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ORIGINAL ARTICLE



# *Clostridium difficile* infection is associated with graft loss in solid organ transplant recipients

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*Clostridium difficile* infection (CDI) is a leading cause of infectious diarrhea in solid organ transplant recipients (SOT). We aimed to assess incidence, risk factors, and outcome of CDI within the Swiss Transplant Cohort Study (STCS). We performed a case-control study of SOT recipients in the STCS diagnosed with CDI between May 2008 and August 2013. We matched 2 control subjects per case by age at transplantation, sex, and transplanted organ. A multivariable analysis was performed using conditional logistic regression to identify risk factors and evaluate outcome of CDI. Two thousand one hundred fifty-eight SOT recipients, comprising 87 cases of CDI and 174 matched controls were included. The overall CDI rate per 10 000 patient days was 0.47 (95% confidence interval ([CI] 0.38-0.58), with the highest rate in lung (1.48, 95% CI 0.93-2.24). In multivariable analysis, proven infections (hazard ratio [HR] 2.82, 95% CI 1.29-6.19) and antibiotic treatments (HR 4.51, 95% CI 2.03-10.0) during the preceding 3 months were independently associated with the development of CDI. Despite mild clinical presentations, recipients acquiring CDI posttransplantation had an increased risk of graft loss (HR 2.24, 95% CI 1.15-4.37; $P = .02$). These findings may help to improve the management of SOT recipients.

**KEYWORDS**
antibiotic: antibacterial, clinical research/practice, complication: infectious, infection and infectious agents – bacterial: *Clostridium difficile*, infectious disease

**Abbreviations:** CDI, *Clostridium difficile* infection; eCRF, electronic case report form; ESCMID, European Society for Clinical Microbiology and Infectious Diseases; ICU, intensive care unit; PH, proportional hazard; PPI, proton-pump inhibitor; SOT, solid organ transplant recipients; STCS, Swiss Transplant Cohort Study.

A. Cusini and C. Béguelin participated equally in this work.

Swiss Transplant Cohort Study authors are listed in the Appendix.

## 1 | INTRODUCTION

*Clostridium difficile* is a leading cause of infectious diarrhea, with a reported incidence rate of 7 cases per 10 000 patient-bed days in Europe.[1] Solid organ transplant (SOT) recipients are at higher risk for CDI than the general population, due to numerous risk factors including severe underlying diseases, immunosuppression, recent surgery, antibiotic treatment, ganciclovir prophylaxis, gastric acid suppression, and prolonged hospital stay.[2-5] A recently published meta-analysis in SOT recipients reported an overall prevalence of 7.4%.[6] The clinical spectrum of CDI ranges from asymptomatic colonization to fulminant pseudomembranous colitis. Knowledge about the severity of CDI and the impact on graft function in SOT is scarce and contradictory; while some authors have described a worse outcome of CDI in SOT recipients,[2,4,5,7,8] a recent Spanish cohort study and two US studies reported a good prognosis of CDI in SOT recipients.[9-11] These aspects are important, as newer treatment guidelines for CDI stratify according to the clinical severity of disease, emphasizing the reduction of recurrence of CDI.[12,13] Indeed, whereas both oral metronidazole and vancomycin were equally effective for treatment of mild CDI, response rates were superior for vancomycin in patients with severe CDI in a randomized trial.[14] Accordingly, the recently updated guidelines of the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and the latest US American guidelines recommend as first option oral metronidazole for patients with nonsevere and oral vancomycin for severe CDI.[12,13] Fidaxomicin achieved significantly lower rates of recurrence of CDI in two clinical trials.[15,16] Accordingly, the ESCMID guidelines recommend the use of fidaxomicin for patient at risk for recurrent CDI.[13]

Our main objective was to determine the outcome of CDI in SOT recipients. Secondary aims were to describe incidence and clinical severity, and to identify risk factors for CDI within this population.

## 2 | METHODS

### 2.1 | Patients and study design

The Swiss Transplant Cohort Study (STCS) is an observational national cohort, enrolling all SOT recipients followed at 6 Swiss university centers. Details on data definitions and the cohort structure have been published previously.[17,18] For the present study, all SOT recipients prospectively enrolled in the STCS between May 2008 and August 2013 with written informed consent were included. The protocol was approved by the ethics committee of all participating centers. Patient's data were collected in the STCS database at enrollment, 6 and 12 months, and yearly after transplantation on standardized electronic case report forms (eCRFs). Clinical data extracted from the STCS database included demographic data, infections, antibiotic and antiviral prophylaxis, induction and maintenance immunosuppressive treatments, as well as medical comorbidities and surgical complications. To analyze risk factors that are not routinely registered in the STCS database, we performed a nested case-control study, applying an incidence density sampling matching 2 controls to each case by age at transplantation (differences ≤10 years), sex, and type of transplant. Controls were defined as SOT recipients without captured CDI in the SCTS database. The nonoccurrence of CDI in these recipients was double checked in the hospital charts and local laboratory databases. For all cases and controls we extracted additional data including type of anti-infective treatment in the 3 months preceding CDI,[19] intake of a proton-pump inhibitor (PPI), as well as hospital and intensive care unit (ICU) stay from the local laboratory databases and hospital charts, and captured them in dedicated eCRF. For cases, we additionally collected the clinical severity of CDI classified in 3 categories (definition see below): hospital and ICU admission due to CDI, peak white blood cell count, platelet nadir, rise in serum creatinine, and the antibiotic treatment for CDI. There were no clinical variables with missing data included in the multivariable analyses.

### 2.2 | Definitions

To ensure homogeneous assessment of the infectious disease events in the STCS, specifically trained infectious diseases specialists at each center record the occurrence of infectious events using standardized definitions.[17] Proven CDI was defined according to the criteria of the STCS Infectious Diseases Working Group as follows: presence of symptoms (diarrhea) and/or clinical signs (evidence of pathologic findings in endoscopy or radiology) together with pathogen isolation (by culture, or antigen) and *Clostridium difficile*–toxin detection. CDI clinical severity was graded (mild-to-moderate/severe disease/severe and complicated disease) as proposed by the American College of Gastroenterology in 2013, without considering the serum albumin level, since this value was available for only a minority of patients.[12] Mild-to-moderate disease was defined as diarrhea with any additional signs or symptoms not meeting criteria of severe or complicated diarrhea. Severe disease included abdominal tenderness or leukocytosis >15 000 cells/mm$^3$. Severe and complicated disease required one or more of the following criteria: ICU admission for CDI, hypotension, fever >38.5°C, paralytic ileus or significant abdominal distension, mental status changes, leukocytosis >35 G/l or leukopenia <2 G/l, serum lactate levels >2.2 mmol/l, and end organ failure. Clinical recurrence was defined as reappearance of diarrhea after the cessation of therapy, isolation of *C. difficile* or its toxin in stool, and need for retreatment. No distinction between relapse and reinfection was possible, since the *C. difficile* strains were not available for further analysis. Infections in the 3 months before CDI were defined according to the criteria of the STCS Infectious Diseases Working Group. A proven bacterial infection required a pathogen isolated together with clinical signs and/or symptoms and treatment given. A proven viral disease required detection of virus replication with corresponding pathology in biopsy tissues. A viral syndrome consisted of detection of virus replication and non–organ-specific clinical symptoms. For fungal infections, we used the EORTC/MSG Consensus Group definitions.[20] We defined graft loss as follows: recurrence of insulin-dependence following pancreas transplant, dialysis post renal transplantation,

retransplantation post heart, liver, or lung transplantation. All-cause mortality, and mortality assumed to be related to CDI were collected separately.

### 2.3 | Statistical analysis

Patients' baseline characteristics are shown descriptively, separated for patients with and without CDI. CDI-specific information for patients with at least one CDI episode, follow-up, and outcome information are also presented. Cumulative incidence rates for the first CDI episode were calculated by transplant type, treating death before CDI as a competing risk. Based on the case-control study, risk factors for CDI post-SOT were investigated in univariate and multivariable conditional logistics regression models. We determined risk exposure either at time of transplantation, or when adequate 3 months prior to first CDI occurrence within the case-control study. Due to the large number of potential risk factors and the relatively low number of CDI events, we used the conservative Bonferroni method to adjust for the multiple testing problem. The final multivariable model, restrained to generic terms, was based both on the univariate analysis and on clinical relevance of potential risk factors, excluding hospitalization because of an excessively large confident interval. We also investigated the probability for recurrent CDI episodes using logistic regression models without further risk adjustment.

We further performed Cox proportional hazard (PH) models to evaluate the effect of CDI on the occurrence of graft loss and death. We applied noninformative censoring about the outcome (death, graft loss). CDI was considered as a time-dependent risk factor in time-to-event analyses. In addition to CDI, we included baseline and time-dependent risk factors (surgical complications, medical problems, rejection, and relevant infections) in the graft loss analysis (Table 5). The PH assumption was verified by plotting Schoenfeld residuals to visualize the effect over time. When the PH assumption was violated, but the effect strong and without change of the direction, no restrictions were included, and the interpretation was not hampered. All statistical analyses were performed using the statistical software R (version 3.2.0; R Foundation for Statistical Computing, Vienna, Austria).

## 3 | RESULTS

### 3.1 | Population characteristics and incidence of CDI

During the observation period, 2400 SOTs were performed in Switzerland. After exclusion of combined transplants and patients who did not sign the informed consent, we included 2158 patients (1261 kidney, 454 liver, 224 lung, 164 heart, and 55 kidney-pancreas recipients) in the present study. Within the study population, we identified 87 patients (cases) with 102 proven CDI episodes corresponding to a crude incidence of 4.0%. CDI was observed in 35 kidney, 23 liver, 22 lung, and 7 heart recipients (Table 1). The cumulative incidence rate for the first CDI episode per patient at 1 year post SOT was 0.09 for lung, 0.05 for liver, 0.04 for heart, and 0.02 for kidney recipients (Figure 1). The overall CDI rate was 0.47 (95% CI 0.38-0.58) per 10 000 patient-days. Lung recipients had the highest (1.48 95% CI 0.93-2.24) and kidney recipients the lowest rate (0.30, 0.21-0.41). Almost all CDI events occurred in the first year following transplantation. The median time-lag from transplantation to CDI was 70 days (interquartile range [IQR] 21-189). Lung recipients had the shortest time-lag (31 days, IQR 7-129), whereas kidneys had the longest (115 days, IQR 38-308). Acquisition of CDI was nosocomial in 49 patients (56%). CDI diagnosis in the 102 CDI episodes was based on culture and toxin detection (N = 60), antigen and toxin detection (N = 24), and exclusively *Clostridium difficile*-toxin detection by PCR (N = 18).

**TABLE 1** Characteristics of 2158 SOT recipients according to CDI

| | Recipients with CDI | Recipients without CDI |
|---|---|---|
| Number of SOT recipients, N (%) | 87 (4.0) | 2071 (96.0) |
| Age at transplantation, mean (SD) | 52.9 (14.6) | 49.6 (16.1) |
| Male, N (%) | 50 (57.5) | 1329 (64.2) |
| Type of transplantation, N (%) | | |
| Kidney | 35 (40.2) | 1226 (59.2) |
| Living donation | 8 (22.9) | 525 (42.8) |
| Liver | 23 (26.4) | 431 (20.8) |
| Living donation | 2 (8.7) | 26 (6) |
| Lung | 22 (25.3) | 202 (9.8) |
| Heart | 7 (8.0) | 157 (7.6) |
| Kidney – Pancreas | 0 (0.0) | 55 (2.7) |
| Diabetes mellitus, N (%) | 20 (23) | 347 (16.5) |
| Follow-up time (years), median [IQR] | 3.3 [1.9, 4] | 2.3 [1.1, 3.8] |
| Graft loss, N (%) | 14 (16.1) | |
| before CDI | 4 (4.6) | 117 (5.6) |
| after CDI | 10 (11.5) | |
| Death, N (%) | 14 (16.1) | 189 (9.1) |

SOT, solid organ transplant; SD, standard deviation; IQR, interquartile range; CDI, C. difficile infection.

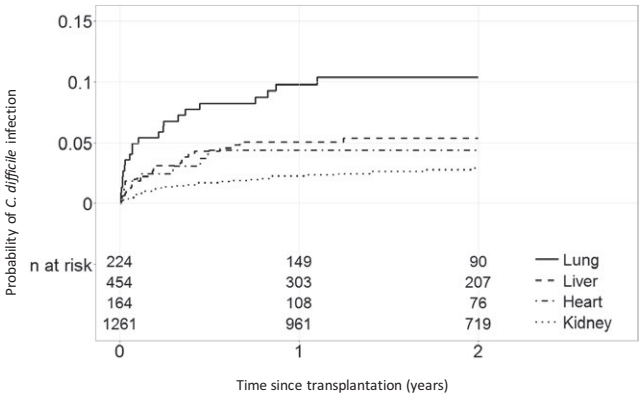


**FIGURE 1** Cumulative incidences of first CDI episodes according to transplant. Shown are the 2-year cumulative incidences of first episodes of *C. difficile* infections in 87 SOT recipients with CDI according to allograft type

**TABLE 2** Risk factors for CDI

| Risk exposure at or within 3 months before CDI | Cases (n = 87) | Controls (n = 174) | Univariate OR [95% CI] adj. *P*-value | Multivariable OR [95% CI] adj. *P*-value |
|---|---|---|---|---|
| Infections, N (%) [Mean number per patient,[a] (min, max)] | 46 (52.9) [1.72, (1,8)] | 35 (20.1) [1.31, (1,4)] | 5.10 [2.70, 9.63] <0.001 | 2.82 [1.29, 6.19] .01 |
| Proven bacterial infections | 36 (41.4) | 26 (14.9) | 4.25 [2.21, 8.16] <0.001 | |
| Proven viral infections & viral syndromes | 13 (14.9) | 8 (4.6) | 3.09 [1.27, 7.49] 0.379 | |
| Proven or probable fungal infections | 7 (8.0) | 4 (2.3) | 3.50 [1.03, 11.96] 1 | |
| Anti-infective prophylaxis, N (%) | 68 (78.2) | 130 (74.7) | 1.37 [0.63, 2.99] 1 | 0.96 [0.36, 2.57] .936 |
| Anti-infective therapy, N (%) | 69 (79.3) | 70 (40.2) | 7.96 [3.72,17.02] <0.001 | |
| Antibiotics, N (%) | 65 (74.7) | 61 (35.1) | 7.41 [3.60, 15.26] <0.001 | 4.51 [2.03, 10.00] <.001 |
| Penicillins, N (%) [duration (days), median (IQR)] | 42 (48.3) [9, (7,14)] | 33 (19) [9, (6,14)] | 4.34 [2.28, 8.28] <0.001 | |
| Cephalosporins, N (%) | 19 (21.8) [7, (5,10)] | 11 (6.3) [10, (5,16)] | 4.47 [1.84, 10.86] 0.028 | |
| Carbapenems, N (%) | 27 (31.0) [9, (6,14)] | 21 (12.1) [9, (9,14)] | 5.80 [2.33, 14.45] 0.005 | |
| Quinolones, N (%) | 24 (27.6) [13, (6,19)] | 14 (8.0) [10, (6,13)] | 4.00 [1.95, 8.22] 0.005 | |
| Glycopeptides, N (%) | 10 (11.5) [15, (7,29)] | 15 (8.6) [9, (6,15)] | 1.26 [0.50, 3.19] 1 | |
| Other antibiotics, N (%) | 19 (21.8) [9, (6,29)] | 15 (8.6) [9, (4,21)] | 2.85 [1.37, 5.92] 0.153 | |
| Antiviral therapy, N (%) | 24 (27.6) | 21 (12.1) | 3.08 [1.51, 6.29] 0.061 | 1.55 [0.65, 3.70] .32 |
| Antifungal therapy, N (%) | 14 (16.1) | 10 (5.7) | 2.97 [1.28, 6.89] 0.341 | 1.12 [0.40, 3.10] .829 |
| Proton pump inhibitor (PPI), N (%) | 84 (96.6) | 155 (89.1) | 3.63 [1.02, 12.92] 1 | 1.25 [0.24, 6.43] 0.788 |
| Hospitalization, N (%) | 85 (97.7) | 125 (71.9) | 41.18 [5.56, 305.10] 0.008 | |
| ICU stay, N (%) | 58 (66.7) | 91 (52.3) | 3.61 [1.57, 8.31] 0.077 | 2.34 [0.83, 6.61] .108 |
| Induction therapy, N (%) | 59 (67.8) | 116 (66.7) | 1.06 [0.59, 1.90] 1 | 0.75 [0.32, 1.72] .491 |
| Immunosuppression at time of CDI | | | | |
| Corticosteroid, N (%) | 76 (87.4) | 146 (83.9) | 1.39 [0.62, 3.14] 1 | |
| Calcineurin inhibitors, N (%) | 82 (94.3) | 154 (88.5) | 2.23 [0.79, 6.33] 1 | |
| Antimetabolites, N (%) | 80 (92.0) | 152 (87.4) | 1.67 [0.69, 4.14] 1 | |
| mTOR-inhibitors, N(%) | 8 (9.2) | 8 (4.6) | 2.11 [0.76, 5.89] 1 | 2.30 [0.63, 8.40] 0.206 |

OR, odds ratio; CDI, *C. difficile* infection; ICU, intensive care unit.
[a]Mean number of infections in patients with at least one infection.

## 3.2 | Risk factors for CDI development

In univariate analysis, infections, especially proven bacterial infections, anti-infective therapy, antibiotic therapy, all ß-lactams and quinolones, and hospitalization in the 3 months preceding the event were associated with the development of CDI (Table 2). In the multivariable analysis, infections (odds ratio [OR] 2.82, 95% CI 1.29-6.19, *P* = .01) and intake of antibiotic treatments (OR 4.51,

**TABLE 3** Clinical variables and course of patients with single and multiple CDI events

| | Single CDI | Multiple CDI | Total | *P*-value[a] |
|---|---|---|---|---|
| Total N | 73 | 14 | 87 | |
| Male, N (%) | 39 (53.4) | 11 (78.6) | 50 (57.5) | .09 |
| Age at transplant, mean (SD) | 52.3 (14.7) | 56.1 (13.8) | 52.9 (14.6) | .37 |
| Transplanted organ, N (%) | | | | .88 |
| Heart | 7 (9.6) | 0 (0) | 7 (8.0) | |
| Kidney | 30 (41.1) | 5 (35.7) | 35 (40.2) | |
| Liver | 19 (26.0) | 4 (28.6) | 23 (26.4) | |
| Lung | 17 (23.3) | 5 (35.7) | 22 (25.3) | |
| Clinical course, N (%) | | | | .76 |
| Mild–moderate | 55 (75.3) | 10 (71.4) | 65 (74.7) | |
| Severe | 16 (21.9) | 3 (21.4) | 19 (21.8) | |
| Severe with complication | 2 (2.7) | 1 (7.1) | 3 (3.4) | |
| WBC (G/l) at first CDI, median [IQR] | 8.9 [5.8, 10.8] | 7.3 [4.2, 19.9] | 8.9 [5.5, 11.3] | .88 |
| out of range 4-10 G/l, N (%) | 34 (46.6) | 9 (64.3) | 43 (49.4) | |
| Treatment of first CDI, N (%) | 71 (97.3) | 14 (100) | 85 (97.7) | .99 |
| Metronidazole | 62 (87.3) | 9 (64.3) | 71 (83.5) | |
| Vancomycin | 8 (11.0) | 2 (14.3) | 10 (11.8) | |
| Metronidazole and vancomycin | 1 (1.4) | 3 (21.4) | 4 (4.7) | |

IQR, interquartile range; SD, standard deviation.
WBC, white blood cells; CDI, *C. difficile* infection.
[a]*P*-values from unadjusted logistic models for probability of second CDI event.

95% CI 2.03-10.00, $P < .001$) during the 3 months preceding the event remained significantly associated with CDI development.

### 3.3 | Treatments

Seventy-one cases (83.5%) were treated with metronidazole for a median duration of 11 days (IQR 10-15), 10 cases (11.8%) were treated with oral vancomycin for a median duration of 11 days (IQR 10-13) and 4 cases (4.7%) received a combined treatment with metronidazole and vancomycin (Table 3). Two cases with a mild course of CDI recovered spontaneously without any treatment. Recurrent CDI was treated by metronidazole in 9, vancomycin in 2 cases, and combination therapy in 3 cases.

### 3.4 | Clinical severity and recurrence

Sixty-five of 87 cases (74.7%) had a mild to moderate, 19 (21.8%) a severe, and 3 (3.4%) a severe complicated course of CDI (Table 3). Seventeen patients (19.5%) required hospital admission and one patient ICU admission for treatment of the CDI. The median white blood cell count at the time of diagnosis was 8.9 G/L (IQR 5.5-11.3), with 43 patients (51%) having a value outside the normal range of 4-10 G/l. Fourteen patients (16.1%) experienced more than a single CDI event, 13 patients had 2 events, and one patient had 3 events. The median time between the first and the second events was 56 days (min 14, max 1127). Ten of 14 recurrent CDI occurred within 8 weeks after the first episode. In univariate logistic regression, we found no significant differences in age, gender, transplant type, clinical severity, and treatment for first CDI between patients with single and recurrent CDI episodes (Table 3).

### 3.5 | Outcome analysis

Two hundred three of 2158 SOT recipients died, including 14 of 87 CDI cases (9.1% vs. 16.1%, Tables 1 and 4). No death was linked directly to CDI. Two deaths occurred within 3 months following CDI. In univariate analysis, patients who died were older (56 vs. 49 years, $P < .001$) and patients transplanted for heart, liver, and lung had a higher risk of death compared to kidney recipients ($P < .001$) (Table 6). This was confirmed in multivariable analysis. CDI increased mortality in univariate analysis (HR 2.31; 95% CI 1.33-3.99, $P = .003$); however, this effect was no longer significant in the multivariable model (HR 1.63, 95% CI 0.94-2.83, $P = .085$) (Table 6).

In univariate analysis for graft loss, baseline recipient characteristics (age, gender, transplant) showed no significant effects ($P > .05$) (Table 6). In contrast, the occurrence of CDI was associated with graft loss (HR 3.72, 95% CI 1.92-7.20, $P < .001$). To confirm

**TABLE 4** Characteristics of 2158 SOT recipients for outcome analysis

| | Recipients with graft loss | Recipients without graft loss |
|---|---|---|
| Number of SOT recipients, N (%) | 131 (6.1) | 2027 (93.9) |
| Baseline characteristics | | |
| Age at transplantation, mean (SD) | 51.5 (14.8) | 49.7 (16.1) |
| Male, N (%) | 85 (63.9) | 1294 (63.8) |
| Type of transplantation, N (%) | | |
| Kidney (incl. Kidney-Pancreas) | 80 (61.1) | 1236 (61) |
| Liver | 26 (19.8) | 428 (21.1) |
| Lung | 13 (9.9) | 211 (10.4) |
| Heart | 12 (9.2) | 152 (7.5) |
| Diabetes mellitus at TX, N (%) | 26 (19.8) | 341 (16.8) |
| Hypertension at TX, N (%) | 72 (55) | 1187 (58.6) |
| Time-dependent characteristics (until death, graft loss or censoring) | | |
| CDI in FUP, N (%) | 10 (7.6) | 73 (3.6) |
| Surgical complications in FUP, N (%) | 29 (22.1) | 127 (6.3) |
| Medical problems in FUP, N (%) | 16 (12.2) | 231 (11.4) |
| Rejections in FUP, N (%) | 64 (48.9) | 715 (35.3) |
| Bacterial/Fungal ID in FUP, N (%) | 46 (35.1) | 593 (29.3) |
| | **Alive** | **Deaths** |
| Number of SOT recipients, N (%) | 1955 (90.6) | 203 (9.4) |
| Baseline characteristics | | |
| Age at transplantation, mean (SD) | 49.11 (16.25) | 56.1 (12.37) |
| Male, N (%) | 1245 (63.7) | 134 (66) |
| Type of transplantation, N (%) | | |
| Kidney (incl. Kidney-Pancreas) | 1248 (63.8) | 68 (33.5) |
| Liver | 395 (20.2) | 59 (29.1) |
| Lung | 176 (9) | 48 (23.6) |
| Heart | 136 (7) | 28 (13.8) |
| Time-dependent characteristics (until death or censoring) | | |
| CDI in FUP, N (%) | 73 (3.7) | 14 (6.9) |

FUP, follow-up; SD, standard deviation; TX, treatment; SOT, solid organ transplant; CDI, *C. difficile* infection.

this effect, we accounted for further time-dependent risk factors potentially associated with graft loss. These included general and transplant-specific surgical complications, medical problems, rejection, and both systemic and transplant specific infections (Table 5). Combined surgical complications, medical problems, relevant infections, as well as both bacterial and fungal infections analyzed alone or in combination, were all significantly associated with graft loss in univariate analyses (Table 6). In contrast, viral infections, including or not CMV, did not increase the risk of graft loss. In a multivariable model, surgical complications (HR 7.22, 95% CI 4.53-11.50, $P < .001$), medical problems (HR 2.35, 95% CI 1.33-4.15, $P = .003$), rejection (HR 7.56, 95% CI 4.70-12.18, $P < .001$), bacterial and fungal infections (HR 3.67, 95% CI 2.22-6.06, $P < .001$), as well as CDI (HR 2.24, 95% CI 1.15-4.37], $P = .02$) remained independent risk factors for graft loss (Table 6).

## 4 | DISCUSSION

We report the results of a nationwide study of 87 cases of *Clostridium difficile* infections among 2158 SOT recipients in the STCS. We identified and confirmed preceding infection and antibiotic use as risk factors for CDI. We showed that despite most episodes being clinically benign, CDI was associated with a 2.2-fold increased risk of graft loss.

The crude incidence of CDI in our prospectively evaluated cohort including >90% of all Swiss SOT recipients was 4% corresponding to an infection rate of 0.47 per 10 000 patient-days. This incidence is low as compared to previous reports, including a meta-analysis comprising 30 studies reporting an overall incidence of 7.4% CDI in SOT recipients.[6] Methodological differences in calculating the occurrence of CDI in SOT might explain such differences. Of note the incidence of CDI in the general Swiss population in hospitals compares to other

**TABLE 5** Definitions and description of time-dependent risk factors

| Risk factor | Definition/composition | Duration |
|---|---|---|
| CDI | Clinical symptoms (diarrhea)<br>+ Clinical signs (pathologic findings by endoscopy or radiology)<br>+ Pathogen isolation (culture, or antigen) and *C. difficile*-toxin | Permanent exposure after occurrence |
| Surgical complications | - Transplant specific vascular complications:<br>  - Liver: arterial or portal vein thrombosis/leak<br>  - Lung: bronchial arterial or venous thrombosis/leak<br>  - Heart: acute ischemia or coronary heart disease<br>  - Kidney: renal artery or venous thrombosis/leak<br>- Transplant specific anastomotic complications:<br>  - Liver: biliary stenosis/leak<br>  - Lung: bronchial stenosis/dehiscence<br>  - Kidney: ureter stenosis/leak<br>- Biopsy related complications<br>- Hemorrhagic complications | Exposure for 6 mo after occurrence |
| Medical problems[b] | - Tumor in transplant (liver/lung)<br>- Arrhythmia or valvulopathy (heart)<br>- Renal failure (not kidney)<br>- Recurrence of initial disease leading to transplant | - Arrhythmia/valvulopathy: exposure for 1 month after occurrence<br>- Tumor, recurrence of initial disease, renal failure: permanent exposure after occurrence |
| Rejection | Biopsy proven and treated rejections | Exposure for 1 month after occurrence |
| Infections | - Bacterial infections:<br>  - proven infections in the transplant<br>  - bacteremia<br>1. Fungal infections:<br>  a fungemia<br>  b all transplants: probable/proven infections due to *Aspergillus* spp./*Zygomycetes* spp.<br>  c Liver: *Candida* spp.<br>  d Lung: *Pneumocystis*<br>1. Proven viral infections in the transplant:<br>  a Liver: hepatitis B/C viruses<br>  b Lung: respiratory viruses[a]<br>  c Kidney: BK polyomavirus<br>Probable/proven CMV disease/CMV syndrome | - *Aspergillus*, *Zygomycetes* hepatitis B and C, BK polyomavirus, and CMV: permanent exposure after occurrence<br>- All other infections: exposure for 1 month after occurrence |

[a]Respiratory viruses: Adenovirus, Influenza, Parainfluenza, Metapneumovirus, Rhinovirus and RSV.
[b]Diabetes and hypertension were analyzed individually.

European countries (4.8 cases vs 4.1 cases per 10 000 patients-days, respectively).[21] In accordance with earlier reports, lung recipients had the highest incidence rate as well as the earliest occurrence after transplantation.[4,5,9,22] Consequently, some authors have suggested to implement metronidazole as prophylaxis early after lung transplantation.[23]

Of major importance is our observation that despite low clinical severity and good therapeutic response, CDI in SOT recipients was associated with an increased risk of graft loss. An impact on graft function has been suggested previously for noninfectious diarrhea,[24] but to our knowledge was never associated with CDI. The therapeutic efficacy of fecal transplantation in the treatment of CDI highlights the importance of CDI as a marker of intestinal dysbiosis.[25,26] It is likely, that whereas the immune dysregulation is the initial insult leading to intestinal dysbiosis in SOT recipients, potentially further worsened by antibiotic treatments,[27] CDI aggravates this intestinal microbial imbalance. Inflammation associated with CDI could also provoke graft rejection via innate immune mechanisms. In such a scenario, dysbiosis or the *C. difficile* itself could enhance helper T cells responses, thereby affecting the graft. However, whether CDI itself or this exacerbated intestinal dysbiosis increases the risk of graft loss is impossible to distinguish at this point. Diarrhea and intestinal dysbiosis could both interfere with adequate absorption and/or the metabolism of immunosuppressive agents, thereby increasing the risk for graft failure. The higher rates of graft loss and death in CDI cases could also be an indirect marker of sicker patients and an unfavorable posttransplantation course, as evidenced by the higher numbers of infections and antibiotic administration in cases compared to controls in this study, rather than a direct effect of the CDI. In line with this hypothesis is the recent report describing a higher number of organ specific complications among SOT recipients.[28] Further prospective studies, analyzing CDI and microbiome changes, as well as immune responses will be needed to decipher the type of association between CDI and graft loss, and its underlying mechanisms.

**TABLE 6** Risk of death and graft loss of SOT recipients with CDI

| | Mortality analysis | | Graft loss analysis | |
|---|---|---|---|---|
| | **Univariate HR [95% CI] *P*-value** | **Multivariable HR [95% CI] *P*-value** | **Univariate HR [95% CI] *P*-value** | **Multivariable HR [95% CI] *P*-value** |
| Baseline characteristics | | | | |
| Recipient's age at SOT (years) | 1.03 [1.02, 1.05] <.001 | 1.04 [1.03, 1.05] <0.001 | 1.01 [1.00, 1.02] 0.16 | |
| Male vs. Female | 1.09 [0.82, 1.46] .55 | | 1.04 [0.73, 1.49] 0.83 | |
| Type of transplant | - [-] <.001[a] | -[-] <0.001[a] | -[-] 0.83[a] | |
| heart vs kidney | 3.90 [2.51, 6.06] <.001 | 4.36 [2.81, 6.79] <0.001 | 1.33 [0.72, 2.44] 0.36 | |
| liver vs kidney | 2.77 [1.95, 3.92] <.001 | 2.81 [1.98, 3.99] <0.001 | 0.99 [0.64, 1.54] 0.97 | |
| lung vs kidney | 4.77 [3.29, 6.90] <.001 | 4.96 [3.41, 7.20] <0.001 | 1.02 [0.57, 1.84] 0.94 | |
| Diabetes | 1.22 [0.80, 1.88] .354 | | | |
| Hypertension | 0.82 [0.58, 1.16] 0.258 | | | |
| Time-dependent risk factors[b] | | | | |
| Clostridium infection (CDI) | 2.31 [1.33, 3.99] 0.003 | 1.63 [0.94, 2.83] 0.085 | 3.72 [1.92, 7.20] <0.001 | 2.24 [1.15, 4.37] 0.02 |
| Surgical complications | | | 11.99 [7.71, 18.64] <0.001 | 7.22 [4.53, 11.50] <0.001 |
| Medical problems | | | 3.54 [2.01, 6.23] <0.001 | 2.35 [1.33, 4.15] 0.003 |
| Rejection | | | 10.58 [6.67, 16.77] <0.001 | 7.56 [4.70, 12.18] <0.001 |
| Relevant infections | | | 3.01 [1.92, 4.71] <0.001 | |
| bacterial/fungal | | | 4.90 [3.01, 8.00] <0.001 | 3.67 [2.22, 6.06] <0.001 |
| bacterial only | | | 2.32 [1.12, 4.81] 0.02 | |
| bacteremia only | | | 8.22 [4.53, 14.92] <0.001 | |
| fungal only | | | 7.05 [2.23, 22.33] <0.001 | |
| fungemia only | | | 7.19 [1.00, 51.89] 0.05 | |
| viral (incl. CMV) | | | 1.58 [0.73, 3.44] 0.25 | |
| viral only | | | 0.75 [0.10, 5.36] 0.77 | |
| CMV only | | | 1.89 [0.82, 4.37] 0.14 | |

[a]Test result from Wald (overall) test without estimates
[b]Risk factors which may be observed multiple times post-SOT over time until death and/or graft loss.
SOT, solid organ transplant; HR, hazard ratio.

All infectious disease events are systematically collected in the STCS. This allowed us to identify previous bacterial, but not viral and fungal, infections that occurred during the preceding 3 months as major risk factors for CDI development. Clearly, we confirm antibiotic treatments, including carbapenems, cephalosporins, quinolones and penicillins, as a significant risk factor (OR ≥ 4) in SOT recipients for

developing CDI. Still, as previously noted, 25% of patients with CDI had not received antibiotics in the last 3 months.[27] In these cases, the development of CDI has been suggested to be linked to immune dysfunction in SOT recipients.[27] As is the case for the Spanish cohort, we found no association with induction therapy or with different immunosuppression regimens.[9]

In our study, 75% of CDI episodes were mild to moderate, 3 cases (3.4%) had a severe complicated course, and no patient required a surgical intervention or died due to CDI. This is a more benign course as compared to previously published data reporting 5.3% complicated cases.[6] The severity of CDI has been shown to depend on the presence of circulating hyper-virulent strains such as ribotype 027.[29] It is likely that the favorable outcome in the present report may be linked to a low prevalence of hypervirulent ribotypes in Switzerland.[30] In correlation with the low clinical severity, most of our cases (83.5%) were treated with metronidazole for a medium duration of 11 days according to current guidelines.[27] CDI recurred in 16.1% cases, less frequently as compared to 19.7% reported in the literature.[6] In the absence of genotyping, differentiation between relapses and reinfections was not possible. However, 59% of the recurrent CDI episodes occurred within 8 weeks after the first, suggesting that these second events were relapses rather than reinfections.

Our study has some limitations. The CDI incidence rate may be underestimated, especially for CDI episodes that could have occurred outside the transplant centers. However, most CDIs occurred early after transplant when SOT recipients were in close contact with the transplant center, and we suppose that only very few events have been missed. The use of different diagnostic tests, including culture, detection of antigens and *Clostridium difficile*-toxin, by enzyme immune assay and PCR might also have affected incidence rates. Despite our study being one of the largest series of CDI in SOT recipients published to date,[6] the number of CDI per transplant remained small and required pooling of specific risk factors in the multivariable analysis.

The strength of our study remains the comprehensive nationwide enrollment of all Swiss SOT recipients, which guaranties highly representative data of the real-life situation in Switzerland.

In conclusion, preceding bacterial infections and antibiotic treatment were risk factors for the development of CDI after SOT. Despite mild clinical presentation, and good clinical responses, SOT recipients with CDI were at increased risk for graft loss. These data support the importance of restrictive antibiotic use in the prevention of CDI and underscores the need for close surveillance of graft function in SOT recipients developing CDI. Further studies are needed to assess the impact of CDI on allograft function.

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## DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

## AUTHOR CONTRIBUTIONS

A.C. was involved in concept, design, data analysis and interpretation, and writing of the article. C.B. contributed to data collection, data analysis and interpretation, and writing of the article. S.S. performed data analysis and statistics. C.V.D. contributed to concept, design, and writing of the article. C.B, K.B., C.G., O.M., P.R.A.M., and N.J.M contributed to data collection and critical revision of the article.

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## APPENDIX

**The members of the Swiss Transplant Cohort Study are**

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