Bacterial substitution of coagulase-negative staphylococci for streptococci on the oral mucosa after hematopoietic cell transplantation

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Abstract

***Purpose*:**

Coagulase-negative staphylococci (CoNS) are frequently isolated from blood cultures of hematopoietic cell transplantation (HCT) patients. Generally, use of central venous catheters is recognized as a significant risk factor for CoNS infection, while the impact of CoNS infection from oral ulcerative mucositis, which occurs frequently in HCT, may be underestimated. Here, we examined bacteria on the buccal mucosa after HCT.

***Methods*:**

Sixty-one patients were examined for bacteria on the buccal mucosa routinely once a week from 1 week before to 3 weeks after allogeneic HCT. Subjects were divided into groups with short and long periods of antibiotic use, and differences in bacterial substitution were evaluated. The relationships between type of HCT (conventional HCT or RIST) and bacterial substitution were also evaluated.

***Results*:**

The changes in detection frequencies of CoNS and α-streptococci from before to 3 weeks after HCT were significant ($P<0.05$, χ^2 test): 14.5%–53.3% and 92.7%–53.1%, respectively. Significant bacterial substitution of CoNS for streptococci was observed in the long-term antibiotic use group ($P<0.05$, χ^2 test), but also occurred in cases with short-term or

no antibiotic use. No relationships between type of HCT (conventional HCT or RIST) were observed.

***Conclusion*:**

Bacterial substitution of CoNS for streptococci occurred frequently on the buccal mucosa after HCT. In addition to antibiotic use, environmental factors may be involved in bacterial substitution. It is important to consider the presence of oral mucositis in CoNS infection after HCT.

Key words: Bacterial substitution, oral mucosa, hematopoietic cell transplantation, coagulase-negative *Staphylococcus*, bacteremia

Introduction

Allogeneic hematopoietic cell transplantation (HCT) is independently associated with increased risk of breakthrough bacteremia, which is an independent predictor of fatal outcome [1]. Coagulase-negative *Staphylococcus* (CoNS) species are the most frequently isolated bacteria from blood cultures of febrile neutropenic patients [2,3]. These organisms are skin commensals, regarded as opportunist pathogens, particularly in association with the use of intravenous catheters [2]. On the other hand, when infections from intravenous catheters are excluded, it is sometimes difficult to identify the focus of infection because of widespread distribution of CoNS over the body surface and their relatively large total population size [2].

Their detection may indicate not only bacteremia but also contamination of the sample [2,4]. Determining the significance of positive blood cultures can be difficult [5–7]. Generally, the presence of clinical signs of bacteremia, identification of a possible source of infection, and/or repeated isolation of the same organism are considered suggestive of bacteremia [2,8–10].

Allogeneic HCT often causes severe oral mucositis [11,12], and we suspected this would be one of the major infection routes. Streptococci are common bacteria in the flora of the oral mucosa. On the other hand, in the HCT period, the bacterial flora on the oral mucosa may change because many antibiotics are used to treat the various infections that occur under

neutropenic conditions. Thus, we speculated that oral mucositis may explain the CoNS infections that excluded contamination.

Previously, we reported the trajectory of oral mucositis in 127 patients undergoing conventional HCT (*n*=63) and reduced-intensity HCT (RIST; *n*=64) patients [12]. In our previous study on the trajectory of oral mucositis, we performed surveillance culture of oral buccal mucosa. Here, we performed a retrospective analysis of the bacteria on the oral mucosa after HCT in 63 of 127 patients enrolled in our previous study and for whom surveillance culture data were available.

Patients and Methods

Subjects

A total of 63 patients (M: 42, F: 21, 43.0±14.3 y) who underwent allogeneic HCT at Okayama University Hospital were included in the present study. The diseases of these patients are shown in Table 1. Conventional allogeneic HCT and RIST were administered to 34 (M: 22, F: 12, 34.7±11.7 y) and 29 (M: 20, F: 9, 52.8±20.3 y) patients, respectively. The times required for engraftment (neutrophil counts >500/μL, continued for 3 days) in each group were 17.5±5.0 and 18.4±9.6 days, respectively.

Previously, we reported the trajectory of oral mucositis in 127 patients undergoing conventional HCT (*n*=63) and RIST (*n*=64). All of the subjects in the present study were included in our previous study. Thus, the trajectory of oral mucositis could be referred to from our previous report [12].

Patients had undergone bacterial examination of the buccal mucosa for oral infection control before and after HCT, and the results were analyzed retrospectively.

Informed consent for examination of oral bacteria was obtained from each subject, and the Ethical Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences approved this study (No. 263).

Conditioning regimens of HCT

Conventional allogeneic HCT:

Most patients with related or unrelated donors received total body irradiation (TBI) at a dose of 12 Gy in six fractions followed by cyclophosphamide (CY) at a dose of 60 mg/kg once daily for 2 days. Alternatively, patients received a combination of busulphan (BU) (4 mg/kg/day ×4 days) and CY (60 mg/kg/day ×2 days). Patients with unrelated cord blood donors were treated with TBI at 12 Gy, CY (60 mg/kg/day ×2 days) and cytarabine (Ara-C; 6 g/m^2/day ×2 days).

RIST:

Patients ineligible for the conventional myeloablative preparative regimen were treated with fludarabine (Flu)-based therapy. Patients with peripheral blood stem cell donors received Flu (25 mg/m^2/day ×5 days) and CY (30 mg/kg/day ×2 days). Patients with bone marrow donors received Flu (30 mg/m^2/day ×6 days) and BU (4 mg/kg/day ×2 days). Patients with unrelated cord blood donors received Flu (30 mg/m^2/day ×6 days), CY (25 mg/kg/day ×2 days) and TBI (2 Gy).

General infection control

All patients were isolated in a room equipped with a laminar airflow system and received trimethoprim-sulfamethoxazole as prophylaxis against *Pneumocystis carinii*. Fluoroquinolone for prophylaxis against bacterial infection and fluconazole for prophylaxis against fungal infection were administered orally. Prophylaxis against herpes virus infection with acyclovir was also given. Neutropenic fever was managed according to the guidelines of

Hughes *et al*. [13]. Briefly, empirical antibiotic therapy was administered promptly in all neutropenic patients at the onset of fever and in afebrile patients who were neutropenic but who had signs or symptoms compatible with infection. A fourth-generation cephalosporin (*e.g.*, cefepime) or carbapenem (*e.g.*, meropenem) was administered intravenously as empirical antibiotic therapy. G-CSF (lenograstim 5 μg/kg/day or filgrastim 300 $\mu g/m^2$) was given intravenously for 60 min starting on day 1 or 5, and was continued until the absolute neutrophil count exceeded 500/μL.

Oral management

All subjects were referred to dentists, and necessary dental treatment was completed before HCT. All subjects received instruction regarding self-management of oral hygiene; tooth brushing after every meal and before going to bed, and oral rinsing with normal saline solution every 3 h during the day was also indicated. In cases in which the patient's condition was poor, nurses, dental hygienists, and dentists performed these oral managements. No antimicrobial rinses were used.

Identification of microorganisms from the oral mucosa

Microbial identification was performed four times (day –7 to –1; day 0 to +6; day +7 to +13; day +14 to +20) for each patient (a total of 252 examinations in 63 patients).

Microbial samples were obtained about 2 h after breakfast by swabbing from the whole surface of the buccal mucosa regardless of whether mucositis was observed. Culture

and identification of microorganisms were performed at the Central Clinical Laboratory of Okayama University Hospital. Microbial samples from mucosal swabs were plated onto brain heart infusion agar plates, and cultured under aerobic conditions at 37°C. Identification of colonies thus obtained was performed using rapid ID 32 STREP API®, rapid ID 32 E API®, or ID 32 GN API® identification kits (Japan bioMérieux, Tokyo, Japan) according to the manufacturer's instructions.

Analysis of relationships between antibiotic use and bacterial substitution

Antibiotic use in all patients (n=63) was examined, and the subjects were divided into two groups: one with short-term use of antibiotics (n=30, antibiotic use for 0–15 days, mean±SD 9.9±4.7 days), and another with long-term use of antibiotics (n=33, antibiotic use for 16–28 days, mean±SD 20.3±3.7 days). The relationships between antibiotic use and bacterial substitution were analyzed by comparing bacterial substitution between these two groups.

Statistical analysis

Differences in detection frequencies of bacteria were compared by χ^2 test using the statistical software StatFlex (Artech, Osaka, Japan). In all analyses, P<0.05 was taken to indicate significance.

Results

Bacterial substitution of oral mucosa after HCT

The bacteria identified on the oral mucosa before and after HCT are shown in Table 2. The detection frequencies of CoNS increased significantly, while those of bacteria that comprise the normal flora, such as *Streptococcus* species, decreased significantly with time after HCT. Significant changes were observed in detection frequencies of CoNS and α-*Streptococcus* species from before to 3 weeks after HCT (14.5% to 53.3% and 92.7% to 53.1%, respectively; both $P<0.05$, χ^2 test). The percentage of subjects carrying bacterial components of the normal flora was significantly decreased, while that of subjects carrying bacteria not normally associated with the normal flora was significantly increased (Fig. 1).

Antibiotic use and bacterial substitution

Detection frequencies of α-*Streptococcus* species and CoNS in the short-term and long-term antibiotic use groups are shown in Fig. 2. Both groups showed significant decreases in α-*Streptococcus* species and increases in CoNS, as shown in Fig. 2 ($*P<0.05$, χ^2 test). According to the course of time, these changes got clear in long-term antibiotic use group, and significant differences between the short-term and long-term antibiotic use groups are observed 7-21 days after HCT ($*P<0.05$, χ^2 test).

Type of HCT (conventional HCT or RIST) and bacterial substitution

Detection frequencies of α-*Streptococcus* species and CoNS in the conventional

HCT and RIST groups are shown in Fig. 3. There were no significant differences in detection frequencies of α-*Streptococcus* species and CoNS between conventional HCT and RIST, as shown in Fig. 3, while significant bacterial substitution was observed in both HCT types, as shown in Table 2 (*P<0.05, χ^2 test).

Discussion

Bacterial substitution of mainly CoNS for streptococci occurs frequently on the oral mucosa after HCT. Approximately 75%–85% of bone marrow transplantation recipients experience mucositis, and in some studies oral mucositis was the most common and most debilitating side effect reported [14,15], which is consistent with our recent findings [12]. As described in the Introduction, the trajectory of oral mucositis of the subjects in the present study could be referred to from our previous report, as the present patient population was a subset of those enrolled in our previous study [12]. The injured mucosa with mucositis may be involved in not only bacteremia caused by bacteria composing the normal flora, such as *Streptococcus* species, but also by those not associated with the normal flora, especially CoNS.

We expected bacterial substitution on the oral mucosa to occur because many different types and amounts of antibiotics are often used after HCT. The results indicated tendencies for bacterial substitution to occur in the high antibiotic use group, while no significant differences were observed in HCT types (conventional HCT and RIST). On the other hand, the differences in bacterial substitution between high and low antibiotic use groups were below our expectations. Interestingly, bacterial substitution was also observed even in two subjects with no antibiotic use. Anticancer treatment regimens, such as chemotherapy and/or irradiation, damage the salivary glands and cause hyposalivation

[16,17]. We reported that HCT also leads to the development of oral dryness [18]. Not only antibiotic use but also oral dryness may contribute to bacterial substitution. Almost all CoNS detected were estimated to be *Staphylococcus epidermidis*, although many of the reports on bacterial identification from our clinical laboratory were limited to the genus level both because of examination capacity and clinical necessity. The conditions of the oral mucosa with hyposalivation may be similar to those of the skin, which would promote such bacterial substitution of CoNS, which are the dominant bacteria composing the skin flora, as streptococci usually comprise the oral mucosal flora.

The widespread use of indwelling central venous catheters (CVC) is recognized as a significant risk factor for infections due to CoNS [19]. Management of CVC in CoNS bacteremia has been discussed previously [20]. On the other hand, DNA analysis demonstrated that the first episode of bacteremia due to *S. epidermidis* originated in the mouth, whereas a second episode involving another strain of *S. epidermidis* was derived from the CVC, recently [21]. Our findings support the suggestion that oral mucositis is a strong predictor of oral streptococci bacteremia and that CoNS bacteremia is clearly associated with mucositis [22]. We agree with the opinion that the impact of damage to the oral mucosal barrier is greatly underestimated [22, 23]. Therefore, we would like to emphasize the importance of infection control on the oral mucosa as intensive oral care in patients undergoing HCT, although the effective use of antibiotics is also important. In the present

study, we also examined the relationship with detection of CoNS in blood culture. However, the detection of CoNS from blood culture was limited to 5 times throughout the present study, and therefore the number of cases was too small to determine the relationships between CoNS detected from the oral environment and blood. Although the reasons for the lower detection rates of CoNS from blood culture are not yet clear, these observations may have been due to our intensive oral care.

In conclusion, bacterial substitution of mainly CoNS for streptococci occurred frequently on the oral buccal mucosa after HCT.

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The authors declare there were no conflicts of interest in this study.

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Table

Table 1. Diseases of patients

Diseases	Type of HCT		Total
	conventional	RIST	
Acute myelogenous leukemia	7	7	14
Acute lymphoblastic leukemia	7	3	10
Chronic myelogenous leukemia	1	8	9
Malignant lymphoma	12	7	19
Aplastic anemia	1		1
Myelodysplastic syndromes	6		6
Myelofibrosis		1	1
Adrenoleukodystrophy		1	1
Paroxysmal nocturnal hemoglobinuria		1	1
Metastatic Renal Cell Carcinoma		1	1
Total	34	29	63

Table 2. Bacteria identified from the oral mucosa and detection frequencies before and after HCT

Bacteria	Detection number and caries frequency			
	Day –7 to –1 (*n*=57)	Day 0 to +6 (*n*=61)	Day +8 to +13 (*n*=60)	Day +15 to +20 (*n*=47)
Bacterial components of the normal flora				
α-*Streptococcus* spp.	54 (91.2%)	54 (88.5%)	34 (56.7%)*	35 (74.5%)*
γ-*Streptococcus* spp.	11 (19.3%)	11 (18.0%)	14 (23.3%)	5 (10.6%)
Neisseria spp.	35 (61.4%)	35 (57.4%)	15 (25.0%)*	8 (17.0%)*
Stomatococcus spp.	15 (26.3%)	14 (23.0%)	16 (26.7%)	14 (29.8%)
Corynebacterium spp.	0 (0.0%)	2 (3.3%)	2 (3.3%)	1 (2.1%)
Bacteria not usually found in the normal flora				
Coagulase-negative *Staphylococcus* spp.	8 (14.0%)	15 (24.6%)	33 (55.0%)*	24 (51.1%)*
Enterococcus spp.	2 (3.5%)	1 (1.6%)	3 (5.0%)	5 (10.6%)
Pseudomonas aeruginosa	1 (1.8%)	1 (1.6%)	1 (1.7%)	1 (2.1%)
Staphylococcus aureus	1 (1.8%)	2 (3.2%)	1 (1.7%)	0 (0.0%)
Bacillus spp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.1%)
Stenotrophomonas maltophilia	0 (0.0%)	0 (0.0%)	1 (1.7%)	1 (2.1%)
Haemophilus influenzae	0 (0.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)
Enterobacter cloacae	2 (3.5%)	1 (1.6%)	0 (0.0%)	0 (0.0%)

(*$P<0.05$, χ^2 test: compared with day –7 to –1)

The bacteria identified on the oral mucosa are shown. Bacterial identification was performed four times (first: day –7 to –1; second: day 0 to +6; third: day +7 to +13, fourth: day +14 to +20) for each patient (total of 252 times for 63 patients). No samples were obtained in 27 of the 252 examinations because of the patients' conditions at these time points. Findings from 225 examinations are shown.

Figure legends

Fig. 1. Bacterial substitution on the oral mucosa after the HCT period.

Carriers of bacterial components of the normal flora (A) and bacteria not usually found in the normal flora (B) according to the days relative to HCT are shown. *P<0.05, χ^2 test: compared with Day –7 to –1.

The percentage of subjects carrying bacterial components of the normal flora was significantly decreased, while that of subjects carrying bacteria not normally associated with the normal flora was significantly increased

Fig. 2. Influence of antibiotic use on bacterial substitution on the oral mucosa before and after HCT.

Detection frequencies of (A): α-*Streptococcus* species and (B): CoNS in the long- and short-term antibiotic use groups, according to the number of days relative to HCT are shown. *P<0.05, χ^2 test.

Both group showed significant decreases in α-*Streptococcus* species and increases in CoNS

According to the course of time, these changes got clear in long-term antibiotic use group, and significant differences between the short-term and long-term antibiotic use groups are observed 7-21 days after HCT (*P<0.05, χ^2 test).

Fig. 3. Influence of HCT type (conventional HCT or RIST) on bacterial substitution on the oral mucosa before and after HCT.

Detection frequencies of (A): α-*Streptococcus* species and (B): CoNS in the conventional HCT and RIST groups, according to the number of days relative to HCT are shown. *P<0.05, χ^2 test.

There were no significant differences in detection frequencies of α-*Streptococcus* species and CoNS between conventional HCT and RIST, while significant bacterial substitution was observed in both HCT types, as shown in other table and figures.

Fig. 1

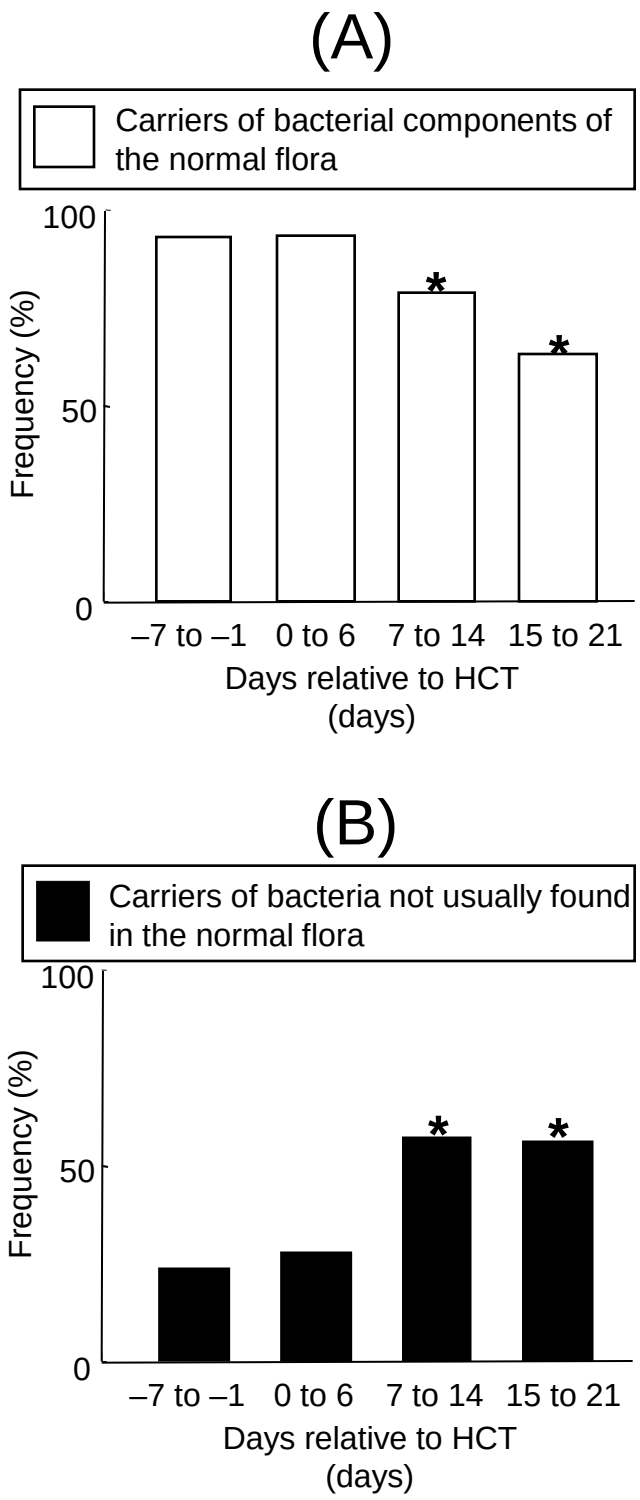

(A)
Carriers of bacterial components of the normal flora
100
50
0
Frequency (%)
*
*
–7 to –1
0 to 6
7 to 14
15 to 21
Days relative to HCT
(days)
(B)
Carriers of bacteria not usually found in the normal flora
100
50
0
Frequency (%)
*
*
–7 to –1
0 to 6
7 to 14
15 to 21
Days relative to HCT
(days)

Fig. 2

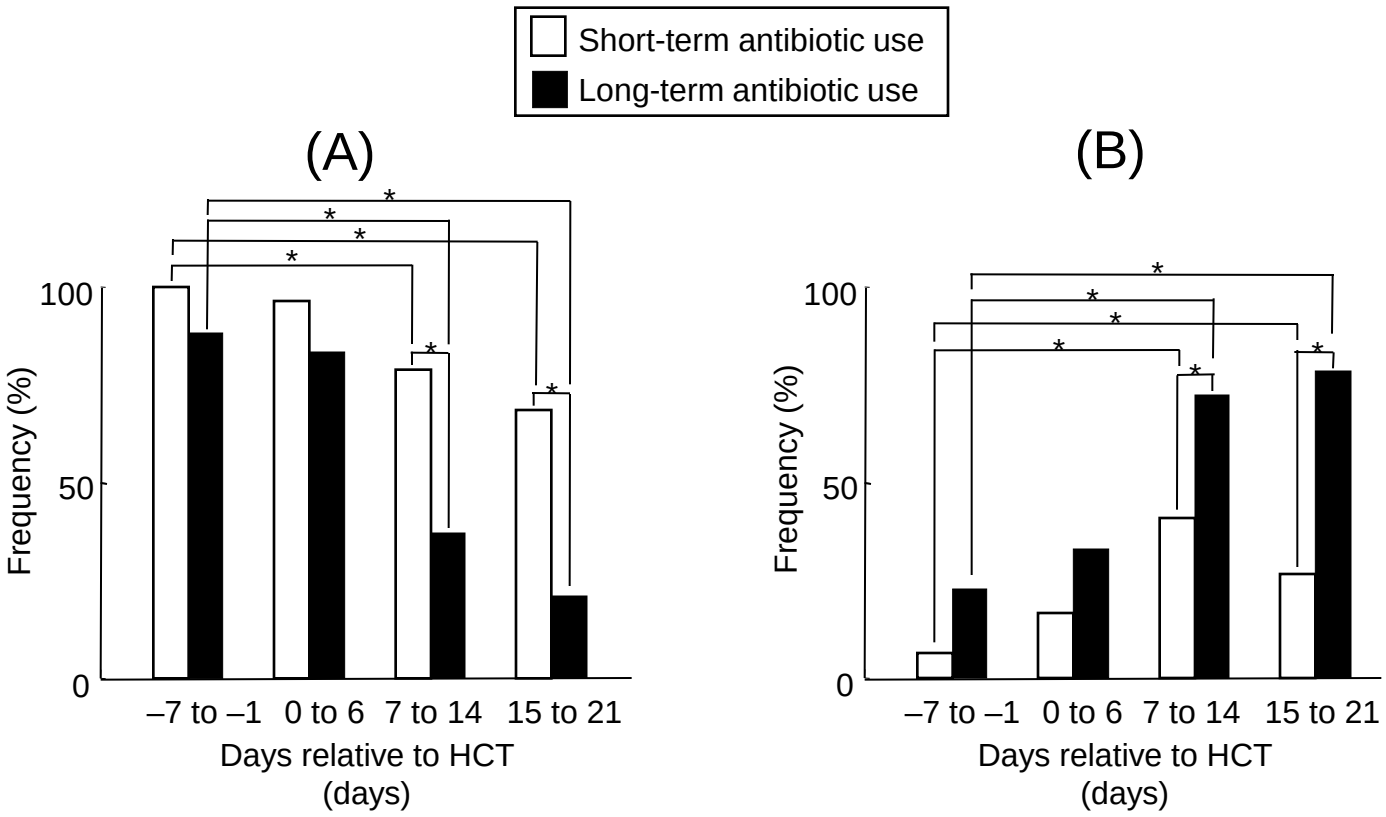
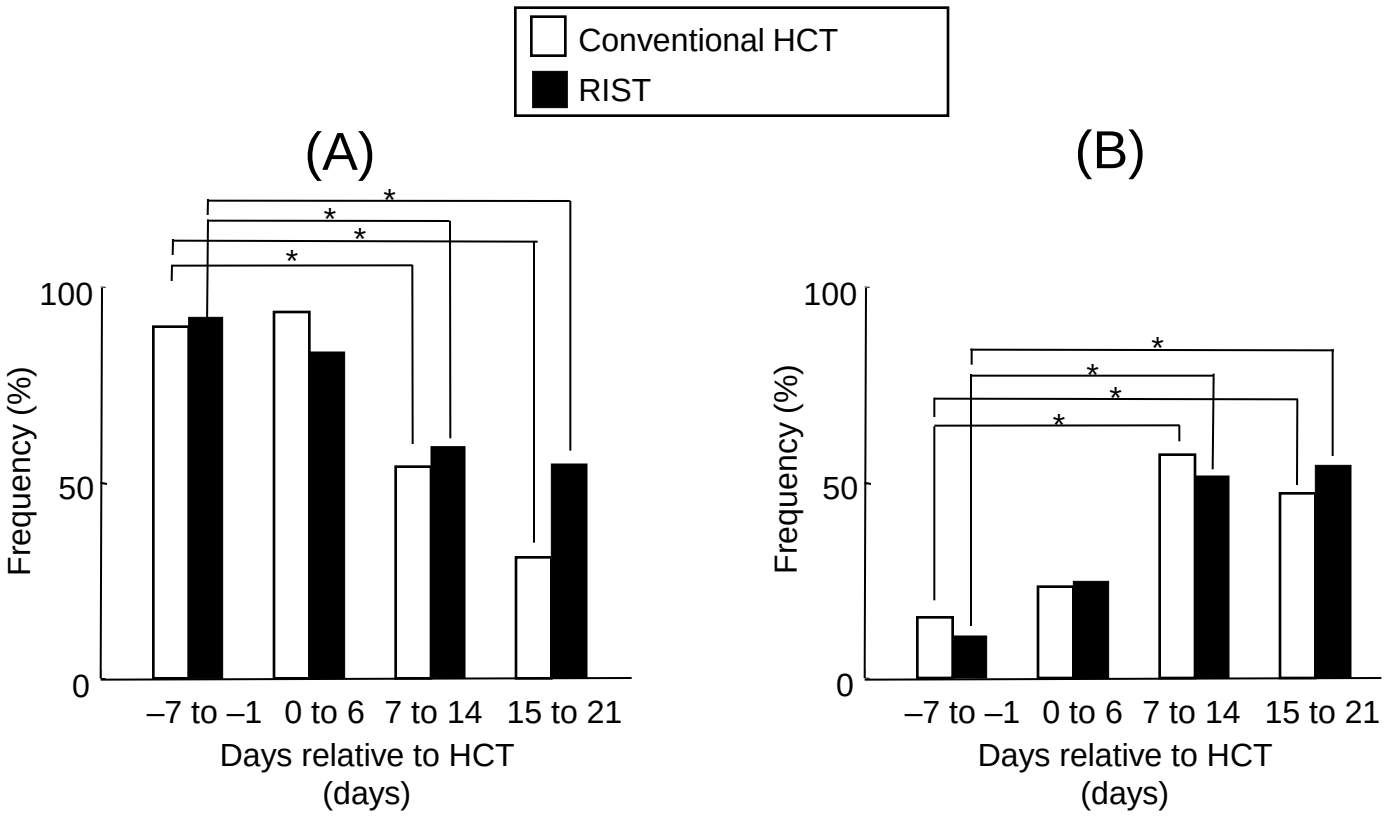

Short-term antibiotic use
Long-term antibiotic use
(A)
(B)
Frequency (%)
100
50
0
–7 to –1
0 to 6
7 to 14
15 to 21
Days relative to HCT
(days)
*

Fig. 3


Conventional HCT
RIST
(A)
(B)
Frequency (%)
100
50
0
*
–7 to –1
0 to 6
7 to 14
15 to 21
Days relative to HCT
(days)