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Early and late onset *Clostridium difficile*-associated colitis following liver transplantation

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Summary

Clostridium difficile colitis (CDC) remains a serious and common complication after liver transplantation (LT). Four hundred and sixty-seven consecutive LTs in 402 individuals were performed between 1998 and 2001 at our center. Standard immunosuppression consisted of tacrolimus, mycophenolate, and steroids. CD toxins A and B were detected by using a rapid immunoassay or enzyme immunoassay. CDC was diagnosed in 32 patients (5–1999 days post-LT), with 93.8% (30/32) of patients developing CDC during the first year post-LT; three individuals had CDC more than 3 years post-LT, one of which also had early CDC. All patients presented with abdominal pain and watery diarrhea. Patients who developed CDC within 1-year post-LT were significantly more likely to have a hemorrhagic, biliary, or infectious complication. Patients who developed CDC within 28 days post-LT had a significantly higher model end-stage liver disease score. Treatment consisted of fluid and electrolyte replacement and metronidazole and no patients developed toxic megacolon, required colonic resection, or died from CDC. CDC represents a potentially severe complication following LT. Most cases occur early post-LT. Development of a hemorrhagic, biliary, or infectious complication is associated with the development of CDC.

Introduction

Liver transplantation (LT) has emerged as an excellent treatment option for acute and chronic end stage liver disease, primary and some secondary hepatic malignancies and some rare metabolic liver based disorders. Improvement in the surgical technique, donor and recipient conditioning, improved intensive care, development of modern immunosuppressive agents, and better control of secondary complications have led to continued improvement in outcomes. However, secondary complications such as renal, cardiovascular and pulmonary issues, neuropsychiatric disorders, metabolic disturbances, malignancies and infections still largely determine graft and patient survival.1 LT recipients have a high risk of developing infectious complications. Debilitating disease, the operative stress, and immunosuppressive therapy cause severe impairment of host defense mechanisms, while peri-operative antibiotics disrupt the natural gut flora [1,2]. Diarrhea is a well-known side effect of immunosuppressive therapy [e.g. mycophenolate mofetil (MMF)], but can also be caused by a variety of infectious agents including

bacteria, fungi, protozoa, and viruses [3]. One of the most common bacterial pathogens is *C. difficile* (CD), a gram positive anaerobic organism. Infection with CD leads to toxin-mediated invasive colitis with characteristic clinical and pathologic features [4]. The clinical spectrum ranges from harmless colonization without symptoms to excessive watery diarrhea and enteritis or even fulminant hemorrhagic pseudomembranous colitis with toxic megacolon [5,6]. This life-threatening condition may require surgical intervention [5,7]. The development of CD colitis has been associated with a variety of antibacterial agents including clindamycin, cephalosporins and extended-spectrum penicillins. In rare cases, CD colitis also can be found in immunocompetent patients who are not exposed to antibiotics. LT recipients are among the group at highest risk for this infection [8]. CD associated diarrhea has been described in all types of solid organ transplantation and after stem cell transplantation [9–16]. Rapid detection of enteric pathogens and their toxins is essential to initiate timely treatment, as diarrhea during the early post-transplant period can cause severe secondary complications [17,18].

The aim of this study was to analyze retrospectively the incidence, epidemiology, and impact of infection caused by CD in a large series of LTs from a single center.

Materials and methods

Patients and transplants

Patient records for all LTs performed at the Mayo Clinic in Jacksonville, Florida, between March 1998 and December 2001 were reviewed retrospectively.

Surgery

Organ procurement was carried out according to standard techniques. Both aortic and portal vein perfusion with 5 l of University of Wisconsin solution was used. All transplants were performed using the piggyback technique without veno-venous bypass [19]. This series includes two patients who had an LT earlier at other institutions. Also, in these two cases, the piggy back procedure was applied.

Fast tracking

Starting in 1999, fast tracking was attempted in all eligible patients that included on-table extubation and bypassing of the intensive care unit. The criteria to perform fast tracking included good general condition of the patient, minimal blood loss, no other intra-operative complications, and no significant comorbid findings on meticulous pretransplant evaluation. The frequency of fast tracking was only 10% in 1998 but in 2001 it reached 50%.

Immunosuppression

Initial prophylactic immunosuppression

Standard immunosuppression consisted of tacrolimus (TAC), MMF and rapidly tapered steroids. MMF was attempted to be withdrawn by the end of the first year. Also steroids were withdrawn at the earliest possible time point. For patients with hepatitis C virus (HCV), this was in most cases the fourth month. Long-term monotherapy with TAC was attempted on patients who included individuals with autoimmune hepatitis and sclerosing cholangitis.

Antirejection

Rejection therapy consisted of bolus steroids and OKT3 (Orthoclone®, Ortho Biotech Products, L.P., Bridgewater NJ, USA) for steroid resistant rejection. Diagnosis of rejection was obtained by biopsy in all cases.

Long-term immunosuppression

Patients were switched to cyclosporine A in case of neurotoxicity or nephrotoxicity. Patients who did not tolerate calcineurin inhibitors were switched to sirolimus. This was performed on an individual basis and no strict protocol was in use. Seventy-five percent of patients were maintained on TAC long term, with the vast majority receiving TAC monotherapy.

Liver biopsies

The liver graft was biopsied 1 h after reperfusion, on day 7, 100 and 365 post-LT and thereafter annually per protocol, with additional biopsies, if clinically indicated.

Acid blocking medication (ABM)

Forty percent of patients had proton pump inhibitors (PPI) therapy pre-LT. Patients received PPIs for 100 days post-LT and if no evidence for peptic ulcer disease (PUD) or gastroesophageal reflux disease (GERD) was found, the agents were planned to be withdrawn. Nevertheless, more than 60% of patients remained on ABM long term [20].

Infection management

Bacterial surveillance and antibacterial prophylaxis

Prior to transplant, no screening of stool for CD carriage was carried out. Standard antibacterial prophylaxis included a 2-day course of a third generation cephalosporin. For patients at excessive risk and those who had pending infection at the time of transplant, a more aggressive antibiotic regimen was used. Individuals at

increased risk for fungal infection received prophylactic amphotericin B lipid complex [21]. A subgroup of individuals enrolled in a randomized trial received pretransplant selective small bowel flora suppression [22]. No non-absorbable antibiotics were given post-transplant. No surveillance cultures were performed per protocol; however, all patients were screened for stool carriage of vancomycin-resistant enterococcus. Stool was sent for detection of pathogens in case of diarrhea or significant abdominal discomfort. An enzyme immunoassay for the detection of toxin A and B (Premier® Toxins A&B by Meridian Diagnostics Inc., Cincinatti, OH, USA) was utilized. Per protocol or our facility, all patients with CD colitis are treated with metronidazole as first-line therapy unless contraindicated. Patients who fail to respond receive either an extended course of metronidazole or receive oral vancomycin.

Cytomegalovirus studies and prophylaxis

Donor and recipient cytomegalovirus (CMV) status was determined by a serologic assay for anti-CMV immunoglobulin G antibodies. For CMV monitoring, the shell vial system and pp65-antigenemia assay were performed on a weekly basis. CMV infection and disease were judged according to previously proposed criteria [23]. Patients with CMV mismatch (donor positive/recipient negative) received prophylactic oral ganciclovir (3000 mg/day) for 3 months [24]. All other patients received acyclovir (400 mg b.i.d. for 30 days) and CMV infection was treated preemptively.

Pneumocystis carinii pneumonia prophylaxis

Trimethoprim/sulfamethoxazole was given three times weekly for a total of 100 days. Patients who were intolerant to this regimen received inhaled pentamidine.

Data collection and statistic analysis

Baseline donor and recipient data were collected prospectively from the donor report, United Network for Organ Sharing (UNOS) reports and pretransplant evaluation sheets. A database was created using MICROSOFT ACCESS and MICROSOFT EXCEL and was supplemented by clinical data, laboratory parameters, complications data, and biopsy results which were collected retrospectively from the hospital records. Follow-up data were available for the entire cohort. This data collection was performed by a surgeon and a hepatologist who both had a large experience with liver transplant recipients. The computerized databases of the transplant unit and the microbiologic laboratory were both cross linked, from which 32 LT recipients with CD were identified. Hospital records of these 32 patients were studied in detail. Data are given as median with minimum–maximum range or mean with SD. Outcome of LT according to the development of CD infection was analyzed. The groups were compared in terms of graft and patient survival, epidemiologic and clinical parameters using SPSS 11.5 (SPSS Inc., Chicago, IL, USA) including chi-squared test; Fisher's exact test, Mann–Whitney *U* and Kruskal–Wallis assay. Survival was calculated using Kaplan–Meier curves with log rank statistics. A *P*-value of <0.05 was considered statistically significant. A subgroup analysis was performed according to the time of onset of CD infection (group 1: early onset, 12 patients (days 1–28 post-LT), group 2: intermediate onset: eight patients (days 29–365 post-LT), group 3: late onset: three patients (after 1-year post-LT).

Results

Between March 1998 and December 2001, 467 LTs in 402 patients were performed at the Mayo Clinic in Jacksonville, Florida. There were 254 men and 148 women with a median age of 52 (range: 15–75) years. HCV associated liver disease accounted for 41.3% (166/402) of all indications, 6.5% (26/402) had hepatitis B virus associated liver disease, 19.6% (79/402) had primary biliary cirrhosis, primary sclerosing cholangitis or autoimmune hepatitis and 19.7% (79/402) had alcoholic liver disease. At the time of transplant 83 patients additionally suffered from primary or secondary hepatic malignancies.

Overall transplant outcome

One-year/5-year graft survival for primary transplants was 78.1%/62.8% and 1/5-year patient survival for first LTs was 85.6%/75.0%. During the entire 5–8 year follow-up, 88 patients were retransplanted and 111 died. Median time to retransplantation was 62 (range: 1–2222) days post-LT and median time to death was 257 (range: 0–2829) days post-LT. Graft survival longer than 1 year occurred in 347, and follow-up was available for all these patients. Data on graft and patient survival was available for the entire cohort to July 2006; however, during follow-up 19 patients were lost to follow-up with regard to some of their comorbidities or other data of planned annual evaluations.

Donor characteristics and epidemiologic data

Only cadaveric grafts were used. There were 25 split grafts, 16 grafts from non-heart beating donors; 57 donors were >70 years and 33 were younger than 15 years. In addition there were 46 grafts from morbidly obese donors, 48 grafts from donors with a serum

sodium >170 mmol/dl and 29 grafts from donors with significantly elevated liver enzymes (aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) > 300U/ml) or total bilirubin (>3 mg/dl). In total 55.9% (261/467) of grafts were considered to have been retrieved from extended criteria donors with 9.0% (42/467) of grafts having multiple risk factors. The median age of all donors was 48.5 (range: 4.1–87.0) years including 270 men (57.8%) and 197 women (42.2%). The median age of the 402 recipients was 52 (range: 15–75) years and there were 254 men (63.2%) and 148 women (36.8%). The median model end-stage liver disease (MELD) score was 15 (range: 6–49); 21 individuals (4.5%) were listed as UNOS 1, 89 (19.1%) as UNOS 2a, 138 (29.6%) as UNOS 2b, 154 (33.0%) as UNOS 3 and 0 as UNOS 4. The median waiting time to LT was 38 (range: 1–600) days. To date, 88 patients (21.9%) underwent retransplantation and 111 patients (27.6%) died. Of the 402 patients, 347 (86.3%) survived longer than 1 year. Rejection rate was 32% for the entire cohort. The median graft survival was 1901 (0–3119) days and the median patient survival was 2043 (0–3119) days.

Clostridium difficile associated disease

Demographic data

During the entire study period, CD was diagnosed in 32 LT recipients (8%). Detailed demographic and clinical characteristics in relationship to development of CD are depicted in Table 1. Patients were included in the study, if they presented with acute colitis and if CD toxin could be detected in stool. Other enteric or opportunistic pathogens were excluded as causative organisms based on the results of repetitive microbiologic cultures or detection assays. Diagnosis of colitis was based on clinical symptoms including diarrhea, abdominal pain, abdominal distension and CD toxin stool assay. Also, the presence of fever, leucocytosis and elevated C-reactive protein levels were taken into consideration. The median onset of first CD infection was 18 (range: 4–1999) days post-LT. 25 patients presented with a single episode and 7 with multiple episodes of CD colitis. Median time to relapse was 62 (range: 22–1259) days. One patient had early CD and a second episode 5 years post-LT, which was not considered a relapse but an independent event and will be reported in detail in this paper. All patients presented with abdominal pain and watery diarrhea. Diagnosis of CD colitis was confirmed by detection of toxin A&B using rapid immunoassay or enzyme immunoassay. Treatment consisted of reduction in immunosuppression, fluid and electrolyte replacement, and metronidazole.

Concerning the incidence of early/intermediate CD cases following LT, the incidence of CDC following LT was 7.1% in 1998 (3/42), 4.7% in 1999 (4/85), 5.0% in 2000 (6/121) and 12.3% in 2001 (19/154). Of the 136 nosocomially acquired cases of CD diagnosed at our hospital during the study period, 30 (22.1%) occurred in the cohort of 402 LT recipients, while two cases developed late, outside the study period. However, the rate of CD infection in all admissions at Mayo Clinic Jacksonville during this time period was 0.4% (136/35948). On the basis of these data, patients who have undergone LT are significantly more likely to develop CD colitis than non-LT patients ($P < 0.001$).

Clinical course

Tables 2 and 3 show detailed demographic and clinical data according to the presence of CD colitis with regard to early or intermediate onset compared to patients without CD colitis. Fig. 1 displays graft survival according to the development of CD infection. Although at 2 years a difference in graft survival (which corresponded to the patient survival) of 15% was observed, this did not reach statistical significance, with the curves approximating at 5 years post-LT. Fig. 2 shows timeline to the development of CD for the 32 identified cases; 93.8% were observed within the first year post-LT. Table 4 summarizes antibiotic exposure prior to the outbreak of CD colitis for the study patients.

Early onset CD colitis (CDC)

Early CDC (onset ≤28 days post-transplant) developed in 18 patients. When compared to patients without CDC, a significant difference was present regarding the number of grafts received ($P = 0.005$) and whether the patient was undergoing a redo liver transplant ($P = 0.013$). The MELD score was significantly higher in this group ($P = 0.007$), but the difference in UNOS status did not reach statistical significance ($P = 0.053$). Patients with early CDC were significantly more likely to have a major intra-abdominal bleed ($P < 0.001$), have a biliary complication ($P = 0.034$), have a bile leak ($P = 0.026$), or have a systemic infection ($P < 0.001$). No significant difference was present regarding patient age, HCV status, presence of diabetes, number of marginal graft risk factors, CMV mismatch, cold ischemic time, presence of rejection, need for whole plasma exchange, or whether they were fast-tracked bypassing the intensive care unit (all $P > 0.05$). One of the main risk factors for early development of CDC was a high MELD score (Fig. 3).

Intermediate onset CDC

Intermediate CDC (onset 29–365 days post-transplant) developed in 12 patients. Comparisons were made between the intermediate CDC group and the non-CDC group (Table 3). Unlike early CDC, no significant differ-

Table 1. Patient and donor characteristics by transplant.

	CDC, %	No CDC, %	*P*-value
Age of patient at transplant median (range)	52.5 (20–69)	52.0 (15–75)	0.89
Patient gender (male)	75.0 (24/32)	62.2 (230/370)	0.15
Patient height (cm) median (range)	173 (157–196)	171 (135–198)	0.22
Patient weight (kg) median (range)	84.0 (47.0–148.0)	81.0 (36.0–166.0)	0.84
Patient BMI median (range)	27.7 (16–51)	27.1 (15–51)	0.84
Diagnosis			
HBV	6.3 (2/32)	6.5 (24/370)	1.00
HCV	40.6 (13/32)	41.4 (153/370)	1.00
ALD	40.6 (13/32)	19.7 (73/370)	1.00
NASH	6.3 (2/32)	9.2 (34/370)	0.76
Cryptogenic	3.1 (1/32)	10.5 (39/370)	0.23
Tumor	12.5 (4/32)	23.0 (85/370)	0.27
PBC	3.1 (1/32)	5.7 (21/370)	1.00
PSC	9.4 (3/32)	7.6 (28/370)	0.73
Autoimmune	3.1 (1/32)	6.8 (25/370)	0.71
A1AT deficiency	0 (0/32)	0.8 (3/370)	1.00
Other liver disease	15.6 (5/32)	6.5 (24/370)	0.07
PNF	9.4 (3/32)	2.2 (8/370)	0.049
Graft loss	3.1 (1/32)	5.4 (20/370)	1.00
MELD score median (range)	18 (7–41)	14 (6–49)	0.010
UNOS status			
1	9.4 (3/32)	4.9 (18/370)	0.029
2A	40.6 (13/32)	20.5 (76/370)	
2B	21.9 (7/32)	35.4 (131/370)	
3	28.1 (9/32)	39.2 (145/370)	
Undergoing non-primary transplantation	28.1 (9/32)	12.9 (56/435)	$P = 0.016$ OR = 2.4 (95% CI 1.2–5.0)
Donor age median (range)	41.5 (7.9–81.2)	48.9 (4.1–87.0)	0.38
Donor BMI median (range)	25.6 (15–66)	24.8 (12–64)	0.58
Donor graft risk factors			
Renal impairment	53.1 (17/32)	32.4 (120/370)	0.018
Split liver	3.1 (1/32)	6.5 (24/370)	0.71
Age > 80 years	0 (0/32)	3.2 (12/370)	0.61
Age > 70 years	3.1 (1/32)	15.1 (56/370)	0.066
Age < 10 years	9.4 (3/32)	5.4 (20/370)	0.41
Age < 15 years	9.4 (3/32)	8.1 (30/370)	0.74
Morbid obesity	18.8 (6/32)	10.8 (40/370)	0.18
Non-heart beating	3.1 (1/32)	4.1 (15/370)	1.00
Hypernatremia	9.4 (3/32)	12.2 (45/370)	0.78
Elevated LFTs	9.4 (3/32)	7.0 (26/370)	0.49
Sum of graft risk factors			
0	50.0 (16/32)	43.7 (190/435)	0.49
≥1	50.0 (16/32)	56.3 (245/435)	
CMV status			
Donor positive	50.0 (16/32)	66.7 (290/435)	0.056
Recipient positive	71.9 (23/32)	69.9 (304/435)	0.81
Mismatch (donor pos./recipient neg.)	6.3 (2/32)	19.3 (84/435)	0.094

CDC, *Clostridium difficile* colitis; BMI, body mass index; HBV, hepatitis B virus; HCV, hepatitis C virus; ALD, alcoholic liver disease; NASH, non-alcoholic steatohepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; A1AT, alpha-1 antitrypsin; PNF, primary non-function; MELD, model end-stage liver disease; UNOS, United Network for Organ Sharing; LFTs, liver function tests; CMV, cytomegalovirus; OR Odds ratio; 95% CI, 95 percent confidence interval.

ence was present regarding the number of grafts received and whether the patient was undergoing a redo liver transplant, nor were the MELD score or UNOS status significantly different ($P > 0.05$). Patients with intermediate CDC were significantly more likely to have a vascular complication ($P = 0.03$), have a biliary complication ($P = 0.01$), have a bile leak ($P = 0.02$), receive either endoscopic retrograde cholangiopancreatography or percutaneous

Table 2. Clinical results by transplant and CDC status.

	CDC, %	No CDC, %	*P*-value
Number of grafts			
1	71.9 (23/32)	87.1 (379/435)	0.031
2	21.9 (7/32)	11.3 (49/435)	
3	6.3 (2/32)	1.6 (7/435)	
Intra-operative event	25.0 (8/32)	26.4 (115/435)	0.86
Hours of cold ischemia median (range)	7.3 (2.5–11.1)	7.3 (0.9–15.1)	0.29
Minutes of warm ischemia median (range)	35.5 (23–70)	34 (12–102)	0.79
Presence of vascular complication	18.8 (6/32)	10.1 (44/435)	0.13
Type of intra-abdominal bleed postoperatively			
None	71.9 (23/32)	93.6 (407/435)	<0.001
Major	25.0 (8/32)	4.4 (19/435)	
Minor or incisional	0 (0/32)	0.2 (1/435)	
Following percutaneous liver biopsy	0 (0/32)	1.4 (6/435)	
Presence of biliary complication	50.0 (16/32)	22.8 (99/435)	0.001 OR 3.06 (95 CI 1.58–5.92)
Type of biliary complication			
Stricture	15.6 (5/32)	8.7 (38/435)	0.20
Biliary necrosis	6.3 (2/32)	3.2 (14/435)	0.30
Bile leak	34.4 (11/32)	14.3 (62/435)	0.002 OR 2.82 (95% CI 1.42–5.62)
Received ERCP or PTC	34.4 (11/32)	11.7 (51/435)	<0.001
Required operative treatment of biliary complication	25.0 (8/32)	12.4 (54/435)	0.043 OR 2.18 (95% CI 1.02–4.63)
Post-operative bacterial infection	62.5 (20/32)	36.5 (135/370)	0.002 OR 2.81 (95% CI 1.41–5.59)
Post-operative systemic infection	37.5 (12/32)	7.6 (33/435)	<0.001 OR 5.62 (95% CI 2.95–10.75)
Development of dehiscence, evisceration, or hernia	31.3 (10/32)	11.6 (43/370)	0.002 OR 2.99 (95% CI 1.50–5.95)
Patient fast-track	21.9 (7/32)	40.5 (176/435)	0.038
Presence of rejection	25.0 (8/32)	32.6 (142/435)	0.37
Whole plasma exchange	15.6 (5/32)	7.4 (32/435)	0.095
Days of graft or patient survival median (range)	1868 (20–3119)	1901 (0–30980	0.79
Days of first graft survival median (range)	1927.5 (20–3119)	2046 (0–3115)	0.16
Time to diagnosis of CDC median (range)	17.5 (4–1999)	NA	NA
Immunosuppressant nephrotoxicity	6.3 (2/32)	6.7 (29/435)	1.00
Long-term comorbidities			
Gastrointestinal	46.9 (15/32)	34.9 (152/435)	0.17
Neurologic/psychiatric	50.0 (16/32)	56.8 (247/435)	0.46
Renal	34.4 (11/32)	38.6 (168/435)	0.63
Diabetes	40.6 (13/32)	38.6 (168/435)	0.82
Hypertension	43.8 (14/32)	49.2 (214/435)	0.55
Pulmonary	18.8 (6/32)	14.3 (62/435)	0.49
Cardiovascular	18.8 (6/32)	24.6 (107/435)	0.46
Died or retransplanted	46.9 (15/32)	40.9 (178/435)	0.51
Retransplanted	12.5 (4/32)	19.3 84/435)	0.48
Died	25.0 (8/32)	23.7 (103/435)	0.87

OR, odds ratio; CI, confidence interval; CDC, *Clostridium difficile* colitis; ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiography; CMV, cytomegalovirus.

transhepatic cholangiography ($P = 0.01$), have a systemic infection ($P = 0.01$), or have a documented bacterial infection ($P = 0.01$). Interestingly, the intermediate CDC group received fewer marginal grafts, but this did not reach statistical significance ($P = 0.31$). No significant difference was present regarding patient age, HCV status, presence of diabetes, CMV mismatch, cold ischemic time, presence of rejection, need for whole plasma exchange, or whether they were fast-tracked bypassing the intensive care unit (all $P > 0.05$).

Late onset CDC

Case 1. This patient received his first transplant in Pittsburgh at age 6 for biliary atresia. At age 20, he underwent retransplantation in December, 1999 at our center, but lost this graft secondary to primary nonfunction and was retransplanted (UNOS status 1; MELD score of 32) 3 days later. The patient experienced no postoperative immunologic, surgical or infectious complications. This patient developed idiopathic thrombo-

Table 3. Clinical and demographic data comparing early and intermediate *Clostridium difficile* (CDC) to no CDC infection.

	No CDC, %	Early CDC, %	*P*-value	Intermediate *C. difficile* colitis, %	*P*-value
Recipient age (median, range)	52 (15–75)	54.5 (36–69)	0.96	53.5 (38–66)	0.66
Number of grafts					
1	87.1 (379/435)	66.7 (12/18)	0.005	83.3 (10/12)	0.77
2	11.3 (49/435)	22.2 (4/18)		16.7 (2/12)	
3	1.6 (7/435)	11.1 (2/18)		0 (0/12)	
Number of MCJ transplants					
1	73.8 (321/435)	61.1 (11/18)	0.47	58.3 (7/12)	
2	19.8 (86/435)	27.8 (5/18)		33.3 (4/12)	
3	6.4 (28/435)	11.1 (2/18)		8.3 (1/12)	
Redo liver transplant	12.9 (56/435)	33.3 (6/18)	0.013	16.7 (2/12)	0.66
Diagnosis of HCV	41.4 (180/435)	38.9 (7/18)	0.83	41.7 (5/12)	1.00
UNOS status					
1	6.4 (28/435)	11.1 (2/18)	0.053	0 (0/12)	0.20
2A	19.8 (86/435)	44.4 (8/18)		41.7 (5/12)	
2B	35.2 (153/435)	22.2 (4/18)		16.7 (2/12)	
3	38.6 (168/435)	22.2 (4/18)		41.7 (5/12)	
MELD score (median, range)	14 (6–49)	19 (7–41)	0.007	16 (9–34)	0.53
Presence of diabetes prior to liver transplant	23.2 (101/435)	22.2 (4/18)	1.00	33.3 (4/12)	0.49
Hours of cold ischemic time (median, range)	7.3 (0.9–15.1)	7.0 (2.5–10.0)	0.27	7.4 (4.9–11.1)	0.81
Presence of major intra-abdominal bleed	4.4 (19/435)	33.3 (6/18)	<0.001	16.7 (2/12)	0.10
Presence of vascular complication	10.1 (44/435)	11.1 (2/18)	0.70	33.3 (4/12)	0.03
Presence of biliary complication	22.8 (99/435)	44.4 (8/18)	0.034	58.3 (7/12)	0.01
Presence of bile leak	14.3 (62/435)	33.3 (6/18)	0.026	41.7 (5/12)	0.02
Use of ERCP or PTC	11.7 (51/435)	27.8 (5/18)	0.058	41.7 (5/12)	0.01
Presence of systemic infection	7.6 (33/435)	44.4 (8/18)	<0.001	33.3 (4/12)	0.01
Presence of bacterial infection	35.4 (154/435)	55.6 (10/18)	0.081	75.0 (9/12)	0.01
CMV mismatch (donor positive/recipient negative)	19.3 (84/435)	5.6 (1/18)	0.22	8.3 (1/12)	0.48
Sum of marginal graft risk factors					
0	43.7 (190/435)	44.4 (8/18)	0.95	58.3 (7/12)	0.31
≥1	56.3 (245/435)	55.6 (10/18)		41.7 (5/12)	
Rejection	32.6 (142/435)	27.8 (5/18)	0.80	16.7 (2/12)	0.35
Whole plasma exchange	7.4 (32/435)	11.1 (2/18)	0.64	25.0 (3/12)	0.059
Fast track	40.5 (176/435)	22.2 (4/18)	0.15	25.0 (3/12)	0.38

MCJ, Mayo Clinic Jacksonville; HCV, hepatitis C virus; UNOS, United Network for Organ Sharing; MELD, model end-stage liver disease; ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiography; CMV, cytomegalovirus.

cytopenia purpura and underwent splenectomy. He was diagnosed with CDC 5.5-year post-transplant secondary to 2 weeks of empiric antibiotic treatment for febrile neutropenia. His symptoms resolved with metronidazole therapy, and he is currently alive and well with a functioning graft.

Case 2. This patient received an LT at age 46 for HCV-related liver cirrhosis (UNOS status 3: MELD score of 14). His post-transplant course was complicated by a biliary stricture, which was managed by stenting. He developed osteomyelitis more than 2 years later with subsequent systemic infection which cultured *Enterococcus faecalis*, *Bacteroides fragilis*, and *Candida tropicalis*. He subsequently underwent left above-knee amputation. Secondary to antibiotic therapy for his systemic infection, CDC was diagnosed 2.6 years post-LT. The enteric infection resolved with metronidazole therapy, and the patient is currently alive and well with a functioning graft.

Case 3. This patient underwent LT in 2001 at age 56 for toxic-mediated liver failure (UNOS status of 2B; MELD score of 15). He experienced no immunologic complication, but had a large hematoma operatively removed from the abdominal wall 6 weeks post-LT. He is the only patient who had both an early and late CD infection. The early CDC was diagnosed 19 days post-LT, which resolved with metronidazole. Following an uncomplicated course, he developed a second CDC infection 3.5 years post-LT after receiving antibiotics for a dental infection. The CDC resolved with metronidazole therapy, and the

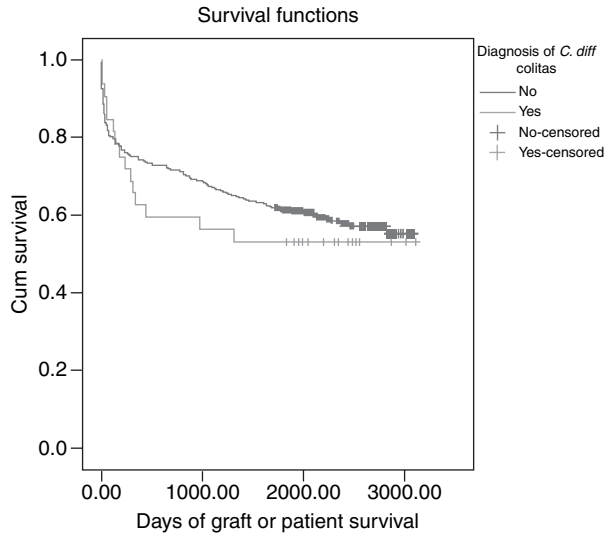

Figure 1 Graft survival according to *Clostridium difficile* infection. Censored refers to either end of follow-up or death.

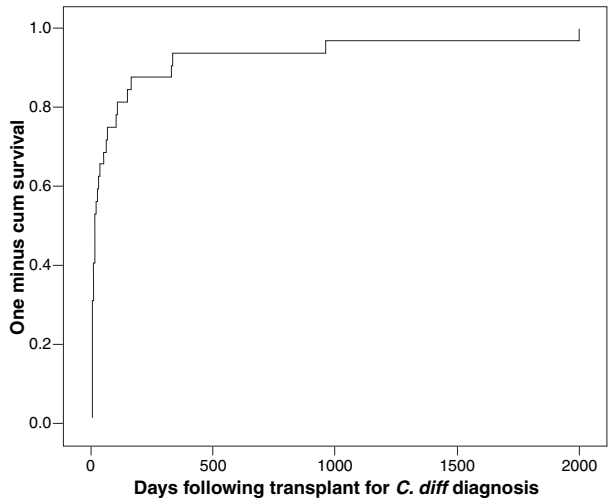

Figure 2 Timeline of days from liver transplantation to onset of first *Clostridium difficile* infection: the vast majority of cases occurred during the first weeks.

patient is currently alive and well with a functioning graft.

Discussion

This report demonstrates that CD remains a significant complication in LT recipients. In this patient population, CDC may be more difficult to diagnose and have a more complicated clinical course than in non-immunocompromized patients [9–16]. It can progress into a life-threatening complication requiring surgical intervention, particularly in lung recipients as also recently reported by Yates *et al.* [15]. Nevertheless, the prognosis is good in most cases if timely diagnosis and treatment are made. In this series, no patient with CDC died. However, CD infection may have contributed to the fatal outcome in some cases, although the difference in survival did not reach statistical significance.

Because of the poor pretransplant condition associated with end stage liver failure, the surgical stress, and the required immunosuppression, LT recipients are at high risk to acquire infectious complications [8]. As a result of repeated courses of antimicrobial therapy pre-, peri- and post-transplant and the prolonged exposure to the hospi-

Table 4. Antibiotic exposure prior to *Clostridium difficile* (CD) colitis.

Antibiotic exposure in addition to prophylaxis	69
Patients with CD colitis during first month	50
Patients with CD colitis after first month	86
Mean (SD) days on antibiotics	15(+/–25) days
On antibiotic therapy at time of transplant	22
Applied antibiotics	
Penicillins	9
Cephalosporins	16
Combination therapy	41
Antifungals	3
No antibiotics	21

Values are provided in percent.

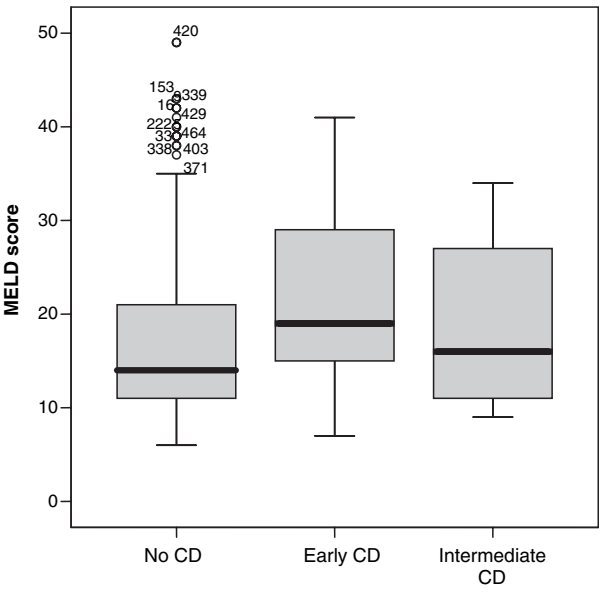

Figure 3 Model end-stage liver disease score according to early/intermediate onset *Clostridium difficile* infection; $P = 0.024$, Kruskal–Wallace test.

tal environment, this patient population is frequently colonized with pathogens in the gastrointestinal tract such as CD. Unfortunately, the efficacy of preoperative screening for CD in an asymptomatic patient is likely to be limited.

Diarrhea is a frequent clinical symptom in transplant patients as the side effect of immunosuppressive therapy [9,25,26]. Diarrhea can be caused by a variety of drugs or enteral nutrition and is associated with antimicrobial therapy. Most importantly, diarrhea originates from bacterial, fungal, viral or protozoal infections [2,4,9].

Eradication of the responsible pathogen from the gastrointestinal tract and removal of toxins is the goal of any therapy. Obtaining a timely, accurate diagnosis is crucial and therefore, rapid detection assays for CD toxins should be used in all solid organ recipients presenting with diarrhea [17,18].

In our series the prevalence of CD infection was the highest during the early post-transplant period, when immunosuppression is the highest. It is well accepted that antibiotic exposure or application of immunosuppressive drugs may disrupt the intestinal flora equilibrium, resulting in an overgrowth of CD [4]. Systemic symptoms associated with CD infection are caused by toxin-induced inflammatory mediators, such as interleukin-8, macrophage-inflammatory protein-2, substance P or tumor necrosis factor-α. These cytokines are released locally within the colon and cause a massive inflammatory reaction, mucosal necrosis and formation of pseudomembranes. Both CD toxins increase vascular permeability because of opening of tight junctions between cells [27].

Nosocomial transmission of CD has been described, especially in the hospital environment, and therefore, preventive strategies such as careful hand washing and disinfection are necessary. Screening for these pathogens and isolation of infected patients may be advisable [17]. All drugs that potentially cause gastrointestinal toxicity must be withdrawn and antibiotics should be discontinued, if possible. First-line treatment today consists of oral metronidazole, which is given for 7–14 days. Stool should be retested if diarrhea does not resolve; oral vancomycin is recommended as a second-line therapy [4,11]. Mortality rates for patients who develop toxic megacolon are extremely high [10,11,28,29]. A significant rise in TAC levels associated with diarrhea caused by CD must be considered and dose reduction is required.

Based upon the findings in this study, CDC has become an important infectious complication associated with LT. Patients who presented with more advanced liver failure, as reflected by the MELD score and inability to bypass the intensive care unit (fast-tracking), appeared to be at higher risk for CDC early post-transplant. In addition, patients who had a complicated postoperative stay, as indicated by vascular and biliary complications requiring intervention or major incisional complication, also had a significantly higher rate of CDC. The purpose of this study was to characterize the other risk factors that may be associated with the later development of CDC following LT. It may be presumed that these patients ultimately received more antibiotics and remained in the intensive care unit for a longer period of time, thus placing them at higher risk. The role of antibiotic use as a risk factor for development of CDC is well established, and the specific utilization of antibiotics was not evaluated in this large cohort. However, it is recognized that all liver transplant patients receive peri-operative antibiotics, with focused use in patients with a diagnosis of bacterial infection or significant risk factors, which would include biliary and other intra-abdominal complications. One may presume that patients with these complications would likely receive more antibiotics. It is important to note that several factors were not shown to be associated with the development of CDC. There was no relationship between any of the patient demographic factors, etiology of their liver disease, CMV mismatch, duration of graft ischemia time, or number of graft risk factors. However, all patients who developed CDC had resolution with appropriate antibiotic treatment and did not exhibit progression to toxic megacolon or need for colectomy.

From the subgroup analysis, it appears that the patients with early CDC and intermediate CDC may represent different patient populations. Patients with a higher MELD score, patients who were not receiving their first graft, patients who had a vascular or biliary complication, and patients who had a systemic infection were more likely to develop CDC early post-transplant. However, the intermediate CDC group did not have a significantly higher MELD score and received significantly fewer marginal grafts. This subgroup was also significantly more likely than patients without CDC to have a vascular or biliary complication requiring intervention or a documented bacterial or systemic infection. On the basis of these analyses, it is the opinion of the authors that patients with more advanced liver disease are at a higher risk early, but the development of a vascular or biliary complication is of great importance in predicting the development of secondary nosocomial complications, such as CDC. This underscores the need for vigilance in evaluating diarrhea and abdominal pain in any patient who has had a postoperative course complicated by biliary or vascular complication within the first year following transplant.

Concerning the high incidence of CD infection in this series, several contributing factors must be considered. Worldwide, the incidence of this infection is reported to be rising [30–33]. For transplant recipients, waiting times are getting longer; therefore, these sicker transplant patients may be at higher risk for this infection. Because of

the more diverse spectrum of infections and higher resistance patterns among many bacteria, more broad-spectrum antibiotics, such as fourth generation cephalosporins, ureidopenicillin/beta-lactamase inhibitor combination and carbapenems, are used. Prophylactic application of trimethoprim/sulfamethoxazol may be an additional factor. This agent has been shown to trigger CD colitis [34,35]. Another important factor may be the intensified immunosuppression that is currently used. Of note, the rejection rates after SOT have dropped from approximately 40% to <20% during the past decade. In this series, the rejection rate was 33% and did not predispose to CD infection, however, patients who required whole plasma exchange had a trend toward increased risk ($P = 0.07$). Some newer immunosuppressive agents may cause severe gastrointestinal side effects and mucosal damage and by that promote or aggravate CD disease. Also outbreaks with other enteric pathogens have been reported [36,37].

Clostridium difficile colitis remains a significant complication after LT. Preventive strategies and guidelines that specifically address this problem in solid organ recipients are urgently needed to prevent this dangerous and costly complication.

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Authorship

JBA, HB, WH: design of the study. JBA, HB, JM, JS, JAM: data collection. JBA, HB, RCD, WH, JN: data analysis. JBA, HB, DK, RH, CH, JN, HC, WH: paper writing.

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