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Increased Bile Duct Complications and/or Chronic Rejection in Crossmatch Positive Human Liver Allografts

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BILE duct complications continue to be a significant cause of bile morbidity after orthotopic liver transplantation, with an incidence of 7% to 34% of patients.[1,2] Bile of duct obstruction, usually due to strictures, biliary leakage, and/or cholangitis, accounts for the majority of these complications. In contrast, although low incidence of chronic rejection has been reported under FK 506-based immunosuppression in early trials of liver transplantation,[3] chronic rejection after liver transplantation occurs in only 5% to 9% of cases, and it remains one of the most common causes of graft loss.[4,5] There have been many conflicting recent reports concerning a relationship between the bile duct-related complications or ductopenic chronic rejection and a positive crossmatch.[6-8]. We investigated the outcome of liver grafts from positive crossmatch donors, focusing on biliary complications and ductopenic chronic rejection, and we compared the results to a consecutive negative crossmatch control group at the same time.

CASE MATERIALS

We analyzed 306 consecutive adults (more than 16 years of age) primary liver transplant cases performed at Presbyterian University Hospital in Pittsburgh between November 13, 1989, and September 26, 1990. Twenty-eight (9.1%) patients were from a donor whose crossmatch test results were positive (more than 50% of donor lymphocytes were killed by dithiothreitol-pretreated recipient serum). Selection of the contemporaneous 278 consecutive control patients whose crossmatch test results were negative when less than 10% of donor lymphocytes were killed, and were doubtfully or weakly positive when 11% to 49% of donor lymphocytes were killed. None of the 306 patients received ABO blood group-incompatible grafts. All donor livers were preserved by University of Wisconsin solution.

The study groups differed in crossmatch status, negative control (group 1, $n = 278$) and positive group (group 2, $n = 28$). There was a generally high degree of illness in both the positive crossmatch group and their controls, as defined by the United Network for Organ Sharing (UNOS) stratification (Table 1).

Table 1. Patients' Profile

	Groups	
	1 ($n = 278$)	2 ($n = 28$)
Crossmatch Status	Negative	Positive
Age	47 (17–75)	53 (33–66)
Male/Female	178/100	10/18*
UNOS score (mean)†	3.5 ± 0.5	3.7 ± 0.5
UNOS 4	78	8
UNOS 4 us	89] 60%	13] 75%
Cold ischemic time (h)	15.8 ± 3.9	14.8 ± 0.7
Preservation solution	UW solution	UW solution

*$P < .01$ vs group 1.
†Mean ± SD.

The Crossmatch Test

Each recipient's serum was drawn immediately before and after liver transplantation and tested for cytotoxic crossmatching activity before and after treatment with dithiothreitol (DTT), which inactivates IgM antibodies.[9] Donor T lymphocytes isolated from lymph nodes using CD3-conjugated dynabeads (Dynal, Great Neck, NY) were used.

The cytotoxicity test was performed according to National Institutes of Health standards with one wash; 1 μL of 2×10^6 mL T lymphocytes was placed into 1 μL of serum, followed by a 1-hour incubation at room temperature. The liter of antibodies present was determined by a 1:2 serial dilution of the sera with RPMI 1640. After one wash, addition of 5 μL of rabbit complement for 1-hour at room temperature produced lysis that was evaluated using trypan blue exclusion. Crossmatch test results were positive when 50% to 79% of donor lymphocytes were killed and were strongly positive when more than 80% of lymphocytes were killed by DTT-pretreated recipient serum.

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Immunosuppression

Between November 1989 and May 1990, intravenous (IV) doses of 0.15 mg/kg of FK 506 in divided dosage were infused over 3 to 4 hours, starting in the operating room. Then, the basement immunosuppression was changed from June 1990 to 0.1 mg/kg to 24 hours' continuous infusion, and repeated every 24 hours until oral intake began. The conversion from IV to oral doses of 0.15 mg/kg every 12 hours was overlapped for 1 day.

Usually, 1 g of IV methylpredonisolone was given after reperfusion. A daily dose of 20 mg methylpredonisolone was started after the operation; the dosage was tapered later in all patients.

Rejection episodes proven by liver biopsy were treated first with a single 1-g bolus of methylpredonisolone. If rejection persisted, the patient received an additional 5-day burst of methylpredonisolone (steroid recycle) or a 3–5 day course of 5 to 10 mg/d of OKT3.

Follow-up Period

The follow-up period was to December 31, 1997, or until death. A median follow-up was 7.7 years, and a minimum follow-up was 7.2 years.

Statistical Analysis

The chi-square analysis was used to compare the incidence of acute rejection, ischemic injury, bile duct complications, and chronic rejection in both groups. Graft survival was determined by the Kaplan-Meier method.

The diagnosis of bile duct complications was according to the protocol with ultrasonography, tube or percutaneous cholangiography to evaluate the causes of cholestatic graft dysfunction and hepatic chemicals. Finally, liver biopsy was performed at the time of graft dysfunction. The diagnosis of chronic rejection was based upon histological findings of disappearing interlobular bile ducts with scant mononuclear portal infiltrates, progressing later to bridging fibrosis with large, expanded portal tracts.[10,11]

RESULTS

There was no difference in patients' profiles of both groups except the ratio of male/female patients. The incidence of female patients was higher in positive crossmatch cases when compared with controls (36% in group 1 vs 64% in group 2, $P < .01$, Table 1). Twenty-six of 28 (92.9%) group 2, patients were strongly positive crossmatch results, which represent more than 80% of the donor lymphocytes killing against recipients' sera in DTT treatment. High titers of the antibodies (more than 1:8 dilution of a recipient's serum) were detected in 15/28 (53.6%) of all positive crossmatch patients.

The actuarial graft survival at 6 months was significantly lower in group 2 patients compared with those of group 1 controls (60.7% vs 84.8%, $P < .001$).

This study, examining bile duct complications and chronic rejection, was undertaken in patients whose first graft had not failed within 3 months after orthotopic liver transplantation (OLTX) in both groups (245 cases in group 1 and 19 cases in group 2).

Table 2. Bile Duct Complications in Negative Control Group 1 Patients (Clinico-Pathological Diagnosis)*

Complication	*N*
Biliary obstruction obstruction with cholangitis	9 (38–2400 days)
Biliary stricture	7 (19–987 days)
Bile leak	3 (37–93 days)
Bile duct stricture following hepatic artery thrombosis	2 (261;661 days)
Stricture of the choledochojejunostomy	2 (265;364 days)
Bile cast syndrome	1 (374 days)
Biliary fistula	1 (112 days)
Total	25/245 (10.2%)

*Patients whose first grafts had not failed within 3 months.

The incidence of major bile duct complications was developed in 10.2% (25/245) of group 1 negative control patients (Table 2). Bile duct obstruction and/or obstruction with cholangitis was observed in nine cases as a major bile duct complication. Biliary strictures were observed in seven cases. Bile leak, bile duct stricture following hepatic artery thrombosis, stricture of the choledochojejunostomy, and biliary fistula were observed as shown in Table 2 of group 1 patients.

Conversely, the incidence of major bile duct complication was significantly high (36.8%, 7/19, $P < .005$) in group 2 patients with the interval of 89 to 664 days after OLTX when compared to group 1 patients (Table 3). In seven cases of major bile duct complications in group 2 patients, bile duct obstruction and/or obstruction with cholangitis was observed in four cases of group 2 (57.1%, 4/7). The others consisted of one case of multiple biliary strictures with ongoing intrahepatic portal thrombi (retransplanted on day 95), one case of biliary stricture and revision (849 days), and one case of focal necrosis of the bile duct and cholestasis (374 days) (Table 4). Additionally, two more positive crossmatch patients experienced late severe vascular complications; one had a portal vein thrombosis on day 582 and was retransplanted; another one had a mycotic aneurysm of the hepatic artery on day 245 after OLTX.

Chronic rejection was diagnosed on the basis of patho-

Table 3. Incidence of Bile Duct Complications in Both Group 1 and Group 2 Patients

	Groups	
Crossmatch Status	1 Negative	2 Positive
Patients whose first grafts had not failed within 3 months	245	19
Incidence of bile duct complications	25 (10.2%)	7 (36.8%)*
Interval (days after OLTx)	10–2400 days	89–664 days

*$P < .005$ vs group 1 in chi-square analysis.

Table 4. Bile Duct Complications in Positive Crossmatch Group 2 Patients (Clinico-Pathological Diagnosis)*

Complication	N
Biliary obstruction	4 (89–1142 days)
Multiple biliary strictures with ongoing intrahepatic portal thromb	1 (95 days)
Biliary stricture and revision	1 (849 days)
Focal necrosis of the bile duct cholestasis	1 (374 days)
Total	7/19 (36.8%)

*Patients whose first grafts had not failed within 3 months.

logical biopsy reports with the importance of assessing findings over time rather than assessing a single needle core in the ultimate prognosis. In principle, the histopathology of the chronic rejection was duct loss, portal fibrosis, and arteriolar thickening of the graft.

Biopsy-proven chronic rejection was observed in 12 cases, with an interval of 185 to 1687 days in group 1 negative control patients (4.9%, 12/245). One patient was retransplanted on day 2130 and was, respectively, dead on day 2148. Two patients died on days 646 and 2009, after OLTX. Only three grafts (1.1%, 3/278) were lost due to chronic rejection. In contrast, chronic rejection was observed in five cases out of 19 patients, with an interval of 125 to 709 days after OLTX in group 2 positive crossmatch patients (26.3%, 5/19). The incidence of chronic rejection was significantly higher than those of negative control group 1 patients (26.3% vs 4.9%, $P < .005$, Table 5). In the five positive crossmatch patients who had a diagnosis of chronic rejection, one patient was retransplanted on day 126 and died on day 141. Also, two patients died on days 95 and 1368, respectively, after OLTX. Three grafts in group 2 positive crossmatch patients were lost by chronic rejection (10.7%, 3/28).

DISCUSSION

The adverse effect of preformed antibody states on the transplanted liver has been increasingly acknowledged.[7,12,13] First was the recognition of the poor prognosis if an ABO-incompatible donor was used[14,15]; second, an increased graft loss in the early phase after liver transplantation was noted in positive cytotoxic crossmatch cases.[13]

However, the impact of lymphocytotoxic antibodies as the cause of humoral rejection after liver transplantation has been confused in the past because of several factors. One factor has been the selection and sensitivity of crossmatch technique. The critical issues that must be decided are which antibodies need to be considered in liver transplantation—IgG, T cell, B cell, HLA class I or HLA class II, anti-endothelial cell. In this series we used the test for cytotoxic antibody activity against T lymphocytes isolated from donor lymph nodes at room temperature (37°C), followed by a 60 minute incubation period with rabbit complement. If the screening test was positive, the recipient serum was pretreated with DTT to inactivate the IgM antibodies. Only two cases out of 28 group 2 patients changed from positive reactivities to positive ones after DTT treatment.

Table 5. Incidence of Chronic Rejection in Both Group 1 and Group 2 Patients*

	Groups	
Crossmatch	1 Negative	2 Positive
Patients whose first grafts had not failed within 3 months	245	19
Incidence of chronic rejection	12 (4.9%)	5 (26.3%)†
Interval (days after OLTx)	185–1687 days	125–709 days

*Duct loss, portal fibrosis, and arteriolar thickening in pathological findings.
†$P < .005$ vs group 1 in chi-square analysis.

In this study, we showed a high incidence of bile duct complications in the presence of a cytotoxic crossmatch group. These biliary complications involve only the donor biliary tree and lead to significant morbidity, the same as the ABO-incompatible group.[16] Although the results of our study imply an immune-mediated pathogenesis, the precise mechanism remains unclear. However, the acute injury appears to be related to mechanical occulusion of microvasculature,[17] and functional narrowing of the arterial tree because of immunologically mediated vasoconstriction has been reported.[11] This injury can manifest as focal intrahepatic infarcts and bile duct necrosis followed later by periductal fibrosis and stricturing. Because of a dual blood supply, which may be one of the causes of resistance to antibody-mediated rejection, the biliary tree may injure because of its arterial-only-type circulation and more conventional arteriolar and capillary network.[11,17]

The incidence of chronic rejection in the positive crossmatch group, which survived more than 3 months after OLTX, was significantly higher than those of negative cases in this series. However, recent reports do not support these observations.[7,8] This may be due to the differences of the sensitivity of the crossmatch test, and differences of the basic immunosuppression regimens. Antibodies to non-HLA target antigens may be important. Immunoglobulin deposition on biliary epithelium and vascular endothelial cells has been reported in chronic rejection of liver transplants.[11,18,19]

Ever since we recognized the increased immunology risk, we routinely include high-dose steroids (1 g methylpredonisolone) immediately after reperfusion and so-called steroid recycle starting postoperative day 1 and/or antileukocyte antibodies after this time period.[20]

The etiology of transplant obstructive arteriopathy or chronic rejection is still unknown. Bile duct injury and chronic rejection may be related to the vasculopathy that is accelerated by chemokines, cytokines, and growth factors released from T lymphocytes and antigen-presenting macrophages, vascular smooth muscle cells, and endothelial cells.[21,22] Further molecular studies of these mechanisms should be investigated to settle the question of the pathogenesis of chronic rejection.

CONCLUSION

We investigated the outcome of liver allografts from positive crossmatch donors, focusing on biliary complications and ductopenic chronic rejection, and we compared the results to a negative crossmatch control group.

2. The major biliary complication developed in 36.8% of positive crossmatch patients, compared to 10.2% of negative controls.
3. Chronic rejection was observed in 26.3% of positive crossmatch patients, and 4.9% of negative control patients. Significantly high incidences of ischemic injuries were observed in all liver biopsy specimens of positive crossmatch patients compared to those of negative controls.

Finally, an immunologic injury to the bile duct epithelium or a bile duct injury due to the reduction of periductal blood supply secondary to the vascular endothelial damage is suspected.

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