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Acute Antibody-mediated Rejection Coexisting With T Cell–mediated Rejection in Pediatric ABO-incompatible Transplantation

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Background. The management and outcome of ABO-incompatible (ABO-I) liver transplantation (LT) has been improving over the past few decades. Recently, the introduction of a pathological evaluation of acute antibody-mediated rejection (AMR) for liver allograft has provided a new recognition of allograft rejection in LT. **Methods.** One hundred and one pediatric ABO-I LTs performed in our institute were retrospectively analyzed. We assessed the clinical manifestations, diagnosis, and treatment of acute AMR, focusing on the recipient age and pathological findings. **Results.** Twelve cases (11.9%) of acute AMR related to ABO-I were observed. Nine cases developed mixed T cell–mediated rejection (TCMR)/AMR. These consisted of 6 patients in the younger age group for whom the preconditioning treatment was not indicated and 4 patients in the older age group to whom rituximab was administered as planned. Two patients in the older age group to whom preoperative rituximab was not administered as planned developed isolated AMR. Acute AMR in the older group required plasma exchange for treatment, regardless of the coexistence of TCMR. In contrast, those in the younger group were successfully treated by intravenous methylprednisolone pulse and intravenous immunoglobulin without plasma exchange, accounting for mild immune reaction. **Conclusions.** Acute ABO-I AMR can develop simultaneously with TCMR, even in young patients with a compromised humoral immune response following ABO-I LT. Establishing the accurate diagnosis of AMR with a pathological examination, including component 4d staining, is crucial for optimizing treatment.

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INTRODUCTION

ABO-incompatible (ABO-I) liver transplantation (LT) must be considered to overcome donor shortages, especially in living-donor LT (LDLT), where donor selection is restricted to family members. The literature in early study periods reported that the graft survival rates of ABO-I LT were 30% to 50% with an increased risk of antibody-mediated rejection (AMR), infection, and consequent vascular and biliary complication.[1,2] In the pediatric population, several studies have reported that the patient and graft survival rate of ABO-I LTs has become comparable to those of ABO-compatible LT, thanks to efforts to overcome acute AMR.[3–5] However, the optimal management approach for pediatric ABO-I LT has not yet been established. A major point of progress regarding ABO-I LT in recent years was the comprehensive update of the pathological diagnosis of AMR in liver allografts, including new recommendations for complement component 4d (C4d) tissue staining and interpretation.[6] The present study assessed the clinical manifestations, diagnosis, and treatment of acute AMR related to ABO-I (acute ABO-I AMR) in pediatric ABO-I LT, focusing on the recipient age and pathological findings.

MATERIALS AND METHODS

Patients

We retrospectively analyzed a database of patients who underwent LT in our institute between 2010 and 2020. During the study period, 542 LTs were performed. Of these cases, 525 involved children under 18 y of age (96.9%). One hundred and one (19.2%) pediatric recipients who received ABO-I grafts were enrolled in the present study. LT was performed under the approval of the Ethics and Indications Committee of the National Center for Child Health and Development. The surgical procedure was performed as described previously.[7] Biliary reconstruction was mainly performed with Roux-en Y hepaticojejunostomy. Three cases were performed via duct-to-duct anastomosis. The patient characteristics, clinical records before and after LT, surgical variables, pathological findings of a liver allograft biopsy, and therapeutic modalities were examined.

This study was approved by the Ethics Committee of the National Center for Child Health and Development (No. 404) and was conducted in accordance with the Declaration of Helsinki (2008). Written informed consents were obtained from the patients involved in this study for publication.

Basic Protocol for Immunosuppression

The basic immunosuppressive regimen consisted of tacrolimus and steroids. Tacrolimus was given by mouth every 12 h starting from the night before the operation. The target trough levels were 10 to 15 ng/mL for the first 2 wk and 5 to 10 ng/mL for the next 2 mo. Methylprednisolone was administered at a dosage of 10 mg/kg after graft reperfusion, followed by a dosage of 1 mg/kg/d for the first 3 d, 0.5 mg/kg/d for the next 3 d, and 0.3 mg/kg on day 7. From day 8, prednisolone was given by mouth starting with a dosage of 0.3 mg/kg/d and tapered during the first 3 to 12 mo after LDLT.

Prophylactic Protocol for Acute AMR in ABO-I LT

The prophylactic protocol for acute AMR in ABO-I LT in our institute is divided into 2 groups according to the indication for the preconditioning treatment, decided by the recipient age (Figure 1). For the comparison analysis in this study, the patients who were not indicated for B-cell depletion therapies were classified into the younger age group (group Y), whereas the patients who were indicated for B-cell depletion therapies were classified into the older age group (group O). In group Y, the immunosuppression protocol was identical to that of ABO-compatible LT and was equivalent to the basic protocol based on the postoperative use of tacrolimus and steroids. The threshold was changed from 2 y old to 18 mo old in 2017, as we experienced a patient under 2 y of age who suffered from intrahepatic biliary complication (IHBC) due to acute AMR combined with T cell–mediated rejection (TCMR) after ABO-I LT.[8] In group O, the preconditioning protocol included rituximab (375 mg/m^2) 1 mo before LT and several sessions of plasma exchange (PE) just before LT if the anti-donor blood group A/B antibody (ADB Ig) titer was >×64. Preoperative PE was repeatedly performed to reduce the ADB Ig titer to ≤×16. The postoperative immunosuppression regimen included tacrolimus, steroids, and mycophenolate mofetil (MMF).

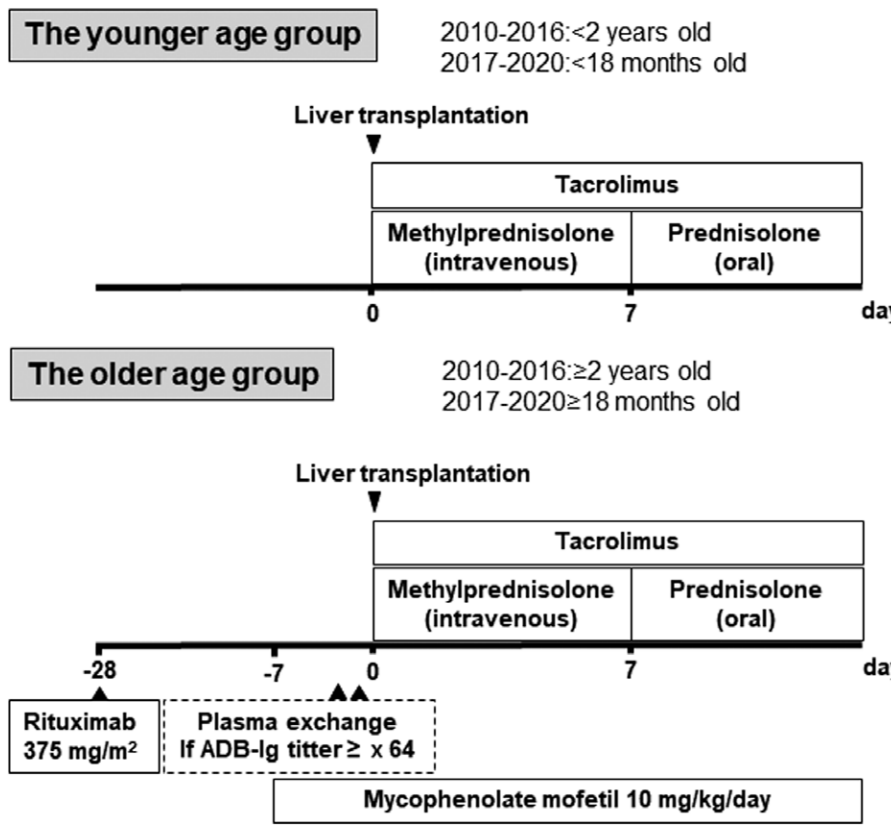

FIGURE 1. Our prophylactic protocol for acute antibody-mediated rejection in ABO-incompatible liver transplantation. ADB-Ig, anti-donor blood group A/B antibody.

Monitoring of ABO Antibodies

A microhemagglutination assay was used to monitor serum levels of ADB Ig. The ADB IgM and IgG titers were measured daily for 2 wk and then twice a week for up to 1 mo.

The Histological Diagnosis

Specimens were obtained by a percutaneous needle biopsy when there was clinical evidence of graft dysfunction. The liver biopsy samples that were diagnosed with acute allograft rejection within 1 mo after LT were evaluated in this study. Paraffin-embedded specimens were subjected to hematoxylin and eosin staining and C4d immunostaining (a polyclonal rabbit anti-human C4d antibody [BI-RC4D]; Biomedica, Vienna, Austria). TCMR was assessed with grading criteria (global assessment); AMR was assessed with C4d score and h score. Mixed TCMR/AMR was defined by the pathological findings including components of AMR and overlapping TCMR. Figure 2 shows the typical histopathological findings of isolated AMR and mixed TCMR/AMR. Histopathological pattern of tissue damage consistent with acute AMR included the following findings: portal microvasculitis, capillary dilatation, portal edema, focal microvascular disruption with fibrin deposition, interstitial hemorrhage, and hepatocyte necrosis. In the cases of isolated AMR, portal inflammation was mild, and neutrophils or eosinophils were conspicuous rather than lymphocytes. Venous endothelial inflammation was not evident in the portal and hepatic venules. Centrilobular inflammation was minimal or absent. In contrast, in the cases of mixed TCMR/AMR, expansion of the triads by a mixed infiltrate containing lymphocytes with neutrophils and eosinophils was observed. Bile ducts were infiltrated by inflammatory cells, and venous endothelial inflammation was observed in the portal and hepatic venules. These findings represent a component of overlapping TCMR. Linear to granular C4d deposition on portal vein and capillary and extension to sinusoids were observed in the patients with both isolated AMR and mixed AMR/TCMR. Three pathologists evaluated the specimens to confirm the diagnosis.

The Diagnosis of Acute ABO-I AMR

The diagnosis of acute ABO-I AMR was made when all of the following criteria were met: (1) clinical evidence of graft dysfunction, such as liver enzyme elevation, increased ascites, or decreased bile excretion (usually within 4 wk after LT); (2) continuous elevation of ADB Ig within 4 wk after LT; (3) histopathological evidence of tissue damage based on portal endothelial hypertrophy, portal capillary dilatation, periportal edema, or necrosis (h score,[6] ≥1); (4) C4d deposition in portal/sinusoidal microvasculature (C4d score,[6] ≥1); and (5) other possible causes excluded. When the flow cytometric lymphocyte crossmatch (FCXM) with T cell was negative before transplantation, preformed donor-specific antibody (DSA) was not evaluated. And evaluating the presence of DSA at the time of the biopsy was performed in the limited cases of severe or refractory acute AMR.

Treatment of Acute AMR After ABO-I LT

Acute ABO-I AMR was treated with the combination of several types of the following therapies: intravenous methylprednisolone pulse, methylprednisolone (10 mg/kg) for 3 d followed by gradual dose reduction, and IVIG (0.3–0.5 g/kg/d) for 3 to 5 d were administered for all cases with acute AMR, and PE was performed until ADB Ig fell to a titer of ≤8 immediately after the diagnosis. If steroid-resistant TCMR coexisted, antithymocyte immunoglobulin (ATG, 1.5 mg/kg/d) was administered for 5 d. Rituximab (375 mg/m^2) was

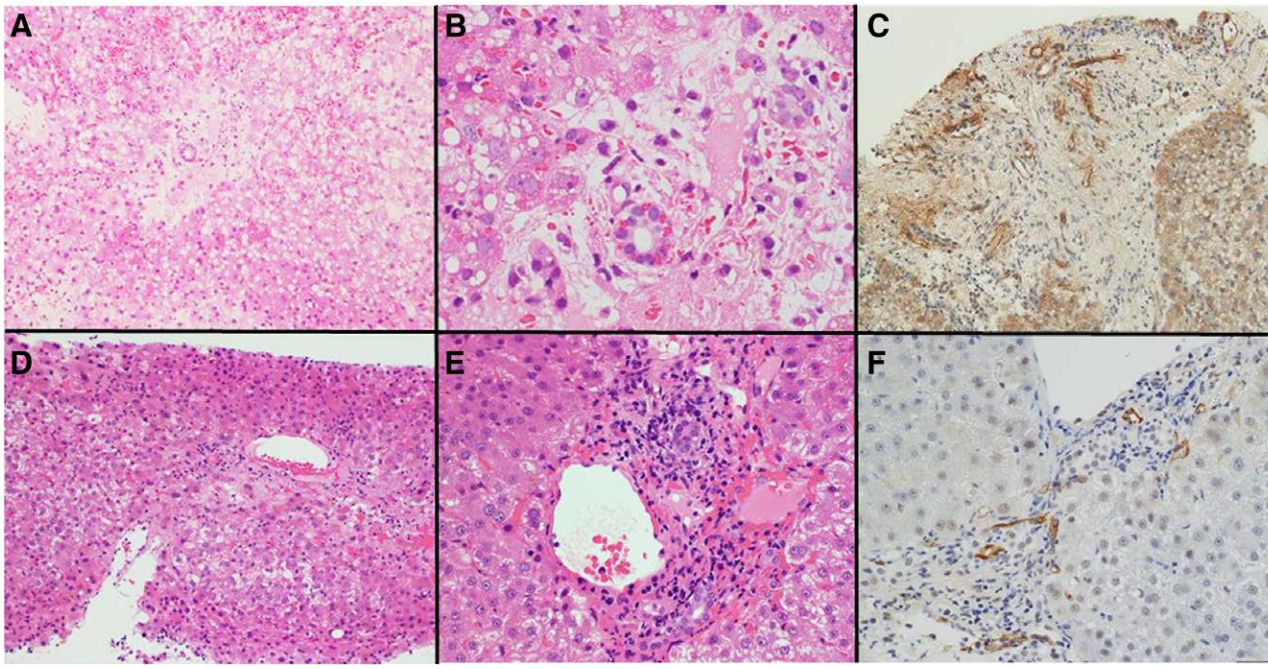

FIGURE 2. Pathological findings of isolated antibody-mediated rejection (AMR) and mixed T cell–mediated rejection (TCMR)/AMR. The upper inset (A–C) shows the pathological findings of isolated AMR, and the lower inset (D–F) shows those of mixed TCMR/AMR. A, B, D, and E, Hematoxylin and eosin staining. Note the portal edema, interstitial hemorrhage, and hepatocyte damage in both types of rejection. A and B, Portal inflammation was mild, and neutrophils are conspicuous rather than lymphocytes. D and E, Portal lymphocytic inflammation and venous endothelial inflammation represent a component of overlapping TCMR. C and D, complement component 4d immunostaining. Diffuse portal microvascular endothelial cell positivity in both types of rejection.

administered when peripheral B cells were present with pathological evidence of acute AMR continuing after the initial treatment. In group Y, MMF (10 mg/kg/d) was added after these treatments to maintain immunosuppression.

Statistical Analyses

Continuous variables are expressed as the median and interquartile range (IQR). The χ^2 test and Wilcoxon test were used in the univariate analyses. Cumulative incidence was shown with Kaplan-Meier curves, and differences in the survival between groups were analyzed using the generalized Wilcoxon test. A P value of <0.05 was considered statistically significant. All statistical analyses were performed using the JMP 14 software program (SAS Institute, Inc, Cary, NC).

RESULTS

Patient Demographics and Characteristics

The preoperative profiles were compared between groups Y and O (Table 1). The number of patients in group Y was 81 and that in group O was 20. The median ages of groups Y and O were 8 mo and 6 y and 1 mo, respectively. Both the preoperative ADB IgM and IgG titers were significantly higher in group O than in group Y (preoperative ADB IgM titer in group Y: ×16 versus group O: ×32; $P < 0.01$; preoperative ADB IgG titer in group Y: ×2 versus group O: ×8; $P = 0.04$). Pretransplant rituximab was omitted for 5 patients in group O because of urgent transplantation for acute liver failure (ALF) or hepatoblastoma and was administered 3 d before LT because of urgent LT for 1 patient with ALF in group O. There were 9 cases for whom PE was performed because of a high ADB IgM titer in group O. After the preconditioning treatment, the ADB IgM and IgG titers in group O decreased to the same extent as in group Y at the time of LT. In group O, the donor age was significantly older (group Y: 32.0 y old versus group O: 37.0 y old; $P < 0.01$) and the graft weight significantly heavier (group Y: 226.0 g versus group O: 278.0 g; $P < 0.01$) than that in group Y. In group Y, the graft-to-recipient weight ratio was significantly higher (group Y: 3.20 versus group O: 1.53; $P < 0.01$) and the amount of bleeding per body weight significantly higher (group Y: 71.9 g/kg versus group O: 33.0 g/kg; $P < 0.01$) than that in group O.

TABLE 1.

Patient profiles in groups Y and O

	Younger age group (n = 81)	Older age group (n = 20)	*P*
Age at LT, mo	8 (5–10)	74 (28–134)	<0.01
Sex (male)	36 (44.4%)	10 (50.0%)	0.80
Original disease	Cholestatic disease 45	Cholestatic disease 3	
	ALF 15	ALF 4	
	Metabolic disease 15	Metabolic disease 2	
	Vascular disease 1	Vascular disease 2	
	Tumor 1	Tumor 4	
	Cryptogenic cirrhosis 1	Fibrocystic disease 5	
	Graft failure 3		
Body weight, kg	6.88 (5.87–8.60)	18.7 (12.0–28.1)	<0.01
Preoperative ADB IgM	×16 (×4–32)	×32 (×16–128)	<0.01
Preoperative ADB IgG	×2 (×1–8)	×8 (×2–32)	0.04
Preoperative rituximab	–	14 (70.0%)[a]	
Preoperative PE for the high titer of ADB IgM	–	9 (45.0%)	
ADB IgM at LT	×8 (×2–32)	×8 (×2–32)	0.67
ADB IgG at LT	×2 (×1–8)	×2 (×1–16)	0.45
Donor type	Living 73	Living 19	
	Deceased 7	Domino 1	
	Domino 1		
Donor age, y	32.0 (29.0–36.0)	37.0 (33.0–39.5)	<0.01
Graft type	Reduced LLS 17	LLS 14	
	LLS 51	Left 5	
	Whole 3	Whole 1	
Combination of blood type (donor to recipient)	A to B: n = 12	A to B: n = 3	
	B to A: n = 4	B to A: n = 5	
	AB to A/B: n = 21	AB to A/B: n = 2	
	Non-O to O = 44	Non-O to O = 10	
Graft weight, g	226.0 (187.0–267.5)	278.0 (240.3–322.8)	<0.01
GRWR, %	3.20 (2.72–3.77)	1.53 (1.04–2.22)	<0.01
Duration of operation, min	451.0 (411.0–519.0)	468.0 (366.3–544.3)	0.82
CIT, min	36.0 (21.0–56.5)	28.5 (22.0–51.5)	0.64
WIT, min	30.0 (26.0–37.5)	29.5 (25.5–35.5)	0.66
Blood loss, g/BW, kg	71.9 (41.9–125.4)	33.0 (24.3–47.6)	<0.01

[a]Pretransplant rituximab was omitted for 5 patients in group O because of urgent transplantation for ALF or hepatoblastoma and was administered 3 d before LT because of urgent LT for ALF in group O.
Continuous variables are shown as the median (IQR). Anti-donor blood type IgM titers are shown as the median.
ADB Ig, anti-donor blood group A/B antibody; ALF, acute liver failure; BW, body weight; CIT, cold ischemic time; GRWR, graft-to-recipient weight ratio; IQR, interquartile range; LLS, left lateral segment; LT, liver transplantation; WIT, warm ischemic time.

Clinical Features of the Cases With Acute ABO-I AMR

Of the 101 total cases, 39 (38.6%) were diagnosed with acute allograft rejection by a pathological examination of liver biopsy samples within 1 mo after LT. The incidence of acute allograft rejection did not differ markedly between groups Y and O (group Y: n = 31 [38.3%]; group O: n = 8 [40.0%]; $P = 1.00$). Of those cases, 12 cases (11.9%) were diagnosed as acute ABO-I AMR, including 2 cases of isolated AMR and 10 cases of mixed TCMR/AMR. One patient who showed positive C4d staining and h score with positive FCXM before LT without elevation of ADB IgM was suspected to have acute AMR due to preformed DSA. The other 27 cases were diagnosed as TCMR. The incidence of acute ABO-I AMR in group O was significantly higher than that in group Y (group Y: n = 6 [7.4%]; group O: n = 6 [30.0%]; $P = 0.01$). The clinical characteristics of the patients who developed acute ABO-I AMR are summarized in Table 2. All patients underwent LDLT with a graft from their parents, and all of them did not show positive FCXM with T cell. Four patients in group O received pretransplant rituximab per protocol. However, case 7 was unable to receive pretransplant rituximab because of an urgent need for LT due to ALF. Case 10 received pretransplant rituximab belatedly (3 d before LT) because of an urgent need for LT due to ALF. Table 3 shows the comparison of the clinical characteristics and laboratory data on the day of the liver biopsy between the patients with acute ABO-I AMR (including isolated AMR and mixed AMR/TCMR) and those with TCMR alone. The median post-LT time to the diagnosis of rejection was significantly shorter in patients with acute AMR than in those with TCMR alone ($P < 0.01$). The body temperature, total bilirubin, aspartate aminotransferase, alanine aminotransferase, and γ-glutamyltranspeptidase were not markedly different between AMR and TCMR patients. However, the ADB IgM and IgG values on the day of the liver biopsy were significantly higher in the patients with acute AMR than in those with TCMR alone ($P < 0.01$). Figure 3 shows the cumulative incidence of acute AMR and TCMR alone separated by groups Y and O. All acute AMR cases were diagnosed within 14 d. The median post-LT time to the diagnosis in the patients with acute AMR was significantly shorter in group O than in group Y (group Y: 9 d versus group O: 6 d; $P = 0.01$). Figure 4 shows the longitudinal increase and decrease data of the median ADB IgM/IgG titers in the patients with acute AMR and TCMR alone separated by groups Y and O. Although the ADB IgM/IgG titers in the patients with TCMR alone did not increase after LT, the ADB IgM/IgG titers in the patients with acute AMR continuously increased during the first week after LT. The peak titers of ADB IgM/IgG were significantly higher in the patients with acute AMR than in those with TCMR alone in group Y (ADB IgM-AMR: 16 [IQR, 8–64] versus TCMR: 2 [IQR, 1–8]; $P = 0.01$; ADB IgG-AMR: 8 [IQR, 4–64] versus TCMR: 2 [IQR, 1–4]; $P = 0.02$). In group O, the peak titer of ADB IgM was significantly higher in the patients with acute AMR than in those with TCMR alone (ADB IgM-AMR: 64 [IQR, 32–6128] versus TCMR: 4 [IQR, 4–4], $P = 0.04$). On comparing the patients with acute AMR in groups Y and O, the elevation of the ADB IgM/IgG titers occurred earlier in group O than in group Y. Although the ADB IgM titer was higher in group O than in group Y, no significant difference was noted in the peak titer of ADB IgM or IgG.

TABLE 2.
Clinical details of the acute ABO-incompatible antibody-mediated rejection cases

Case	Age, y	Sex	Blood type (recipient/donor)	Original disease	XM (T/B)	Pre-LT treatment	Graft type (GRWR)	Peak titers of ADB Ig				
								Pre-LT IgM	Pre-LT IgG	IgM at LT	IgG at LT	Post-LT IgM	Post-LT IgG
1	8 mo	F	O/A	BA	–/–	–	LLS (2.89)	32	2	–	–	16	4
2	8 mo	M	O/B	BA	–/–	–	LLS (3.72)	8	2	–	–	8	2
3	8 mo	F	A/B	BA	–/+	–	LLS (3.58)	128	16	–	–	64	32
4	10 mo	M	B/A	ALF	–/–	–	LLS (2.92)	32	2	–	–	16	4
5	11 mo	M	O/B	LC	–/+	–	LLS (4.09)	16	2	–	–	4	4
6	1 y 8 mo	F	O/B	BA	–/–	–	LLS (1.88)	128	8	–	–	128	128
7	3 y 1 mo	M	O/B	ALF	–/–	PE	LLS (1.53)	32	32	2	4	64	8
8	6 y 1 mo	F	O/A	ALF	–/–	Rituximab[a] + PE	Left (1.40)	64	64	2	8	64	32
9	7 y 1 mo	M	B/A	Caroli	–/–	Rituximab + PE	Left (1.42)	64	8	2	<2	128	16
10	8 y 6 mo	F	O/B	Caroli	–/–	Rituximab + PE	Left (0.93)	256	32	1	<2	128	32
11	9 y 6 mo	F	A/B	CHF	–/–	Rituximab + PE	Left (1.44)	32	2	<1	<2	8	4
12	15 y 3 mo	F	A/B	CHF	–/–	Rituximab + PE	Left (1.15)	128	4	1	<2	64	8

[a]Case 8 received pretransplant rituximab 3 d before liver transplantation because of urgent transplantation for ALF.
ADB Ig, anti-donor blood group A/B antibody; ALF, acute liver failure; BA, biliary atresia; CHF, congenital hepatic fibrosis; F, female; GRWR, graft-to-recipient weight ratio; LC, liver cirrhosis; LLS, left lateral segment; LT, liver transplantation; M, male; PE, plasma exchange; XM, lymphocyte crossmatch.

TABLE 3.

A comparison of clinical manifestations between AMR and TCMR

	AMR (n = 12)	TCMR alone (n = 27)	*P*
Day of liver biopsy (days after LT)	6.5 (5.25–10.0)	14.0 (10.0–17.0)	<0.01
Body temperature, °C	38.4 (37.6–38.6)	37.7 (37.0–38.4)	0.10
T.Bil, mg/dL	2.03 (1.02–4.28)	0.88 (0.44–2.49)	0.08
AST, IU/L	76 (51–120)	70 (49–127)	0.65
ALT, IU/L	98 (69–247)	99 (45–177)	0.27
GGT, IU/L	62 (52–140)	79 (44–116)	0.70
ADB IgM on the day of biopsy	×16 (×8–64)	×4 (×1–8)	<0.01
ADB IgG on the day of biopsy	×4 (×4–8)	×2 (×1–4)	0.01

ADB Ig, anti-donor blood group A/B antibody; ALT, alanine aminotransferase; AMR, antibody-mediated rejection; AST, aspartate aminotransferase; GGT, γ-glutamyltranspeptidase; LT, liver transplantation; T.Bil, total bilirubin; TCMR, T cell–mediated rejection.

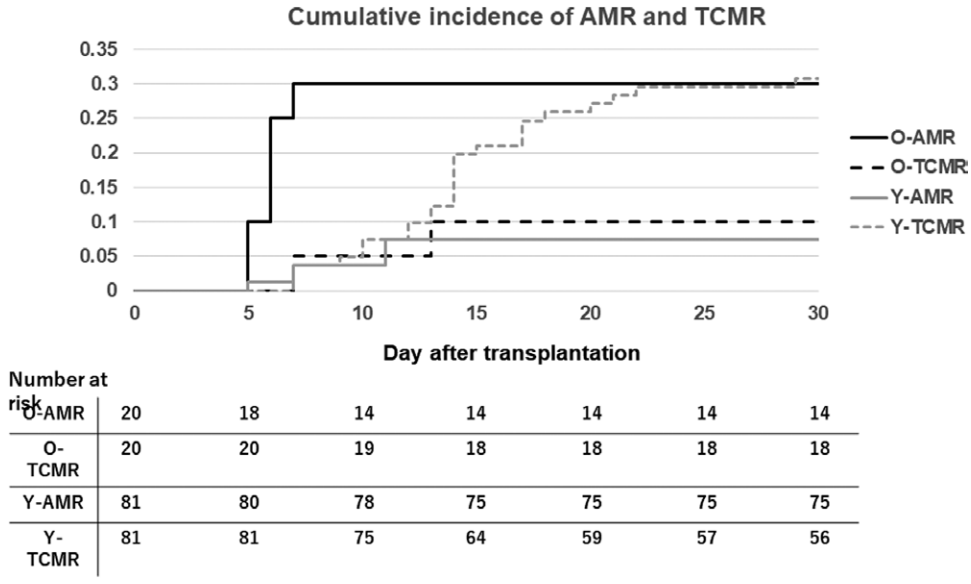

FIGURE 3. The cumulative incidence of acute antibody-mediated rejection (AMR) and T cell–mediated rejection (TCMR) alone.

Histopathology of Acute ABO-I AMR Cases

The histopathologic findings of acute ABO-I AMR are summarized in Table 4. All 6 patients in group Y showed mixed TCMR/AMR. In group O, 4 patients showed mixed TCMR/AMR, all of whom received pretransplant rituximab at the protocol-appointed timing. In contrast, 2 patients who did not receive protocolized rituximab showed isolated AMR (cases 7 and 8). Except case 6, the h score at the first biopsy was 1 in 5 patients in group Y. C4d deposition on the portal vein and capillary was observed in all cases and scored according to the Banff 2016 criteria. Focal extension to sinusoids was observed in 3 cases with C4d score, 2 in group Y (cases 1, 5, and 6). Portal stromal C4d deposition was focally seen in 1 case (case 5). There was no positive correlation between C4d score, h score, and the severity of TCMR.

Treatment and Outcomes of Acute ABO-I AMR

The patients who developed acute AMR were treated by several therapies (Table 5). Four patients who developed mixed TCMR/AMR in group Y were treated with the combination of intravenous methylprednisolone pulse and IVIG followed by MMF. Case 4 was initially diagnosed as TCMR at the time of the biopsy but was later corrected to mixed TCMR/AMR. He was initially treated with intravenous methylprednisolone pulse without IVIG, and then MMF was initiated because of the prolonged rejection. Case 6, who developed severe mixed TCMR/AMR leading to IHBCs and whose DSA was negative, was treated with intravenous methylprednisolone pulse, IVIG, PE rituximab, and ATG.[6] One patient in group O was successfully treated without PE, whereas 5 in group O required PE for 5 to 8 d with the combination of intravenous methylprednisolone pulse and IVIG. Case 11 underwent second biopsy for reelevation of liver enzyme at 25 d after LT. The liver biopsy showed alleviation of the tissue damage and C4d deposition by AMR; however, severe TCMR was observed to be treated with ATG. Four patients who underwent follow-up biopsies showed improvement of AMR tissue damage and C4d deposition in parallel as shown in Table 3 and Figure 5. Ten of the 12 patients recovered from acute AMR without sequelae. Case 6 suffered from IHBCs. Case 4 died of graft failure after strangulation ileus in the late LT period. The Kaplan-Meier curves for the graft survival are shown in Figure 6. The 3-y graft survival rates in group Y were 83.3% in the patients with acute AMR and 91.9% in

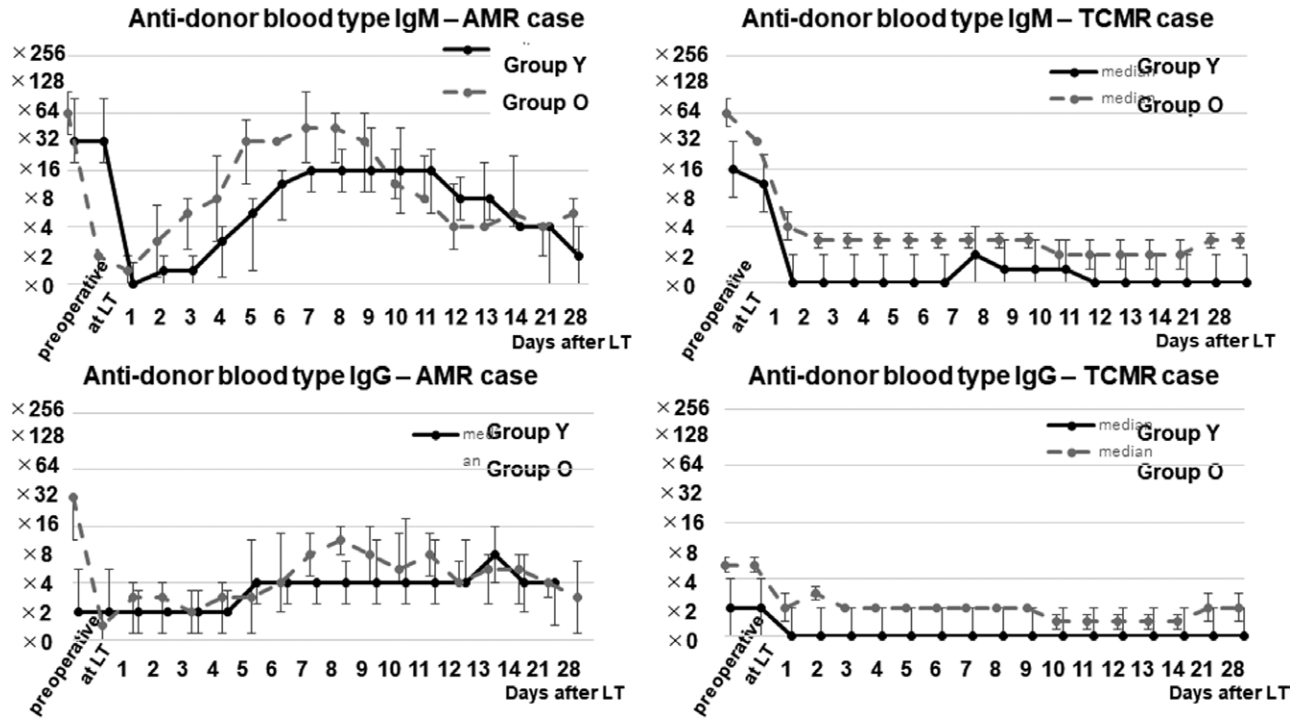

FIGURE 4. Changes in anti-donor blood group IgM and IgG titers. Median values and interquartile range of anti-donor blood group IgM and IgG titers in patients with acute antibody-mediated rejection (AMR) and T cell–mediated rejection (TCMR) comparing between groups Y and O through 1 mo after ABO-incompatible liver transplantation (LT).

TABLE 4.

Histopathology of acute ABO-AMR cases

Case	Biopsy day	Diagnosis	Assessment of acute AMR		Assessment of coexisting TCMR
			h score	C4d score	
Group Y					
1	POD11	TCMR/AMR	1	2	Mild
2	POD5	TCMR/AMR	1	2	Severe
3	POD7	TCMR/AMR	1	1	Moderate
	POD120		0	0	Indeterminate
4	POD12	TCMR/AMR	1	2	Moderate
5	POD11	TCMR/AMR	1	2	Moderate
	POD32	TCMR	0	1	Mild
6	POD7	TCMR/AMR	3	3	Severe
	POD11	TCMR/AMR	1	2	Severe
	POD25	TCMR	0	1	Moderate
	POD94	TCMR	0	0	Severe
Group O					
7	POD5	AMR	3	1	–
8	POD7	AMR	1	3	–
9	POD6	TCMR/AMR	3	1	Mild
10	POD5	TCMR/AMR	1	1	Moderate
11	POD6	TCMR/AMR	3	3	Mild
	POD25	TCMR/AMR	1	2	Severe
	POD69	TCMR	0	1	Moderate
12	POD6	TCMR/AMR	3	2	Moderate

AMR, antibody-mediated rejection; C4d, complement component 4d; POD, postoperative day; TCMR, T cell–mediated rejection.

the patients without acute AMR (Wilcoxon test, $P = 0.25$). The 3- and 5-y graft survival rates in group O were 100% in the patients with acute AMR and 92.9% in the patients without acute AMR (Wilcoxon test, $P = 0.43$). When the 3-y graft survival rates were compared between groups Y and O, they were similarly good (91.9% in group Y and 95.0% in group O; Wilcoxon test, $P = 0.72$). Importantly, those outcomes were comparable to the ABO-compatible LT recipients, whose 3-y graft survival rates were above 90% in our institution.

TABLE 5.

Treatment and outcomes of acute AMR cases

Case	Treatment of AMR				Outcome	Follow-up period
	mPSL pulse	IVIG	PE	Others		
Group Y						
1	+	+			Alive	3.5 y
2	+	+			Alive	3.2 y
3	+	+			Alive	1.3 y
4[a]	+				Dead (liver failure due to ileus)	10 mo
5	+	+			Alive	1.8 y
6	+	+	8 d	ATG, rituximab	Alive (IHBC)	4.4 y
Group O						
7	+	+			Alive	6.6 y
8	+	+	8 d		Alive	4.2 y
9	+	+	5 d		Alive	1.5 y
10	+	+	7 d		Alive	1.3 y
11	+	+	3 d	ATG	Alive	10 mo
12	+	+	6 d		Alive	1.6 y

[a]Case 4 was initially diagnosed as TCMR at the time of the biopsy but was later corrected to mixed TCMR/AMR.
AMR, antibody-mediated rejection; ATG, antithymocyte immunoglobulin; IHBC, intrahepatic biliary complications; mPSL, methylprednisolone; PE, plasma exchange; TCMR, T cell–mediated rejection.

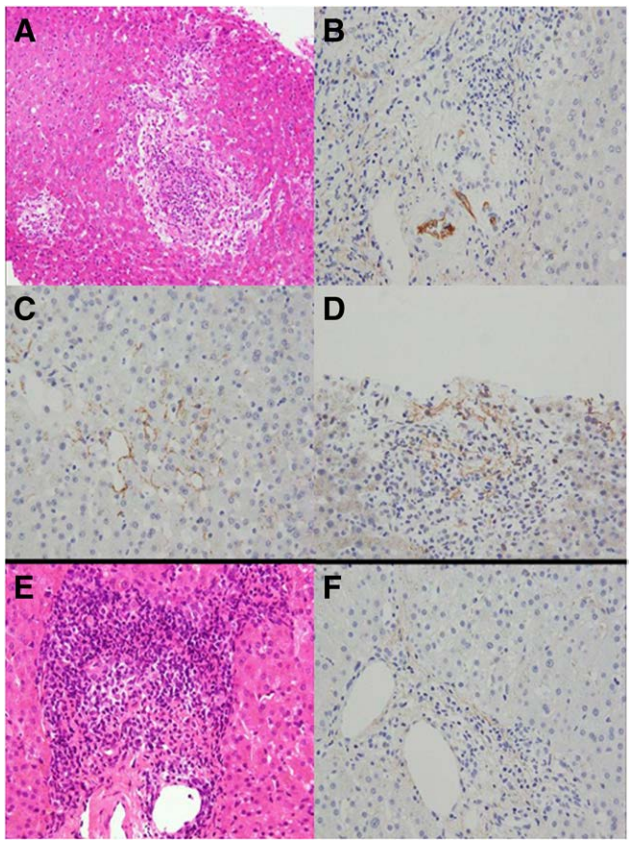

FIGURE 5. Histopathological improvement of acute antibody-mediated rejection (AMR). Histopathology of liver biopsies in case 5. The upper inset (A–D) shows the histopathology on the day of diagnosis of acute AMR, and the lower inset (E and F) shows the histopathology of the follow-up biopsy. A, Hematoxylin and eosin staining. Portal inflammation indicating T cell–mediated rejection (TCMR) and periportal edema indicating acute AMR were observed. complement component 4d (C4d) deposition on the portal capillaries (B), sinusoids (C), and portal stroma (D). E, Portal inflammation indicating TCMR was still observed; however, periportal edema was improved with diminishing C4d deposition (F).

DISCUSSION

In ABO-I LT, acute AMR is mediated by preformed ABO blood group antibodies combining with antigens in vascular endothelium, although the liver is a privileged organ that could be transplanted with a relatively lower prevalence of acute rejection than those associated with the kidney or heart. ADB Ig reacts with ABO blood group antigens on endothelial cells in the graft, leading to complement activation and neutrophil exudation and resulting in vasculitis, IHBCs, intrahepatic disseminated coagulation, activation of fibrinolysis, and eventual hemorrhagic necrosis of the graft.[9] However, it has been commonly accepted that the patient and graft survival rates of ABO-I LT are good in children under 2 y of age.[5,10] Several factors may be related to this. Anti-ABO blood group antibody (anti-ABO Ab) levels show an age-dependent increase physiologically. Anti-ABO Abs are present at birth because of the transplacental transport of maternal antibodies (IgG) but not as a result of self-production. The newborn starts producing anti-ABO Abs of its own at approximately 8 to 12 wk of age. The proportion of infants producing detectable IgM blood group antibodies increases up to 8 mo of age, with all infants 8 mo of age and older producing detectable levels of IgM blood group antibodies.[11] However, the maturation of their ability to produce antibodies is not complete until approximately 18 mo of age, and these titers reach adult levels by 10 y of age.[12,13] The physiological development of the anti-ABO Abs antibody supports our findings: the ADB IgM titer was lower in group Y than in group O, which may contribute to the lower incidence of acute AMR. In addition to the immaturity of antibody production at a young age, there may be several explanations for the low incidence of acute AMR in group Y: large-for-size grafts may be responsible, as they dilute the antibody and complement binding across a larger endothelial cell surface than smaller grafts, resulting in a reduced incidence of acute AMR in group Y[14]; a young donor age may correlate with less endothelial damage than an older age; and a large amount of bleeding and large transfusion volume might have removed any preformed antibodies.

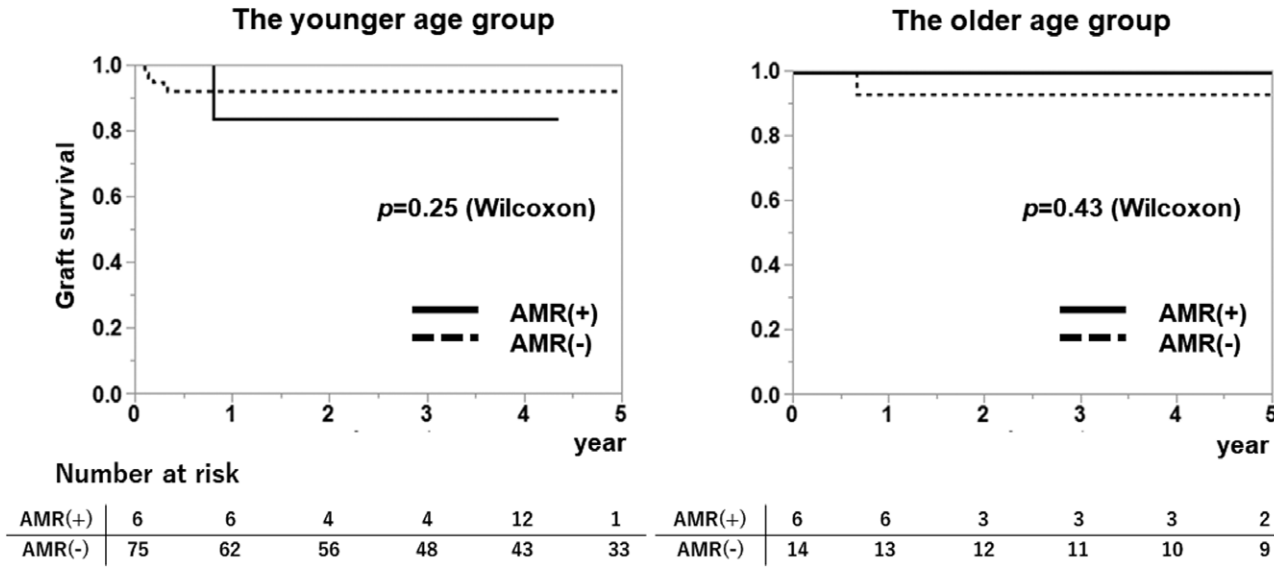

FIGURE 6. The Kaplan-Meier curves for the graft survival. AMR, antibody-mediated rejection.

A major point of progress regarding ABO-I LT in recent years was the comprehensive update of the pathological diagnosis of AMR in liver allografts, including new recommendations for C4d tissue staining and interpretation.[6] Histopathology of acute ABO-I AMR shows portal microvascular endothelial cell enlargement, focal fibrin deposition, portal edema, periportal hepatocyte necrosis, and red blood cell congestion and hemorrhage.[15] The histological features typifying acute AMR are collectively characteristic but not specific, and diagnosing acute ABO-I AMR has been a challenge.[9] Morphologic features of acute AMR of the liver overlap with various conditions such as preservation/ischemic injury, acute cellular rejection, and bile duct obstruction.[16] It is important to recognize whether acute AMR is occurring in ABO-I LT. The highlight of this study was the fact that acute AMR was accompanied by TCMR in patients whose ability to produce antibodies was immature (group Y) or medically suppressed by preconditioning treatment using rituximab. Synergistic actions between humoral and cellular rejection have been reported in renal transplantation and LT in some studies.[17–19] In addition, the complement system is activated in the vasculature during inflammatory conditions, facilitating complement deposition on the endothelial surface.[20] Based on these reactions, TCMR can promote the development of acute AMR. In some studies, C4d deposits were directly proportional to Banff TCMR grade, suggesting that mixed TCMR represents severe episodes.[21] However, the C4d score showed no correlation with TCMR grade in this study. It was recently reported that follicular helper T cells promote AMR in mixed TCMR/AMR in renal transplantation.[22,23] Identifying the role of CD4+ T cell in the onset of acute AMR and understanding the therapeutic target of specific helper T-cell subtype is key to eradicating acute AMR in this era using rituximab for ABO-I LT. Another important issue is C4d staining. C4d staining facilitates an AMR diagnosis. Portal vein and capillaries and sinusoidal endothelial cell C4d staining is reportedly most specific for acute AMR.[6] Haga et al reported that portal C4d stromal staining seems to be associated with severe acute ABO-I AMR.[24] However, the same group noted later that endothelial C4d staining alone is adequate because only endothelial staining has been used as the standard for other solid organ transplants and stromal staining alone is often difficult to differentiate from nonspecific staining.[25] In our present study, C4d deposition on the portal vein and capillary was observed in all cases with acute ABO-I AMR. Extension to sinusoids was observed in 3 cases, and portal stromal C4d deposition with portal capillary and sinusoid staining was seen in 1 case. Sites of C4d deposition had no relation with the severity of tissue damage (h score). However, all 13 liver biopsies that showed portal microvascular damage (positive h score) simultaneously showed portal vein and capillary endothelia C4d deposition in this study. And C4d score and h score were improved in parallel after treatment. Those results indicated that endothelial C4d deposition was adequate and that C4d deposition could strongly support the proof of antibody reaction. It was considered important that both portal microvascular damage and portal/sinusoidal endothelia C4d deposition were observed. Although we have been less likely to experience severe acute AMR in this era of preconditioning using rituximab, careful evaluation must be taken to find even mild acute AMR based on the combination of portal microvascular damage and portal capillary C4d deposition. Salah et al suggested that postoperative ADB Ig titer monitoring may be practical and that the routine application of C4d immunostaining in ABO-I LT may not be necessary for detecting acute AMR.[25] However, because the ADB Ig titer varies greatly depending on the age, it is plausible that early recognition of AMR through histopathological evaluation for tissue damage and C4d deposition might prompt therapy tailored to humoral rejection in children. In diagnosing acute ABO-I AMR, the elevation of ADB Ig titer is essential, as well as histopathology. However, defining the cutoff value of elevation of ADB Ig is difficult because the ability to produce antibody varies greatly depending on age. Therefore, continuous elevation of ADB Ig, usually within 2 wk after LT, was defined as an elevation of titer in this study. Twelve of 15 cases who showed the elevation of ADB IgM after LT were diagnosed as acute ABO-I AMR with histopathological evidence in this study. When diagnosing acute AMR, other types of acute AMR caused by anti-HLA antibodies should always

be considered. Although diagnosing AMR should not depend solely on the presence of DSA,[16] it should be evaluated if acute AMR with positive lymphocyte crossmatch before LT, acute AMR without the elevation of ADB IgM, or refractory AMR is observed in ABO-I LT. And we recommend ensuring that liver allograft biopsies are performed when allograft rejection is suspected during the first 2 wk after ABO-I LT, whether the elevation of ADB IgM is observed or not, even in young children. Diagnosis of AMR in liver allografts depends on strong clinical, serologic, and morphologic suspicion.

Several limitations associated with the present study warrant mention. This was a retrospective study, having a bias regarding the indication for the liver biopsy. Several patients were treated for suspected acute allograft rejection without a liver biopsy, which may have led to the underestimation of the incidence of acute allograft rejection. Our study also has limitations for the analysis of DSAs. Preoperative analysis of DSA other than FCXM was not performed, and postoperative DSA assays at the time of treatment were not performed in most cases. Although the negativity of preoperative FCXM suggests that DSAs that were possibly present in diagnosing acute AMR were de novo DSAs, definitive data are lacking in this study.

In conclusion, ABO-I LT is a feasible option that can be offered to pediatric patients with end-stage liver disease. However, close attention should be paid to acute AMR, which is often accompanied by TCMR. Establishing the accurate diagnosis of acute AMR based on clinical, serological, and definitive pathological findings, especially using C4d staining, is crucial, as AMR is treatable with therapies with anti–B-cell and anti–T-cell strategies. Further studies concerning the immunological status will allow for the validation of these observations.

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REFERENCES

1. Demetris AJ, Jaffe R, Tzakis A, et al. Antibody-mediated rejection of human orthotopic liver allografts. A study of liver transplantation across ABO blood group barriers. *Am J Pathol*. 1988;132:489–502.
2. Gugenheim J, Samuel D, Reynes M, et al. Liver transplantation across ABO blood group barriers. *Lancet*. 1990;336:519–523.
3. Heffron T, Welch D, Pillen T, et al. Successful ABO-incompatible pediatric liver transplantation utilizing standard immunosuppression with selective postoperative plasmapheresis. *Liver Transpl*. 2006;12:972–978.
4. Kasahara M, Umeshita K, Inomata Y, et al; Japanese Liver Transplantation Society. Long-term outcomes of pediatric living donor liver transplantation in Japan: an analysis of more than 2200 cases listed in the registry of the Japanese Liver Transplantation Society. *Am J Transplant*. 2013;13:1830–1839.
5. Honda M, Sugawara Y, Kadohisa M, et al. Long-term outcomes of ABO-incompatible pediatric living donor liver transplantation. *Transplantation*. 2018;102:1702–1709.
6. Demetris AJ, Bellamy C, Hübscher SG, et al. 2016 comprehensive update of the Banff Working Group on liver allograft pathology: introduction of antibody-mediated rejection. *Am J Transplant*. 2016;16:2816–2835.
7. Tanaka K, Uemoto S, Tokunaga Y, et al. Surgical techniques and innovations in living related liver transplantation. *Ann Surg*. 1993;217:82–91.
8. Narumoto S, Sakamoto S, Sasaki K, et al. ABO-incompatible liver transplantation for children under 2 years of age: a case report and a single-center review. *Pediatr Transplant*. 2019;23:e13308.
9. Demetris AJ, Jaffe R, Tzakis A, et al. Antibody mediated rejection of human liver allografts: transplantation across ABO blood group barriers. *Transplant Proc*. 1989;21:2217–2220.
10. Egawa H, Oike F, Buhler L, et al. Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. *Transplantation*. 2004;77:403–411.
11. Fong SW, Qaqundah BY, Taylor WF. Developmental patterns of ABO isoagglutinins in normal children correlated with the effects of age, sex, and maternal isoagglutinins. *Transfusion*. 1974;14:551–559.
12. Wuttke NJ, Macardle PJ, Zola H. Blood group antibodies are made by CD5 + and by CD5 - B cells. *Immuno Cell Biol*. 1997;75:478–484.
13. Eastlund T. The histo-blood group ABO system and tissue transplantation. *Transfusion*. 1998;38:975–988.
14. Astarcioglu I, Cursio R, Reynes M, et al. Increased risk of antibody-mediated rejection of reduced-size liver allografts. *J Surg Res*. 1999;87:258–262.
15. Egawa H, Shirase T, Miyagawa A, et al. Periportal edema and necrosis as diagnostic histological features of early humoral rejection in ABO-incompatible liver transplantaion. *Liver Transpl*. 2004;10:16–27.
16. Ali S, Ormsby A, Shah V, et al. Significance of complement split product C4d in ABO-compatible liver allograft: diagnosing utility in acute antibody mediated rejection. *Transpl Immunol*. 2012;26:62–69.
17. Sun Q, Liu ZH, Cheng Z, et al. Treatment of early mixed cellular and humoral renal allograft rejection with tacrolimus and mycophenolate mofetil. *Kidney Int*. 2007;71:24–30.
18. Bellamy CO. Complement C4d immunohistochemistry in the assessment of liver allograft biopsy samples: applications and pitfalls. *Liver Transpl*. 2011;17:747–750.
19. Bu X, Zheng Z, Yu Y, et al. Significance of C4d deposition in the diagnosis of rejection after liver transplantation. *Transplant Proc*. 2006;38:1418–1421.
20. Karpman D, Ståhl AL, Arvidsson I, et al. Complement interactions with blood cells, endothelial cells and microvesicles in thrombotic and inflammatory conditions. *Adv Exp Med Biol*. 2015;865:19–42.
21. Dankof A, Schmeding M, Morawietz L, et al. Portal capillary C4d deposits and increased infiltration by macrophages indicate humorally mediated mechanisms in acute cellular liver allograft rejection. *Virchows Arch*. 2005;447:87–93.
22. Gaughan A, Wang J, Pelletier RP, et al. Key role for CD4 T cells during mixed antibody-mediated rejection of renal allografts. *Am J Transplant*. 2014;14:284–294.
23. Louis K, Macedo C, Bailly E, et al. Coordinated circulating T follicular helper and activated B cell responses underlie the onset of antibody-mediated rejection in kidney transplantation. *J Am Soc Nephrol*. 2020;31:2457–2474.
24. Haga H, Egawa H, Fujimoto Y, et al. Acute humoral rejection and C4d immunostaining in ABO blood type-incompatible liver transplantation. *Liver Transpl*. 2006;12:457–464.
25. Salah A, Fujimoto M, Yoshizawa A, et al. Application of complement component 4d immunohistochemistry to ABO-compatible and ABO-incompatible liver transplantation. *Liver Transpl*. 2014;20:200–209.