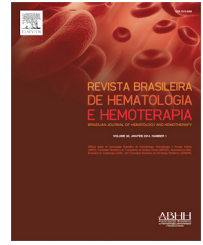




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Original article

Clostridium difficile infection in Chilean patients submitted to hematopoietic stem cell transplantation



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ABSTRACT

Introduction: Patients submitted to hematopoietic stem cell transplantation have an increased risk of *Clostridium difficile* infection and multiple risk factors have been identified. Published reports have indicated an incidence from 9% to 30% of transplant patients however to date there is no information about infection in these patients in Chile.

Methods: A retrospective analysis was performed of patients who developed *C. difficile* infection after hematopoietic stem cell transplantations from 2000 to 2013. Statistical analysis used the Statistical Package for the Social Sciences software.

Results: Two hundred and fifty patients were studied (mean age: 39 years; range: 17–69), with 147 (59%) receiving allogeneic transplants and 103 (41%) receiving autologous transplants. One hundred and ninety-two (77%) patients had diarrhea, with 25 (10%) cases of *C. difficile* infection being confirmed. Twenty infected patients had undergone allogeneic transplants, of which ten had acute lymphoblastic leukemia, three had acute myeloid leukemia and seven had other diseases (myelodysplastic syndrome, chronic myeloid leukemia, severe aplastic anemia). In the autologous transplant group, five patients had *C. difficile* infection; two had multiple myeloma, one had amyloidosis, one had acute myeloid leukemia and one had germinal carcinoma. The overall incidence of *C. difficile* infection was 4% within the first week, 6.4% in the first month and 10% in one year, with no difference in overall survival between infected and non-infected groups (72.0% vs. 67.6%, respectively; p -value = 0.56). Patients infected after allogeneic transplants had a slower time to neutrophil engraftment compared to non-infected patients (17.5 vs. 14.9 days, respectively; p -value = 0.008). In the autologous transplant group there was no significant difference in the neutrophil engraftment time between infected and non-infected patients (12.5 days vs. 11.8 days, respectively; p -value = 0.71). In the allogeneic transplant group, the median time to acute graft-versus-host disease was similar between the two groups (p -value = 0.08), as was the incidence of grades 1–4 acute graft-versus-host disease (40% vs. 48%; p -value > 0.05).

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Conclusion: The incidence of *C. difficile* infection after hematopoietic stem cell transplantation was low, with a significant number of cases occurring shortly after transplantation. Allogeneic transplants had a three-time higher risk of infection compared to autologous transplants, but this was not associated with increased mortality, decreased overall survival or higher risk of acute graft-versus-host disease.

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Introduction

Clostridium difficile (CD) is an anaerobic Gram-positive spore-forming bacillus, responsible for nearly 20% of antibiotic-associated diarrhea in developed countries. The clinical presentation is varied but may include mild to moderate diarrhea, toxic megacolon with perforation or death.¹ *C. difficile* infection (CDI) has an impact on length of hospital stay, costs, morbidity and mortality in adult patients and children,² and it is a major concern for patients who are immunosuppressed, especially after hematopoietic stem cell transplantation (HSCT).^{3–5} The emergence and dissemination of a high virulent strain (NAP-1/027) has changed the epidemiology of CDI, with high mortality and morbidity as well a high risk of recurrence, and potential impact on certain patient subgroups including HSCT recipients.^{6–8}

The days surrounding HSCT are usually accompanied by gastrointestinal (GI) tract disorders mainly due to mucositis, with elevated predisposition to diarrhea and CDI.⁹ Clinical manifestations of CDI in this setting may vary from asymptomatic colonization to fulminant colitis.^{10,11} The high risk of CDI in HSCT patients is due to prolonged hospitalization, chemotherapy-related damage of the enteric mucosal barriers,¹² use of broad spectrum antibiotics (prophylactic and therapeutic),¹³ use of proton-pump inhibitors,^{14,15} and in some cases due to infection prior to the HSCT hospitalization.¹⁶

The incidence of CDI in cancer patients receiving chemotherapy is 3–7%, with approximately 8% developing a more severe disease.¹⁷ There are data that show the rates of CDI in HSCT are nine-fold higher than general patients (24.0 vs. 2.6 per 10,000 patients-days, respectively), and 1.4-fold higher compared with other oncology patients (24.0 vs. 16.8 per 10,000 patients-days, respectively).¹⁸ Large epidemiologic studies show an overall incidence of CDI of 9.2% at one year after transplant.¹⁹ In patients submitted to allogeneic HSCT (allo-HSCT), the incidence varies between 15% and 30%,^{18,20} and has been associated with the development of acute graft-versus-host-disease (aGVHD) of the GI tract and non-relapse mortality.²¹

In Chile there are few reports of this infection in hospitalized patients, mainly due to lack of surveillance in many centers, with only one retrospective study showing an overall incidence of CDI of 5.3 cases/1000 discharges per year, mainly in kidney disease patients,²² with no cancer or HSCT recipient patients included. Since no CDI data in HSCT recipients is available in Chile, the aim of this study is to describe the features of CDI in HSCT recipients compared to published

international data, in order to improve all the measures to prevent this complication in a resource-limited setting.

Methods

Patients

A retrospective analysis was performed of all the patients submitted to HSCT from January 2000 to June 2013 at the Hospital Clínico Universidad Católica, in Santiago, Chile. This institution is a 468-bed teaching hospital with a 24-bed hematology unit, and Hematology and Infectious Diseases teams dedicated to patient care. Data were obtained from the HSCT database and from electronic and paper patient records. The data collected included demographics, diagnosis, type of transplant and conditioning regimen, presence and type of aGVHD in patients with CDI, time to CDI, antibiotics and proton-pump inhibitor prescription prior to and during the HSCT admission. The study was approved by the Ethics Committee and by the Medical Investigation Center Committee at the Hospital.

Transplantation procedure

The patients were divided in two groups, allo-HSCT and auto-HSCT. The conditioning regimens utilized in allo-HSCT were myeloablative, including cyclophosphamide and total body irradiation (TBI), busulfan plus cyclophosphamide, and reduced intensity conditioning (RIC), including fludarabine plus cyclophosphamide, fludarabine plus busulfan and busulfan plus cyclophosphamide. Patients submitted to auto-HSCT were conditioned using Melphalan 140 mg/m², Melphalan 200 mg/m² and ifosfamide, carboplatin and etoposide (ICE). All patients were hospitalized in private rooms with a protective environment (double door rooms with a positive air pressure system and high efficiency particle arresting filters). Prophylaxis for standard infectious diseases using levofloxacin, fluconazole and acyclovir was started on Day –1 and continued until discharge. Omeprazole was used in all patients during hospitalization. Filgrastim was started on Day +5 after transplantation until neutrophil engraftment. After HSCT, all patients were studied for any sign of infection (tachycardia, fever, hemodynamic instability), including at least blood cultures, urine cultures, chest X-ray and stool samples in cases of diarrhea. If febrile neutropenia was documented, empiric antibiotic therapy (ceftazidime, amikacin and vancomycin) was started until the source of infection was identified using specific microbiologic tests. The time to

neutrophil engraftment, defined as the first of three consecutive days of neutrophil count with 0.5×10^9 cells/L was obtained for all patients as was time to platelet engraftment, defined as the first of five consecutive days with counts of 20×10^9 cells/L without transfusion support. The presence and type of aGVHD was diagnosed and graded according to the Glucksberg criteria and the International Bone Marrow Transplant Registry Database (IBMTR) severity Index.^{23,24} All aGVHD was documented for infected patients, if the information was available, with day of diagnosis and its correlation with CDI.

C. difficile infection

The definition of CDI in HSCT patients was an episode of diarrhea with a positive test for *C. difficile* toxin at any time from the start of the conditioning regimen until one year after the transplantation (Day -7 until Day +365). Documentation of CDI was performed using two diagnostic methods available in the institution: Enzyme Immunoassay (EIA) for *C. difficile* toxins A and B from January 2000 to February 2012 and then polymerase chain reaction (PCR) toxin assay (X-pert™ *C. difficile*, GeneXpert Technology, Cepheid Company, Sunnyvale, CA, USA) until the end of the study. Samples were obtained at the bedside and sent directly to the laboratory for immediate analysis. The severity of the disease was graded based on the consensus of the Chilean Society of Infectious Disease and Chilean Society of Gastroenterology. Classification included mild to moderate disease (non-severe CDI), complicated CDI (change in mental status, megacolon, shock, increased levels of lactic acid >2.2 mmol/L) and severe CDI (leukocytes $>20 \times 10^9$ cells/L, hypoalbuminemia <3 g/dL, creatinine >2.2 mg/dL or increased more than 50% of baseline value). Patients with CDI confirmed by a positive toxin test were put in contact isolation, managed with hand hygiene using soap and water, avoiding alcohol-based hand sanitizers and treated with metronidazole (500 mg q8 PO) or vancomycin (125-250 mg q6 PO), depending on the severity of the disease. Antibiotic therapy for CDI was continued until resolving the diarrheal episode and the neutropenia. In mild to moderate cases, metronidazole was preferred over vancomycin as the first-line treatment. In cases of intolerance to metronidazole, oral vancomycin was used as the second line therapy. In patients with more symptoms attributable to infection, but not sufficient to fulfill more severe criteria, or in cases of recurrent CDI, vancomycin was preferred as first-line treatment. Data for known risk factors was documented for patients with CDI, including the use of prophylactic and therapeutic antibiotics in the previous three months, use of proton-pump inhibitors (omeprazole), use of enteral or parenteral nutrition and previous episodes of CDI.

Statistical analysis

The statistical analysis was performed using the IBM Statistical Package for the Social Sciences software version 21 (IBM Company, Armonk, New York, USA). Variables are reported as numbers and percentages. Survival curves were obtained using the Kaplan-Meier method and were compared with the Log-Rank Test, the t test was used for independent samples

Table 1 – Patient characteristics.

	Allo-HSCT	Auto-HSCT	Total
n	147	103	250
Age - years (range)	36 (17-61)	45 (18-69)	39 (17-69)
Gender (male - %)	60%	64%	60%
Conditioning - n (%)			
MA	132 (90%)	102 (99%)	234 (94%)
RIC	15 (10%)	1 (1%)	16 (6%)
Main indication for transplant - n (%)			
AML	48 (33%)	8 (8%)	56 (22%)
ALL	47 (32%)	1 (1%)	48 (19%)
MM	0 (0%)	36 (35%)	36 (14%)
HL/NHL	6 (4%)	43 (42%)	49 (20%)
Others	43 (29%)	18 (17%)	61 (24%)

Allo-HSCT: allogeneic hematopoietic stem cell transplantation; Auto-HSCT: autologous hematopoietic stem cell transplantation; MA: myeloablative; RIC: reduced intensity conditioning; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MM: multiple myeloma; HL: Hodgkin's lymphoma; NHL: non-Hodgkin lymphoma; CDI: *Clostridium difficile* infection.

and Fisher exact test for analysis between groups in terms of CDI and type of transplantation and conditioning.

Results

Patient characteristics

During the study period, 250 HSCT were performed, including 147 allo-HSCT and 103 auto-HSCT. Patient characteristics are summarized in Table 1. The median age of patients submitted to allo-HSCT was 36 years (range: 17-61 years) and in the auto-HSCT group, it was 45 years (range: 18-69). The main indications for HSCT were acute leukemia ($n = 104$; 42%), lymphoma ($n = 49$; 20%) and multiple myeloma ($n = 36$; 14%); 93% ($n = 234$) of patients received myeloablative conditioning (MA).

Of the 250 patients studied, diarrhea was seen and documented in 192 (77%) cases, of which 25 (10%) had CDI, 20 (80%) in the allo-HSCT group (Table 2). All of the infected patients had mild to moderate disease, and no deaths were attributable to this infection. In the CDI group, only three (12%) patients received vancomycin as first-line therapy, with the majority of the patients (22 patients; 88%) receiving metronidazole, with a median time to treatment of two weeks (range: 7-24 days). Apart from one patient who received intravenous vancomycin for the empiric management of febrile neutropenia, all patients were managed with oral metronidazole or vancomycin until resolution of the infection. No side effects of CDI therapy were reported.

Incidence of *C. difficile* infection

The overall incidences of CDI in the first week, month and year after transplantation were 4.0%, 6.4% and 10%, respectively, with a median time from HSCT to CDI of 20 days. For patients submitted to allo-HSCT, the cumulative incidences of CDI in the first week, month and year after the procedure were 5.4%, 8.8% and 13.4%, respectively (median time to infection

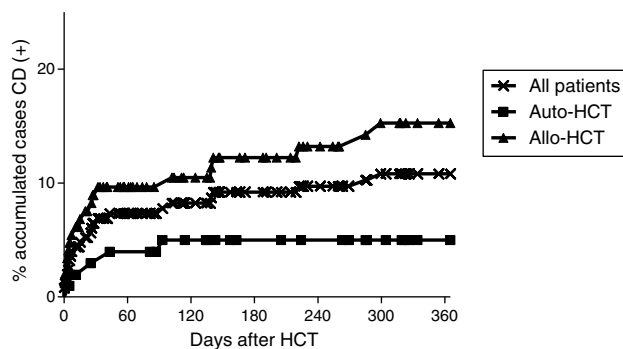
Table 2 – Clostridium difficile infection in each subgroup of patients.

	Allo-HSCT (n = 147)	Auto-HSCT (n = 103)	Total (n = 250)
Total infection	20 (14)	5 (5)	25 (10)
AML	3 (15)	1 (20)	4 (16)
ALL	10 (50)	0	10 (40)
MM	–	2 (40)	2 (8)
HL/NHL	0	0	0
CML	4 (20)	0	4 (16)
MDS	1 (5)	0	1 (4)
SAA	2 (10)	0	2 (8)
Others	0	2 (40)	2 (8)

Data presented as n (%).

Allo-HSCT: allogeneic hematopoietic stem cell transplantation; Auto-HSCT: autologous hematopoietic stem cell transplantation; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; MM: multiple myeloma; HL: Hodgkin's lymphoma; NHL: non-Hodgkin lymphoma; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; SAA: severe aplastic anemia.

Others included one case of amyloidosis and one case of germinal cancer.

**Figure 1 – One-year cumulative incidence of Clostridium difficile infection in patients submitted to hematopoietic stem cell transplantation.**

18 days). After auto-HSCT, the cumulative incidences of CDI in the first week, month and year were 1.9%, 2.9% and 4.9%, respectively (median time to infection 21 days) (Figure 1). Forty percent of cases occurred before Day +7 after transplantation, and the remaining cases occurred during the follow-up, until Day +365. Twenty-two patients with CDI took oral metronidazole (500 mg t.i.d.). Three patients received oral vancomycin as first-line therapy. Only four patients in the CDI group (16%) had a second episode of CDI after the transplant (three between Day +40 and Day +60 and one after Day +200), all of whom were successfully treated with oral metronidazole. There were no differences in incidence of other infections in patients with and without CDI.

Risk factors for *C. difficile* infection

In the risk factors analysis, all infected patients had received proton-pump inhibitors (omeprazole 20 mg per day) and all received standard infectious disease prophylaxis (levofloxacin, sulfamethoxazole trimethoprim, acyclovir and

Table 3 – Multivariate analysis of risk factors for Clostridium difficile infection.

Characteristic	HR	95% CI	p-Value
Age	1.164	0.354–15.96	0.372
Gender	1.051	0.99–1.10	0.873
Type of transplant	0.775	0.44–28.90	0.951
Disease status at time of transplantation (CR vs. no CR)	0.399	0.07–2.57	0.182
Proton-pump inhibitors	NS	NS	NS
Type of conditioning regimen	1.21	0.45–2.36	0.92
Non-infectious diarrhea	0.987	0.01–10.23	0.87

CR: complete remission; HR: hazard ratio; CI: confidence interval.

fluconazole). Eighty-one percent of the infected patients (n=21) used antibiotics other than prophylaxis during the three months before transplantation, mainly for febrile neutropenia, and pulmonary, urinary and central venous catheter-related infections, and other related infections. No information about antibiotic use before the transplant was available in the majority of patients without CDI. The antibiotics used primarily as treatment of these conditions were cephalosporins, amikacin, vancomycin and carbapenems. None of the infected patients received enteral or parenteral nutrition before the CDI. Patients transplanted for acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) represented 40% (10/25) and 16% (4/25) of CDI cases, respectively (p-value=0.041). Multivariate analysis found no correlation between CDI with the analyzed risk factors, including age, gender, disease status at transplantation, type of transplant, type of conditioning regimen, presence of non-infectious diarrhea and the use of proton-pump inhibitors (Table 3).

C. difficile infection and acute graft-versus-host disease

Incidence of grades 1–4 aGVHD was similar between the two groups; 40% of patients with CDI and 48% of patients without CDI. Grades 3–4 aGVHD was also similar between the two groups (22%). The median time until this complication was 35 days in infected patients and 30 days in non-infected patients (p-value=0.08).

C. difficile infection and time to neutrophil and platelet engraftment

In patients submitted to allo-HSCT, the median time to neutrophil engraftment of patients with CDI was longer than in non-infected patients (17.5 days vs. 14.9 days, respectively; p-value=0.008). On the other hand, in patients submitted to auto-HSCT, the median time to neutrophil engraftment in patients with CDI versus non-infected patients was 12.5 and 11.8 days, respectively (p-value=0.71). In terms of time to platelet engraftment, there were no significant differences between the study groups. The median time to platelet engraftment was 32.2 days in patients with CDI vs. 25.7 days in non-infected patients in the allo-HSCT group (p-value=0.48) and 31.5 days in patients with CDI vs. 28.1 days in non-infected patients of the auto-HSCT group (p-value=0.91) (Table 4).

Table 4 – Median time to neutrophil and platelet engraftment.

Engraftment	Allo-HSCT			Auto-HSCT		
	CDI (+)	CDI (–)	p-Value	CDI (+)	CDI (–)	p-Value
Neutrophils (days)	17.5	14.9	0.008	12.5	11.8	0.71
Platelets (days)	32.2	25.7	0.48	31.5	28.1	0.91

Allo-HSCT: allogeneic hematopoietic stem cell transplantation; Auto-HSCT: autologous hematopoietic stem cell transplantation; CDI (+): *Clostridium difficile* infection; CDI (–): no *Clostridium difficile* infection.

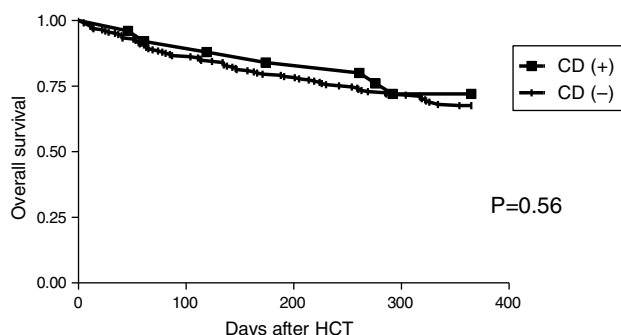


Figure 2 – Overall survival at one year in patients submitted to hematopoietic stem cell transplantation with and without *Clostridium difficile* infection.

C. *difficile* infection and survival

There was no statistically significant difference in overall survival (OS) between the group with CDI and non-infected patients at one year after the transplant (72% vs. 67.6%, respectively; p -value = 0.56) (Figure 2).

In the auto-HSCT group, 1-year OS was 77% for patients with CDI and 60% for patients without CDI (p -value = 0.34). In the allo-HSCT group, 1-year OS was 75% for patients with CDI and 61% for patients without CDI (p -value = 0.19).

Discussion

This is the first study reporting CDI outcomes in a HSCT population in Chile; the overall incidence was similar to previously published data.^{16–18} There was a higher incidence of CDI in patients submitted to allo-HSCT compared to patients submitted to auto-HSCT, a difference that was seen in other studies.^{19,25–27} No correlation was found between CDI and increased frequency of other infections during the transplantation period, and this study was not able to demonstrate any correlation between the severity of the CDI and known risk factors, which may be due to the ubiquity of these traditional measures in patients submitted to HSCT.^{28,29}

In other studies, some clinical characteristics are associated with increased risk of CDI, such as the disease status at transplantation, age, gender or conditioning regimen.^{28,30} However, this study did not demonstrate any correlation with these characteristics, possibly due to the small sample size of patients with CDI. All the patients with CDI received at least two types of antibiotic families (cephalosporins,

broad-spectrum penicillin, carbapenems, vancomycin and quinolones) within the three months before the procedure, but with no further risk for the development of the disease in comparison to non-infected patients.

In terms of the transplantation procedure, CDI only occurred in patients submitted to myeloablative conditioning regimens, a finding that was concordant with studies that show that these type of regimens are more toxic, increasing endothelial damage, affecting the integrity of the intestinal mucosa³¹ and increasing immunodeficiency.^{12,17} The use of TBI is, according to some authors, the main risk factor for the development of CDI during the first week of conditioning.²⁷ However, in this study, the conditioning regimens did not affect the CDI rate, probably because only 16 out of 240 patients received RIC (p -value = 0.24).

The peak of CDI in the current study occurred during the first week after transplantation (40% of cases was diagnosed before Day +7), which could be related to the intestinal toxicity peak after TBI. After the initial peak of CDI in the first week after transplant, the cumulative incidence remained stable over time especially after auto-HSCT. Interestingly it seems that the incidence of CDI after allo-HSCT continues to increase almost ten months after transplantation and then stabilizes, probably due to longer immunosuppression in these patients secondary to GVHD and delayed immune reconstitution compared to auto-HSCT.

Previous studies have shown that CDI is a risk factor for the development of GVHD, with a variable incidence of CDI with aGVHD in different studies, and increased frequency and severity of episodes and new onset of GI aGVHD associated with CDI until Day 180 after transplant.^{19,21,32–34} This study, however, did not demonstrate this association, with no difference in terms of aGVHD and CDI, possibly because the number of infected patients with aGVHD was small.

This study also found that CDI was statistically more frequent in patients transplanted for ALL compared to AML, a finding that could be explained by the fact that the underlying disease requires more intensive and longer chemotherapy, prior to the HSCT. Other possible explanations for this finding are the greater immunodeficiency in ALL patients, higher rates of antibiotic use for frequent febrile neutropenia episodes, with more hospitalizations for complications related to therapy or other unknown factors.

The fact that all cases of CDI were mild to moderate is probably related to the high index of suspicion, which favors the early establishment of appropriate therapeutic measures, thus avoiding the most severe forms of CDI. The good response to metronidazole observed in this study was compatible with

previous evidence that this antibiotic is the best alternative as first-line therapy for mild to moderate CDI.^{35,36} In this study, only three patients received vancomycin as a first-line therapy, two patients receiving oral vancomycin for GI intolerance of metronidazole and one patient due to concomitant treatment of febrile neutropenia, with a good response in all cases.

In this patient population, there were no deaths associated with CDI, a result that is compatible with previous reports.²⁷ While other studies have found increased mortality after CDI in allo-HSCT patients, this has been associated with the diagnosis of the more severe forms of the disease.^{19,34,37} Only four patients (16%) had a second episode of CDI, all of which occurred after Day +40, were of mild to moderate severity and responded to metronidazole, suggesting that this therapy remains efficient even in relapsed cases of CDI.²⁷ The recurrence rate of CDI observed in this population was lower than that observed in previous reports that described a mean incidence of recurrence of 20% in patients treated with metronidazole or vancomycin.³⁸ No specific reasons for this low relapse rate of CDI can be concluded from this study but possibly it could be related to factors such as the different diet and intestinal flora compared to developed countries, lower antibiotic use in the post-transplant setting, less restrictive diet after transplant, specific characteristics of the *Clostridium* species or local practices in the hospital. On the other hand, CDI infection resulted in a statistically significant delay in neutrophil engraftment in allo-HSCT patients. This observation is concordant with the literature that shows that certain infections can complicate the procedure and cause engraftment failure, but until now, the main infectious diseases associated with engraftment failure are viral infections³⁹ such as CMV,^{40–42} and human herpes virus 6 (HHV-6).^{43,44} To date, there is no available data indicating an association between engraftment delay or failure and diarrhea associated with CDI, especially of mild to moderate severity.

The presence of CDI did not have an impact in OS during the first year after transplant, a finding that is in agreement with other reports found in the literature.^{27,34} Possible explanations for this finding include the rapid identification of the infection due to the high index of suspicion, absence of severe cases, good sensitivity of *C. difficile* strains to metronidazole and vancomycin, good performance status at the moment of the infection and other factors that were not elucidated in the present study.

The main limitations of this study include its retrospective nature and the lack of data on some patients especially those without CDI, but despite this, the results compare favorably to what has been previously published from developed countries.

Conclusions

These findings emphasize the importance of measures to prevent CDI, including early suspicion, the appropriate use of antibiotics, use of contact precautions and treatment according to the severity of the disease. It is important to assess the role of the environment and cleaning protocols of the unit, as the highest incidence of CDI occurs within the first week after transplantation.

Conflicts of interest

The authors declare no conflicts of interest.

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