



ORIGINAL ARTICLE

Liver-inclusive intestinal transplantation results in decreased alloimmune-mediated rejection but increased infection

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Abstract

Background and aims: A co-transplanted liver allograft has been thought to protect other organs from rejection-mediated injury; however, detailed analyses of co-transplanted liver on intestinal allograft outcomes have not been conducted to date. The aim of the study was to compare immune-mediated injury, causes of graft failure and clinical outcomes between recipients who underwent either a liver-inclusive intestinal transplant (LITx) or liver-exclusive intestinal transplant (LETx).

Methods: Between May 2000 and May 2010, 212 adult patients undergoing LITx ($n = 76$) and LETx ($n = 136$) were included. LITx underwent either liver combined intestinal or full multivisceral transplantation. LETx underwent either isolated intestinal or modified multivisceral transplantation.

Results: During 44.9 ± 31.4 months of follow-up, death-censored intestinal graft survival was significantly higher for LITx than LETx (96.9%, 93.2% and 89.9% vs 91.4%, 69.3% and 60.0% at 1, 3 and 5 years; $p = 0.0001$). Incidence of graft loss due to rejection was higher in LETx than in LITx (30.9% vs 6.6%; $p < 0.0001$), while infection was the leading cause of graft loss due to patient death in LITx (25.0% vs 5.1%; $p < 0.0001$). Despite similar immunosuppression, the average number (0.87 vs 1.42, $p = 0.02$) and severity of acute cellular rejection episode (severe grade: 7.9% vs 21.3%; $p = 0.01$) were lower in LITx than in LETx. Incidence of acute antibody-mediated rejection was also significantly lower in LITx than in LETx (3.6% vs 15.2%; $p = 0.03$). Incidence of chronic rejection was reduced in LITx (3.9% vs 24.3%; $p = 0.0002$).

Conclusions: Intestinal allografts with a liver component appear to decrease risk of rejection but increase risk of infection. Our findings emphasize that LITx has characteristic immunologic and clinical features. Lower immunosuppression may need to be considered for patients who undergo LITx to attenuate increased risk of infection.

Key words: Intestinal transplant; immunosuppression; rejection; infection

Introduction

Intestinal transplantation (ITx) has become an effective treatment option for patients with irreversible intestinal failure [1,2].

Due to the unique structure and immunologic properties of the intestine, it has been deemed one of the most difficult organs to transplant [3]. Despite improvements in short-term outcomes,

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long-term survival of both patient and graft after ITx is still poor compared to other solid-organ transplants, with a 5-year graft survival around 50–60% [1,4]. Rejection and infection continue to be the leading causes of graft loss after ITx [5,6]. Finding the delicate balance between rejection and infection remains one of the primary challenges of managing intestinal allograft recipients [7,8].

It is well known that liver allografts can spontaneously be accepted across the full major histocompatibility complex barrier in many animal models [9,10]. Clinically, a transplanted liver appears to be more resistant to alloimmune-mediated damage than other organ transplants and some liver allograft recipients who develop “operational tolerance” can successfully be withdrawn from chronic immunosuppressive medications [11,12]. Compelling evidence shows that, in the setting of highly sensitized recipients, a co-transplanted liver can provide immunoprotection for kidney or cardiac grafts [13–15]. Several mechanisms have been hypothesized to explain the better outcomes associated with a co-transplanted liver [16]. The immunoprotective property of a co-transplanted liver on intestinal allografts has been described earlier [17]. So far, the largest series of 500 cases of ITx have been reported regarding the evolution of surgical techniques, immunosuppression and management strategies but lack a detailed analysis of the effect of co-transplanted liver allografts on immune-mediated damage and outcomes [18].

Traditionally, ITx is divided into four main categories for clinical analyses: an intestinal transplant alone, a combined liver-intestinal transplant, a modified multivisceral transplant without a liver and a full multivisceral transplant [19,20]. Owing to the unique immunoprotective features of co-transplanted liver allografts, we hypothesize that different mechanisms and causes for intestinal graft loss exist between a liver-inclusive intestinal transplant (LITx) and liver-exclusive intestinal transplant (LETx). The purpose of this study was to retrospectively compare the incidence of immune-mediated injury, causes for graft failure and clinical outcomes between LETx and LITx to develop a better therapeutic strategy toward more balanced immunosuppression.

Methods

Patient selection

During a 10-year period from May 2000 to May 2010, a total of 236 consecutive adult ITx procedures were performed at the University of Pittsburgh Medical Center (UPMC). Of these, 212 patients with primary transplants were included in the final analysis; the results from 24 patients with secondary transplants were reported earlier [21]. Patient data were retrieved from computerized databases, flow-charts and medical records. Graft failure was defined as a patient returning to total parenteral nutrition (TPN), receiving an antrectomy or death. The average time of follow-up from date of transplant to date of graft failure or death was 44.9 ± 31.4 months.

All donors were cadaveric and ABO blood type-identical. Donor grafts were allocated based on blood group matching, size match and clinical urgency. Donor and recipient human leukocyte antigen (HLA) match were random. Immunological testing, including HLA tissue typing, panel reactive antibody (PRA), HLA antibodies and complement-dependent cytotoxicity cross-match (CDC-XM), was previously described in detail [18]. All 212 patients received a complete CDC-XM and PRA analysis; 154/212 (72.6%) patients underwent a complete HLA antibody

assessment via either LAT Single Antigen ELISA or Lab Screen Single antigen bead Luminex assays.

Transplant types

Based on the unique immunoprotective features of the liver, intestinal graft recipients were divided into two categories in this study: liver-exclusive or liver-inclusive transplants. Liver-exclusive transplants underwent either isolated intestinal or modified multivisceral transplantation; liver-inclusive transplants underwent either liver combined intestinal or full multivisceral transplantation.

Post-transplant management

The immunosuppressive regimens used in our program have previously been described in detail [18]. In brief, 212 patients received either induction therapy including Daclizumab (Zenapax) ($n=20$), rabbit anti-thymocyte globulin (r-ATG) ($n=43$) and alemtuzumab ($n=117$) or no induction therapy ($n=32$). The maintenance immunosuppressive agents were mainly based on tacrolimus (Prograf; Astellas Pharma, Deerfield, IL) and steroids. Tacrolimus doses were targeted to achieve a trough level of 15–20 ng/ml within the first 3 post-operative months, 10–15 ng/ml between 4 and 12 months and 5–10 ng/ml thereafter. Similar immunosuppressive regimens and targeted tacrolimus levels were used between LETx and LITx.

For infection prophylaxis, the patient received piperacilline/tazobactam plus vancomycin, amphotericin B lipid complex (5 mg/kg/day for 1 week) and then fluconazole or voriconazole for 3 months, co-trimoxazole (80 mg every 2 days for 6–12 months) and ganciclovir (5 mg/kg/day q12hr for 2 weeks) and valganciclovir (900 mg for 3–6 months thereafter).

Diagnosis of rejection

Surveillance endoscopy was routinely performed twice per week for the first 2–3 weeks after transplantation and then weekly thereafter, with increased frequency as clinically indicated by increased stomal output, fever, abdominal pain or other symptoms. The histological criteria for a diagnosis of acute cellular rejection (ACR) were as described previously [22]. A diagnosis of acute antibody-mediated rejection (ABMR) is based on clinical evidence of graft dysfunction, high PRA levels with circulating donor-specific antibody (DSA) and pathological evidence of tissue injury and C4d deposition [23]. A new rejection episode is defined as the occurrence of new clinical symptoms with characteristic pathologies and at least one normal mucosal biopsy between two rejection events. A diagnosis of chronic rejection is based on clinical manifestations, confirmed with a full-thickness specimen of a totally or partially removed intestinal allograft to show evidence of vasculopathy and mesenteric lymphoid depletion with mesenteric sclerosis [24].

Statistical analysis

Data are presented with continuous variables as means \pm standard deviation (SD) and with categorical variables as counts and proportions. Comparisons between groups were made using Student's t-test and Fisher's exact test for continuous and categorical variables, respectively. Time-to-event data (e.g. death, graft survival, rejection) are described using Kaplan-Meier curves and compared using log-rank test. Cox proportional hazards regression was used to assess univariate association of independent variables with chronic rejection. For graft survival

analysis, all patient deaths were considered as graft failure regardless of graft functioning status at the time of patient death. For death-censored graft survival, all patient deaths were defined as lost to follow-up. Recipient variables (age, sex, cause of graft failure, positive CDC-XM, preformed DSA, *de novo* DSA, number of surgical procedures prior to transplant and duration of TPN), donor variables (age, sex, positive cytomegalovirus [CMV] status and cold ischemia time) and co-transplanted liver allograft were used for the analysis. Multivariate analyses were performed using a backward elimination method and a significance level of 0.10. Results of the Cox models are presented as odds ratios (ORs) and 95% confidence intervals (CIs). A *p*-value <0.05 was considered to be statistically significant.

Results

Baseline patient characteristics

A total of 212 adult patients undergoing primary ITx were included in this study. Of these, 136 (64.2%) received a liver-exclusive allograft including an isolated intestine (*n* = 106) and a modified multivisceral allograft without a liver (*n* = 30); 76 (35.8%) received a liver-inclusive allograft including combined liver-intestine (*n* = 28) and a full multivisceral allograft (*n* = 48).

At the time of transplant, LETx and LITx did not differ significantly in age, number of previous abdominal operations, durations of TPN or number of HLA mismatches. The proportion of female patients was higher in LETx than in LITx (65.4% vs 47.4%; *p* = 0.01). The rate of mesenteric thrombosis-related intestinal failure was significantly lower in LETx than in LITx (27.2% vs 27.3%; *p* = 0.0001). The proportion of patients who had splenectomy was significantly higher in LITx than in LETx (5.9% vs 65.8%; *p* < 0.0001). The proportion of patients who received no

induction therapy was significantly higher in LITx than in LETx (27.6% vs 8.1%; *p* = 0.0001). The proportion of patients who underwent Campath-1H induction was higher in LETx than in LITx (61.7% vs 43.5%; *p* = 0.01). The donor age was 25.3 ± 9.6 years in LETx and 26.2 ± 11.4 years in LITx. The cold ischemia times were shorter in LETx than in LITx (7.4 ± 1.4 vs 8.6 ± 1.5 hours; *p* < 0.0001). The rate of positive CMV donors was significantly higher in LITx than in LETx (61.8% vs 44.1%; *p* = 0.01) (Table 1).

Patient and graft survival

Overall survival rates at 1, 5 and 10 years were 94.0%, 65.2% and 61.3% for patients with LETx and 81.6%, 55.6% and 52.1% for those with LITx, respectively. Although patient survival was not significantly different between the two groups, it approached statistical significance (*p* = 0.061; Figure 1A). The death-censored intestinal graft survival rate was significantly higher for LITx compared to LETx: 96.9% at 1 year, 93.2% at 3 years and 89.9% at 5 years vs 91.4% at 1 year, 69.3% at 3 years and 60.0% at 5 years (*p* = 0.0001; Figure 1B).

In LETx, a total of 56 recipients (41.2%) lost an intestinal graft with an average follow-up of 45.7 ± 32.2 months, and the leading cause of graft loss was irreversible rejection. Of the 136 LETx recipients, 42 (30.9%) developed uncontrolled rejection (ACR in 7, acute ABMR in 2 and chronic rejection in 33) (Table 2). Lethal infections occurred in seven (5.1%): bacterial in six and viral in one with no documented fungal-caused death. The locations of infection included intra-abdominal in three, respiratory in two and bloodstream in two. Three (42.9%) infections occurred within the first 6 months after transplantation and four (57.1%) occurred thereafter (Table 3). An opportunistic infection was

Table 1. Clinical characteristics of the liver-exclusive versus liver-inclusive allograft

Clinical characteristics	All patients (<i>n</i> = 212)	Liver-exclusive (<i>n</i> = 136)	Liver-inclusive (<i>n</i> = 76)	<i>p</i> -value
Mean age, years	43.9 ± 12.1	42.1 ± 11.9	47.1 ± 11.9	NS
Female sex, <i>n</i> (%)	125 (59.0)	89 (65.4)	36 (47.4)	0.01
Prior abdominal operations	5.6 ± 5.5	5.7 ± 5.2	5.5 ± 5.9	NS
Durations of TPN, months	30.3 ± 43.1	30.3 ± 42.1	30.3 ± 44.9	NS
<i>Primary diagnoses, n (%)</i>				
Mesenteric thrombosis	76 (35.8)	37 (27.2)	39 (51.3)	0.0001
Crohn's disease	38 (17.9)	29 (21.4)	9 (11.9)	NS
Motility disorders	24 (11.3)	17 (12.5)	7 (9.2)	NS
Gardner's syndrome	14 (6.7)	12 (8.8)	2 (2.6)	NS
Radiation enteritis	8 (3.8)	7 (5.1)	1 (1.3)	NS
Gastric bypass	10 (4.7)	8 (5.9)	2 (2.6)	NS
Others	42 (19.8)	26 (19.1)	16 (21.1)	NS
Cold-ischemic times, hours	7.8 ± 1.5	7.4 ± 1.4	8.6 ± 1.5	<0.0001
Positive CMV donor, <i>n</i> (%)	107 (50.5)	60 (44.1)	47 (61.8)	0.01
Splenectomy, <i>n</i> (%)	58 (27.4)	8 (5.9)	50 (65.8)	<0.0001
Donor BMTx, <i>n</i> (%)	32 (15.1)	22 (16.2)	10 (13.2)	NS
Donor bowel irradiation, <i>n</i> (%)	21 (9.9)	17 (12.5)	4 (5.3)	NS
<i>Donor/recipient, n (%)</i>				
Sex mismatch	99 (46.7)	65 (47.8)	34 (44.7)	NS
Race mismatch	40 (18.9)	28 (20.5)	12 (15.8)	NS
<i>Induction, n (%)</i>				
None	32 (15.1)	11 (8.1)	21 (27.6)	0.0001
Zenapax	20 (9.4)	13 (9.6)	7 (9.2)	NS
Thymoglobulin	43 (20.3)	28 (20.6)	15 (19.7)	NS
Campath-1H	117 (55.2)	84 (61.7)	33 (43.5)	0.01
Follow-up, months	44.9 ± 31.4	45.7 ± 32.2	43.6 ± 29.9	NS

TPN, total parenteral nutrition; CMV, cytomegalovirus; BMTx, bone marrow transplantation; NS, not significant.

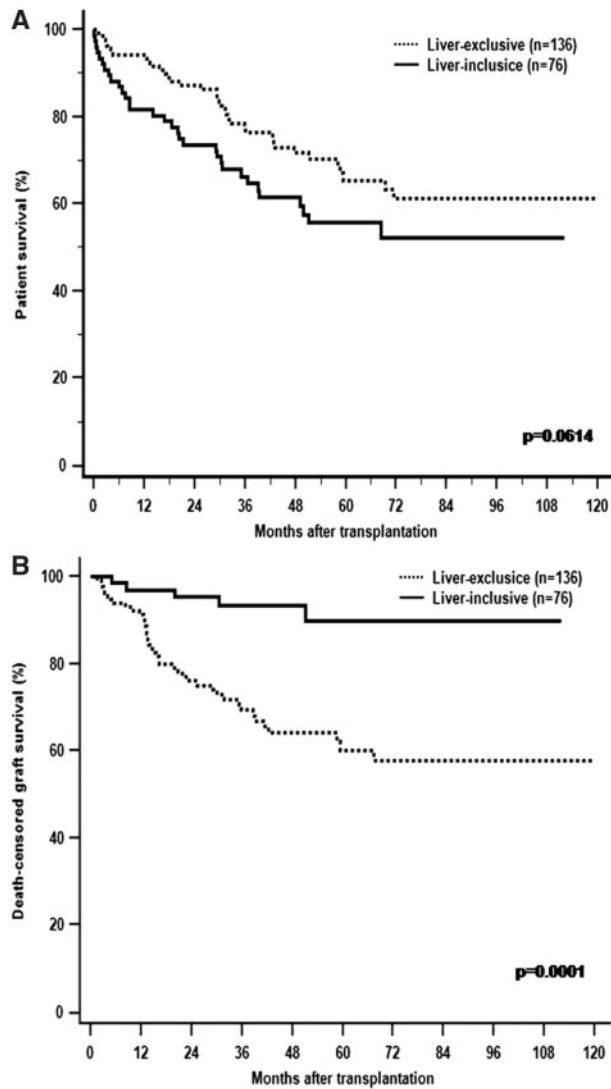


Figure 1. The Kaplan–Meier patient (A) and death-censored graft (B) survival for liver-exclusive transplants (dotted line) and liver-inclusive transplants (solid line). Although the trend of overall patient survival was lower in LITx than LETx, death-censored intestinal graft survival after LITx is superior to LET.

documented in one case (EBV-related post-transplant lymphoproliferative disease [PTLD]). Other causes of graft failure due to patient death included technical failure by intra-abdominal bleeding ($n=1$), cardiovascular event ($n=1$), motor vehicle accident ($n=1$), intra-cranial bleeding ($n=1$) or undefined etiology ($n=3$). In this group, 22 (16.2%) patients underwent retransplantation. A total of eight (5.9%) patients died within the first year after transplantation (Table 2).

In LITx, 33 (43.4%) recipients lost grafts with an average follow-up of 43.6 ± 29.9 months. Five cases suffered irreversible rejection (ACR in two and chronic rejection in three). The rate of rejection-caused graft loss in LITx was significantly lower compared to LETx (6.6% vs 22.2%; $p < 0.0001$) (Table 2). In contrast to LETx, lethal infection was the leading cause of graft loss in 19 LITx recipients: bacterial in 15, fungal in 3 and viral in 1. The locations of infection included nine respiratory infections, six abdominal abscesses (including two arterial graft pseudoaneurysms) and four bloodstream infections. Five (26.3%) infections occurred within the first 6 months after transplantation and 14 (73.7%) occurred after 6 months (Table 3). The lethal infection rate was

significantly higher in LITx when compared to LETx (25.0% vs 5.1%; $p < 0.0001$). Opportunistic infections were diagnosed in five cases including *Nocardia* ($n=1$), fungal ($n=3$) and EBV ($n=1$). The incidence of opportunistic infections was significantly higher in LITx than that in LETx (6.6% vs 0.7%; $p = 0.014$). In this group, three patients died peri-operatively from technical complications, including intra-cardiac clot ($n=1$) and arterial graft rupture ($n=2$). Other causes of graft loss included primary graft non-function ($n=1$), lymphogiosarcoma ($n=1$), graft-versus-host disease (GVHD) ($n=1$) or undefined etiology ($n=3$). Two patients (2.6%) underwent retransplantation. Overall, a total of 14 (18.4%) patients died within the first year after transplantation and the mortality rate within the first year in LITx was significantly higher than in LETx ($p = 0.004$) (Table 2).

Acute cellular rejection

Of the 76 LITx patients, the incidence of ACR (\geq mild grade) within the first year was 51.3%, which was similar to that seen in LETx patients (56.6%). The average number of episodes of ACR experienced per patient within the first year was significantly lower for LITx than LETx (0.87 [range 0–5] vs 1.42 [range 0–9]; $p = 0.02$). In general, there were similar proportions of a mild grade ACR between LITx and LETx (43.4% vs 42.6%), whereas the LITx patients showed a less moderate (15.8% vs 30.9%; $p = 0.02$) and severe grade of ACR (7.9% vs 21.3%; $p = 0.01$) (Figure 2). The median onset of the first episode of ACR in LITx was 1.6 months (range 0.1–31.3 months) post transplantation versus 0.8 months (range 0.5–35.4 months) in LETx ($p = 0.58$) (Table 2).

Acute ABMR and DSA

In the 154 patients (99 in the LETx and 55 in the LITx) with a complete HLA antibody assessment both before and after transplantation, we identified 17 cases (11.0%) that fulfilled all the criteria for a diagnosis of acute ABMR proposed by the National Conference. The incidence of acute ABMR in LITx was 3.6%—significantly less than 15.2% in LETx ($p < 0.03$). A detailed description of acute ABMR patients was given in our previous publication [21].

A preformed DSA was present in 44/154 (28.6%) cases. Fourteen (25.5%) LITx patients had a preformed DSA at time of transplantation: 11 (78.5%) to HLA class I only, 1 (7.2%) to HLA class II only and 2 (14.3%) to both HLA class I and class II. Similarly to LITx, 30 (30.3%) LETx patients had a preformed DSA: 17 (56.7%) to HLA class I only, 2 (6.7%) to HLA class II only and 11 (36.6%) to both HLA class I and class II (Table 2). There was no difference in the proportion of positive CDC-XM between LITx and LETx (27.6% vs 25.0%).

Post-operatively, resolution of a preformed DSA occurred in 71.4% (10/14) of LITx but only 33.3% (10/30) of LETx ($p = 0.01$). A newly formed DSA was observed in 7/55 (12.9%) of LITx and 28/99 (28.3%) of LETx ($p = 0.03$) (Table 2). In both groups, these *de novo* DSAs were predominantly against class II HLA both in 6/7 (85.7%) for LITx and in 25/28 (89.3%) for LETx. The average time to detect a *de novo* DSA was 16.7 ± 15.1 months for LITx versus 18.9 ± 15.3 month for LETx.

Chronic rejection

With a mean follow-up of 44.9 months, a total of 36 (16.9%) allografts developed pathology-confirmed chronic rejection. Of these, only 3/76 (3.9%) of LITx had chronic rejection (simultaneous liver and intestinal allograft chronic rejection in 2 and

Table 2. Immunological parameters and clinical outcomes of the liver-inclusive versus the liver-exclusive allograft

Clinical characteristics	All patients (n =212)	Liver-exclusive (n =136)	Liver-inclusive (n =76)	p-value
<i>Number of HLA mismatches</i>				
Class I (A, B locus)	2.9 ± 1.0	2.9 ± 1.0	2.8 ± 1.1	NS
Class II (DR locus)	1.6 ± 0.5	1.6 ± 0.5	1.6 ± 0.5	NS
PRA HLA class I (%)	21.8 ± 32.1	20.7 ± 30.7	23.8 ± 34.6	NS
PRA HLA class II (%)	16.2 ± 29.6	16.8 ± 30.4	15.1 ± 28.3	NS
Positive cross-match, n (%)	55 (25.9)	34 (25.0)	21 (27.6)	NS
Preformed DSA, n (%)	44/154 (28.6)	30/99 (30.3)	14/55 (25.5)	NS
De novo DSA, n (%)	35/154 (22.7)	28/99 (28.3)	7/55 (12.9)	0.03
Acute rejection (≤ 360 days), n (%)	133 (62.7)	92 (67.6)	41 (53.9)	0.05
Acute ABMR, n (%)	17/154 (11.0)	15/99 (15.2)	2/55 (3.6)	0.03
ACR, n (%)	116 (54.7)	77 (56.6)	39 (51.3)	NS
Mild	91 (42.9)	58 (42.6)	33 (43.4)	0.91
Moderate	54 (25.5)	42 (30.9)	12 (15.8)	0.02
Severe	35 (16.5)	29 (21.3)	6 (7.9)	0.01
Median first acute rejection, months	1.0 (0.1–35.4)	0.8 (0.5–35.4)	1.6 (0.1–31.3)	NS
Chronic rejection, n (%)	36 (17.0)	33 (24.3)	3 (3.9)	0.0002
Causes of graft failure, n (%)	89 (42.0)	56 (41.2)	33 (43.4)	NS
Rejection	47 (22.2)	42 (30.9)	5 (6.6)	<0.0001
Infection	26 (12.3)	7 (5.1)	19 (25.0)	<0.0001
Technical	4 (1.9)	1 (0.7)	3 (3.9)	NS
Primary-non-function	1 (0.5)	0	1 (1.3)	NS
Graft-versus-host disease	1 (0.5)	0	1 (1.3)	NS
Neoplasm	1 (0.5)	0	1 (1.3)	NS
Others	4 (1.9)	3 (2.2)	0	NS
Unknown	6 (2.8)	3 (2.2)	3 (3.9)	NS
Retransplantation, n (%)	24 (11.3)	22 (16.2)	2 (2.6)	0.003
Mortality ≤360 days	22 (10.4)	8 (5.9)	14 (18.4)	0.004

PRA, panel reactive antibody; HLA, human leukocyte antigen; DSA, donor-specific antibody; ACR, acute cellular rejection; ABMR, antibody-mediated rejection; NS, not significant.

Table 3. Type and timeline of post-transplant fatal infections in the liver-exclusive versus the liver-inclusive allograft

Type of infections	Timeline of infections		
	<1 month	2–6 months	>6 months
<i>Liver-exclusive</i>			
Bacterial	1	2	3
Fungal	0	0	0
Viral	0	0	1
Total	1	2	4
<i>Liver-inclusive</i>			
Bacterial	1	3	11
Fungal	0	1	2
Viral	0	0	1
Total	1	4	14

solitary intestinal chronic rejection in 1), which was significantly less frequent compared to 33 of 136 (24.3%) LETx ($p = 0.0002$; **Table 2**). The time to develop chronic rejection for three LITx recipients was 16.1, 30.5 and 40.0 months, respectively. The average time to chronic rejection for LETx was 23.1 ± 19.5 months. Freedom from chronic rejection at 6 months, 1 year and 5 years was 95%, 90% and 30% for LITx and 60%, 50% and 30% for LETx, respectively (**Figure 3**).

The results of the univariate and multivariate analyses of risk factors associated with the development of chronic rejection are shown in **Table 4**. While a *de novo* DSA (OR 25.42, 95% CI: 7.72–89.73; $p < 0.0001$), a higher number of acute rejection episodes (OR 1.93, 95% CI: 1.46–2.57; $p = 0.04$) and a positive CMV

donor (OR 2.72, 95% CI: 1.68–13.24; $p = 0.003$) were independently associated with chronic rejection, simultaneous liver transplantation reduced this risk significantly (OR 0.21, 95% CI: 0.04–0.79; $p = 0.03$).

Discussion

In this retrospective study, we compared the incidence of immune-mediated injury, causes of intestinal graft loss and patient outcomes between LETx and LITx to ascertain the immunoprotective role of a co-transplanted liver. Our results showed that: (i) although the trend of overall patient survival was lower in LITx than LETx, death-censored intestinal graft survival after LITx is superior to LETx; (ii) rejection is the leading cause of graft loss in LETx, while infection is the leading cause of graft loss in LITx; (iii) the frequency and severity of ACR and acute ABMR are reduced after LITx; (iv) the incidence of chronic rejection is lower after LITx. Our results indicate that LITx appears to render the graft more tolerable to rejection, but increases the risks of post-transplant infections. Our findings emphasize that LITx has characteristic immunologic and clinical features. Accordingly, ITx recipients should be managed mainly based on either liver-inclusive or liver-exclusive transplants. Different degree of immunosuppression between LITx and LETx may be used to better control rejection and prevent post-transplant serious infections.

In the current larger series of patients, we show decreased frequency of ACR and lower incidences of moderate to severe ACR after LITx, supporting the immunoprotective effect of liver allografts on simultaneously transplanted intestine.

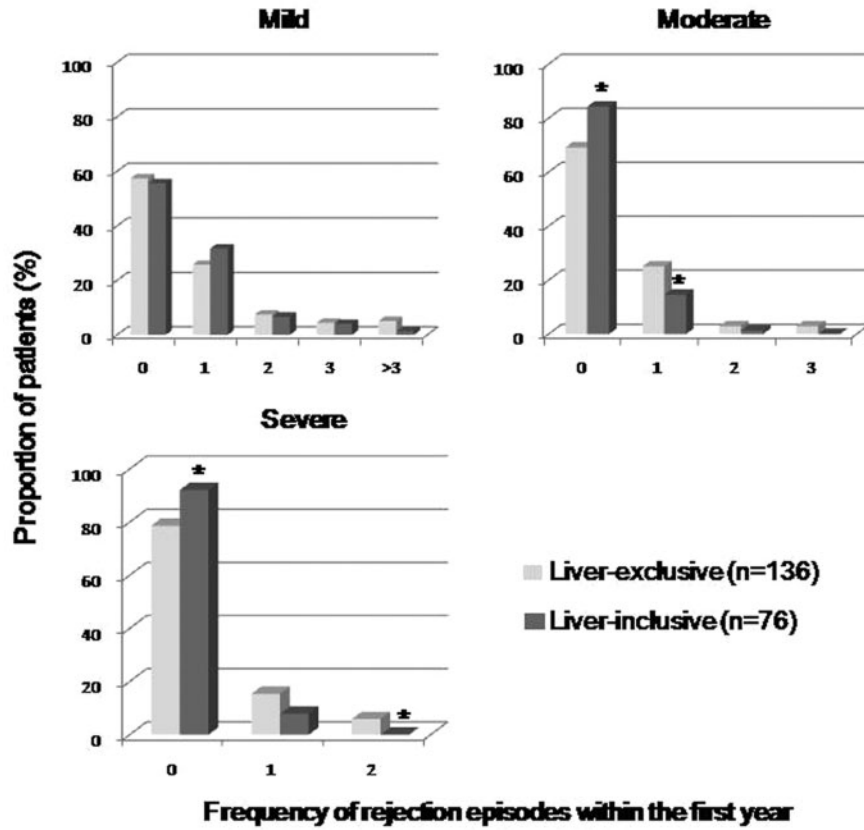


Figure 2. The proportion of patients who experienced frequency of acute rejection episodes within the first year after transplantation. The LITx patients (heavy gray) showed a less moderate and severe grade of ACR than the LETx patients (light gray).

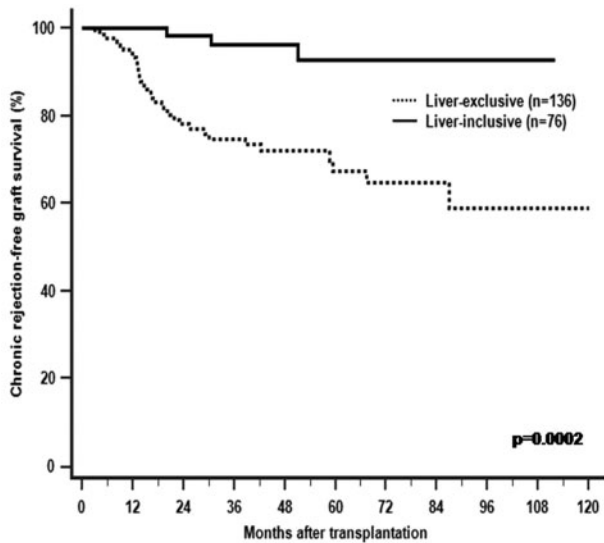


Figure 3. The Kaplan–Meier chronic rejection-free graft survival for liver-exclusive (dotted line) and liver-inclusive transplants (solid line). The incidence of chronic rejection is significantly lower after liver-inclusive transplantation.

Selvaggi *et al.* reported 209 ITx recipients and found an increased incidence of overall rejection and severity in recipients of the isolated intestinal and combined liver-intestinal allograft compared to the multivisceral allograft with a liver or without a liver [25]. The discrepancy between two observations may be related to the differences in the patient population and in the

time span to study the frequency of rejection. In their study, pediatric patients accounted for 61%, which may contribute less frequency and severity of acute rejection after transplantation of a larger amount of lymphoid tissue. The relative immature immune system in pediatric patients may be more “immunologically educable” at a central or peripheral level than the matured immune system in adults [25,26]. In addition, our study focused on ACR within the first year after ITx to make our two study groups more comparable. Several potential mechanisms have been proposed to explain the reduced cellular immune response in transplant recipients. It has been noted that the liver allograft harbors donor-derived hematopoietic cells, which migrate out of the liver graft at the time of graft implantation to establish a state of chimerism [27]. Persistent chimerism can lead to clonal depletion of host alloreactive T cells, which has been speculated as one of the major factors that help promote long-term graft survival or tolerance induction after transplantation [28,29]. Recent data indicate that chimerism is associated with a lower incidence of rejection after intestinal and multivisceral transplantation [30].

Our earlier study examined the risk factors for acute ABMR and showed that the presence of a liver component was associated with a lower risk of developing acute ABMR after ITx [21]. In this observation, we further compared the recipients between LITx and LETx to confirm a protective effect of liver allografts against humoral rejection. There were no significant differences between LETx and LITx in terms of the proportions of a positive CDC-XM, pre-existing DSA and the levels of PRA, indicating that these two groups might face a similar immunologic challenge pre transplantation. Given that a positive CDC-XM was not

Table 4. Univariate and multivariate analysis of risk factors associated with chronic rejection

Clinical characteristics	Univariate		Multivariate	
	Odd ratio (95% CI)	p-value	Odd ratio (95% CI)	p-value
<i>Recipient</i>				
Age (years)	1.08 (1.01–1.16)	0.03	1.04 (0.99–1.09)	0.09
Male	1.07 (0.13–8.63)	0.95		
PRA HLA class I	1.09 (0.96–1.03)	0.93		
PRA HLA class II	0.99 (0.97–1.02)	0.78		
Positive cross-match	0.74 (0.07–7.87)	0.80		
Preformed DSA	0.75 (0.07–7.63)	0.81		
<i>De novo</i> DSA	76.49 (8.83–97.55)	0.0001		
Duration of TPN (mos)	1.01 (0.99–1.02)	0.22		
Number of surgery	1.21 (0.71–1.98)	0.75		
Rejection number ≤ 360 days	3.21 (1.05–5.73)	0.03		
<i>Donor</i>				
Age (years)	0.99 (0.91–1.08)	0.88	2.72 (1.68–13.24)	0.003
Male	0.66 (0.19–2.26)	0.51		
Cold-ischemic times, minutes	1.47 (0.81–2.66)	0.20		
Positive CMV	5.87 (1.41–24.42)	0.02		
Liver allograft	0.04 (0.00–0.53)	0.014		

PRA, panel reactive antibody; HLA, human leukocyte antigen; DSA, donor-specific antibody; TPN, total parenteral nutrition; CMV, cytomegalovirus.

contraindication to ITx, similar immunosuppression was used while B-cell-targeted therapies were not used, this observation can better explain the unique immunoprotective effect of liver allografts on ABMR. It is also worth noting that there were two cases (2.6%) of acute ABMR after LITx, implying that the liver is not always protected. Caution should be taken when transplanting highly sensitized patients. Several potential mechanisms for the immunoprotective effect of the liver have been hypothesized to play a role. It has been speculated that the transplanted liver may promote the clearance of alloantibodies via absorption by either surface-bound or soluble alloantigens. Recent evidence by Taner *et al.* further showed that the immunoprotective effect of the liver involves more than simply absorbing donor-specific antibodies, with a shift in the pattern of gene expression in kidneys away from proinflammatory toward preservation of tissue integrity [31].

During average follow-up of 44 months, chronic rejection occurred as high as 16.9% of the recipients. Our results showed that the presence of co-transplanted liver allografts significantly reduced the incidence of chronic rejection. Several donor and recipient factors have been reported to be associated with the development of chronic rejection [32,33]. Based on our findings, a post-transplant *de novo* DSA was closely related to the development of chronic rejection. Several pieces of evidence suggest that the major target of HLA antibodies appears to be the graft endothelium, which can be activated and injured via a complement-dependent or complement-independent pathway [34–36]. The mechanisms by which HLA antibodies lead to chronic rejection are not well understood, and whether the presence of antibodies is an initiating event or merely a response to tissue damage remains to be defined. We also identified a higher frequency of ACR episodes as an independent risk factor for chronic rejection. It has been speculated that repeated immune-mediated damage exhausts the recipient's natural repair mechanism, leading to chronic rejection. Lower incidence of chronic rejection in LITx may be a secondary effect of the immunoprotection conferred by the donor liver on the intestinal allograft.

Our data show that a significant reduction in rejection did not improve patient survival and that infection accounts for the

majority of patient deaths after LITx. In this study, infections during the early period appear to be related to the high morbidity of intestinal recipients at the time of transplantation and surgical complications. The type, timeline and site of infections were consistent with previous report [37–40]. In our previous report, we showed that overall infection-caused graft failure was around 11% and was the second most common cause of graft loss. Recent data by Silva *et al.* showed that infection-related graft loss was the major cause of mortality after ITx. But they did not exam specific contributions of the liver to infection [40]. Our findings indicate that the LITx recipient is more vulnerable to infections than the LETx when exposed to a similar degree of immunosuppression. The LITx candidates may have more comorbidities that can result in multiple admission, prolonged lengths of stay and many surgical procedures. Most of the LITx recipients underwent splenectomy at the time of transplantation, which may decrease the risk of humoral and cellular-mediated rejection but may increase the risk of infection. The liver is roughly 10 times larger than the heart or kidney, and its large transplanted tissue mass has been hypothesized to dilute cytokines and alloreactive T lymphocytes, leading to exhaustion of the recipient immune response. The higher co-morbidities, an asplenic state, the use of relatively heavy immunosuppressive agents, a heavy burden of alloantigens, etc. might make recipients' immune systems over-suppressed, as reflected by *de novo* malignancy, GVHD and increased incidences of severe infections. Our findings indicate that lower immunosuppression may need to be considered for the patients who undergo LITx to attenuate increased risk of infection. Finding a delicate balance between over- and under-immunosuppression remains as a major challenge for the optimal management of ITX recipients.

Our study is a retrospective analysis at a single center and has several important limitations. Although the size of the cohort is relatively large given the low case volumes of intestinal transplant procedures performed worldwide, it has the limitations of a small sample size. Due to its retrospective nature, the patients in the two groups were not matched and higher comorbidities of LITx recipients might exist at the time of transplantation. As the assessment of HLA antibodies was not

routine in our program, more detailed analyses of the DSA including C1q-binding capacity were not performed. These will need to be included in future studies, to better understand the role of the liver in reducing antibody-mediated immune injury.

In summary, intestinal allografts with a liver component appear to decrease the risk of rejection but increase the risk of infection. Lower immunosuppression may need to be considered to address the increased risk of infection associated with LITx.

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