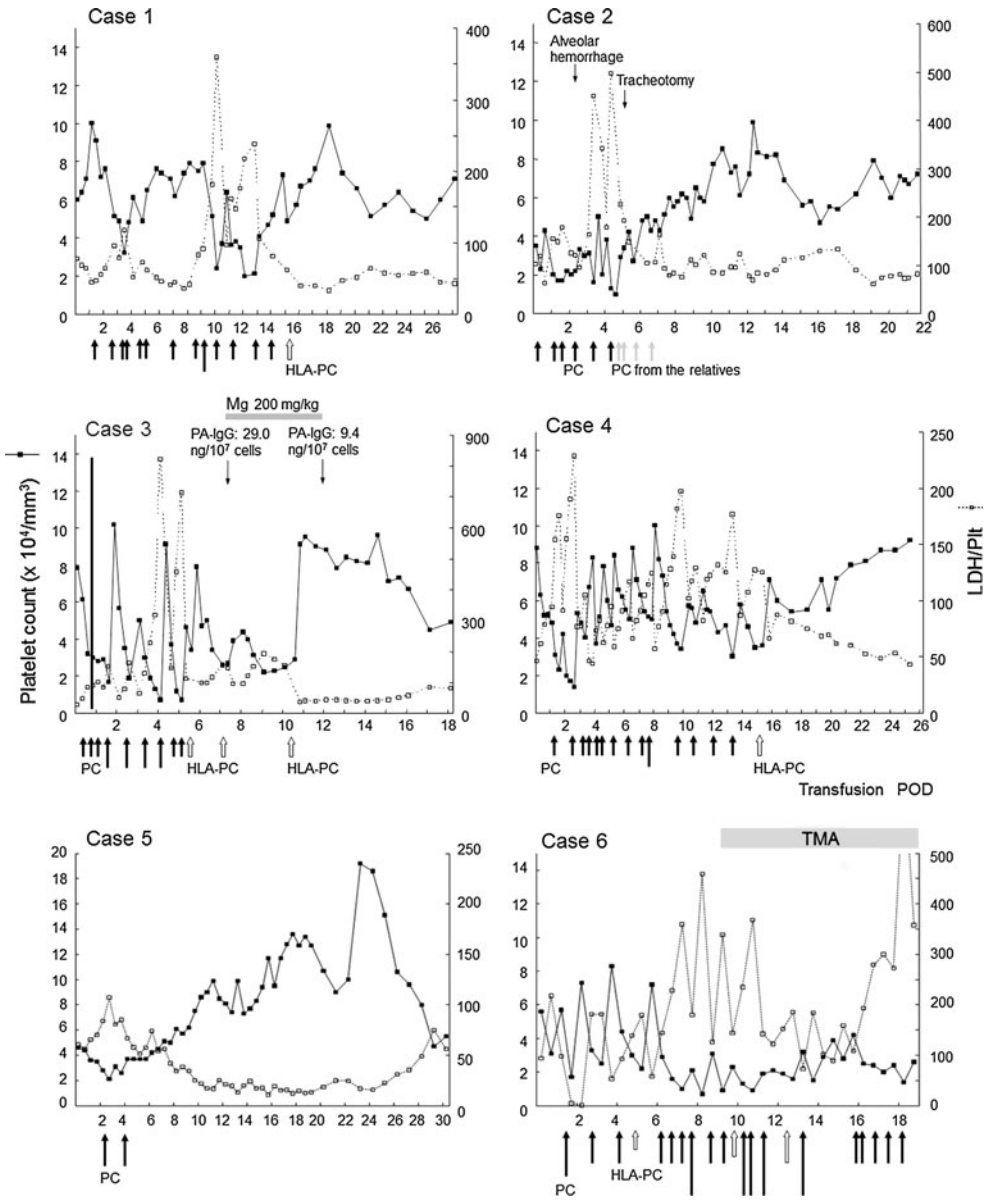

Fig. 1 Post-transplant platelet counts and LDH/Plt ratios in the six cases. *Black arrows* indicate random-donor PC transfusion. The *arrow length* represents the amount of PC; *short arrows* indicate 10 units of PC and *long arrows* represent 20 units of PC. HLA-PC is indicated by the *outlined arrow*. In Case 3, PA-IgG values improved significantly after administration of IVIg and response to HLA-PC recovered concurrently. *POD* postoperative day, *PC* platelet concentrate, *IVIg* intravenous immunoglobulin, *PA-IgG* platelet-associated IgG

difficult to obtain a sufficient quantity of HLA-matched PC; also, it cannot be prepared for emergency cases. Because fatal hemorrhagic complications (Cases 2 and 6) or severe PTR requiring several sessions of HLA-matched PC transfusions (Case 3) were observed in the present series, it seemed better to routinely prepare as much HLA-matched PC as possible preoperatively. Other than for elective LDLT cases, however, liver transplantation is usually an emergency operation. Therefore, the anti-HLA antibody screening should be completed before registering the patient on a waiting list, and the preparation of HLA-matched PC should begin immediately after the first call giving deceased donor information.

Until the HLA-matched PC is available, random-donor PC is usually used to maintain the platelet count. A continuous 24-h slow platelet transfusion might be an effective strategy to decrease platelet destruction in severe PTR [5, 16]. In critical situations in which severe hemorrhagic complications progress under shortage of HLA-matched PC, as in Case 2, however, transfusions from patient's relatives might be an optional strategy to keep the patient alive. Because we have at least half the HLA types in

common with our lineal blood relatives, a PC transfusion from a close relative can be a substitute for HLA-matched PC in emergency cases. Additionally, administration of IVIg, antifibrinolytic agents [17], or recombinant activated factor VII [18, 19] might also reduce platelet destruction and bleeding. Regarding IVIg, a past placebo-controlled trial reported that the effectiveness of IVIg was confirmed only at 1-h after transfusion and no significant increase in platelet count was achieved at 24-h [20]. In the present study (Case 3), however, the platelet-associated IgG titer was significantly decreased and the response to the HLA-matched PC was apparently improved by administration of IVIg for severe PTR with a slight decrease in titer of IgG attacking the platelets. These results imply that IVIg might block immune-mediated platelet destruction, and therefore, in some cases, IVIg may have an additive effect to HLA-matched PC for the treatment of PTR.

As for the other immune-mediated complications, graft rejection was unexpectedly infrequent (Cases 1 and 5) and relatively mild in the present series. The incidence of TMA, however, was extremely high (67%, 4/6) and all of these patients died, refractory to intensive care, once the disorder had been induced. Post-transplant TMA is relatively rare in liver transplantation and several etiologies have been proposed [21]. Although immune reactions in ABO-incompatibility are a reported risk factor for TMA [22, 23], the association between anti-HLA antibody and post-transplant TMA has not been clarified so far. Recently, we have reviewed our 470 LDLT cases and found that 30 patients (6.4%) had TMA postoperatively [24]. Although sensitization against HLA was thought to be a risk factor for TMA in multivariate analysis, the actual pathological mechanism and the reason for the high mortality in patients with anti-HLA antibodies are still unknown.

Although allele specificity or titers of anti-HLA antibodies were not available in the present study, evidence for reduced platelet destruction or a decrease in anti-HLA antibodies attacking platelets after administration of HLA-matched PC or IVIg were indirectly confirmed by improvements in LDH/Plt ratios or decreases in PA-IgG. To prove the actual effect of these therapies and dynamic immunochemical activities of anti-HLA antibodies in patients undergoing LDLT, further investigations are required.

In summary, PTR is the most common problem observed in LDLT recipients who are already sensitized against HLA. Although administration of HLA-matched PC is the best strategy for severe PTR, adequate amounts of HLA-matched PC are usually difficult to prepare in time for emergency surgery. PC transfusions from close relatives or IVIg administration might be a substitute for or at least a support for the HLA-matched PC in critical situations due to PTR. Under adequate immunosuppression, acute graft rejection was infrequent and relatively mild in the present series. Susceptibility to TMA seemed to be increased in the presence of anti-HLA antibodies, and accordingly, long-term survival might be negatively influenced in patients immunized against HLA.

Acknowledgments This study was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science of Japan.

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