Clinical Utility of Oral Management in Allogeneic Hematopoietic Stem Cell Transplantation Recipients: Microbiological Evidence Based on Molecular Analysis of Oral Bacteria

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Abstract

Purpose: This study aimed to clarify the clinical utility of oral management to prevent bloodstream infections by oral bacteria microbiologically in patients undergoing allogeneic hematopoietic stem cell transplantation (ASCT).

Methods: Ten consecutive patients with hematological malignancies undergoing ASCT were enrolled in this study. We implemented dental treatments before transplantation, if required, and carried out oral hygiene instructions and oral management every other day after transplantation. Molecular analysis of bacterial DNA for seven oral species using a polymerase chain reaction (PCR) assay was performed for oral samples and peripheral blood once a week for 3 weeks after transplantation.

Results: Periodontitis was found in all 10 patients (mild grade in 3 and middle grade in 7) for whom basic periodontal therapy was conducted. Necessary dental procedures, including tooth extraction of the tooth were performed in 5 patients. After transplantation, oral mucositis occurred in 10 patients (grade 1 in 3, grade 2 in 2, and grade 3 in 5) for whom oral hygiene instruction and oral care were continued every other day. PCR-identified three to six bacterial species in oral samples from nine patients, but none in peripheral blood from any patient during the observation period.

Conclusions: Oral management prevented blood stream infections by oral bacteria in ASCT recipients despite the existence of periodontitis or oral mucositis. Its utility was confirmed by microbiological evidence based on molecular data.

Introduction

Allogeneic hematopoietic stem cell transplantation (ASCT) is an important treatment strategy for hematological malignancies. However, bloodstream infections are a major cause of morbidity and mortality in patients undergoing ASCT [1,2]. Accordingly, successful management of these infectious complications is crucial to improve the clinical outcomes of ASCT patients. Bloodstream infections commonly develop early after ASCT. This is because of neutropenia and mucosal injury due to the adverse effects of preconditioning with total body irradiation (TBI) and cytotoxic drugs such as high-dose cyclophosphamide and/or cytarabine, and post-transplant methotrexate (MTX). Therefore, the oral mucosa is damaged quite frequently, and the oral cavity becomes an important port of entry for systemic infections. Periodontal diseases are the most common oral diseases found in ASCT recipients. In recent years, oral management has been increasingly reported to be useful in reducing oral mucosal lesions in such patients [3-8]. However, there have been no reports on the effectiveness of oral management in the prevention of systemic infection by orally derived pathogens based on microbiological evidence.

In this study, we prospectively applied a polymerase chain reaction (PCR) analysis of bacterial DNA to identify sensitively causative pathogens in the oral samples and peripheral blood (PB) from patients with hematological malignancies undergoing ASCT. Furthermore, we evaluated the clinical utility of our oral management to reduce the occurrence of bloodstream infection by oral bacteria based on microbiological evidence.

Materials And Methods

The clinical characteristics of the patients are summarized in **Table 1**. Ten consecutive patients (median age, 40 years; range, 20-58 years) with hematological malignancies undergoing ASCT at the Mie University Hospital between January and December 2014 were enrolled. The underlying diseases were acute myeloid leukemia in four patients, acute lymphoblastic leukemia in three, chronic myeloid leukemia, adult T-cell leukemia/lymphoma, and non-Hodgkin lymphoma in one each. This prospective study was approved by the Human Research Ethics Committee of Mie University Hospital (Tsu, Japan) and was conducted in accordance with the current version of the Helsinki Declaration. Written informed consent was obtained from all patients recruited. Nine patients received a TBI-based conventional preconditioning regimen, and one received reduced-intensity preparative regimens. Sources of hematopoietic stem cells were bone marrow in 3 patients, peripheral blood in 3, and cord blood in 4. Graft-versus-host disease (GVHD) prophylaxis was either cyclosporine or tacrolimus, which was combined with a short course of MTX. All patients were housed in rooms equipped with a high-efficiency particulate air system, and standard precautions were used for all patient care. They received antibacterial prophylaxis with fluoroquinolone and antifungal prophylaxis with mold-active azoles. Prophylaxis against herpes virus infection was also provided with acyclovir. For febrile neutropenia, which was defined as a single axillar temperature $\geq 37.5^{\circ}C$ in patients with PB neutrophil counts $\leq 500/\mu l$, empirical antibacterial therapy was initiated with broad-spectrum β-lactam antibiotics [cefepime, meropenem (MEPM), doripenem (DRPM), or piperacillin/tazobactam (PIPC/TA)] with or without arbekacin (ABK), and when a fever refractory to these antibiotics persisted, a glycopeptide [vancomycin (VCM) or teicoplanin] was added. For febrile episodes, blood cultures were performed as necessary. Blood samples were cultured in an automated system (BacT/Alert 3D, BioMerieux, France).

Oral assessment

Dental status was assessed, and treatment of the tooth that was the source of infection and basic periodontal therapy were performed before the start of ASCT in all patients. The severity of periodontitis was defined as follows: a proving pocket depth $\leq$3 mm as mild grade, 4-5 mm as middle grade, and $\geq$6 mm as severe grade. The severity of oral mucositis was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events Scales (NCI-CTCAE Ver4.0).

PCR analysis of oral bacteria

PCR-based molecular detection of bacterial DNA was designed to detect representative seven species of oral pathogenic bacteria: *Porphyromonas gingivalis*, *Treponema denticola,Tannerella forsythia*, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Streptococcus* species, and *Lactobacillus*. Limited oral bacteria are recognized as the ‘red complex’ and the ‘orange complex’, which are highly correlated with aggressive periodontitis. *P. gingivalis*, *T. denticola*, and *T. forsythia* constitute the red complex, and *P. intermedia and F. nucleatum* are grouped into the orange complex. *Streptococcus* species and *Lactobacillus* spp. are major pathogens related to dental caries. These seven species have also been reported to cause bloodstream infections. Oral samples were obtained from

the subgingival plaque or gingival crevicular fluid in the periodontal pocket of the lower right first molar. We set the transplant date to day 0, and oral samples were collected on days -21, -1, 7, 14, and 21, and PB was taken on days 7, 14, and 21 (**Figure 1**). Bacterial DNA was extracted and purified from the samples using a Mora Extract (Kyokuto Seiyaku Co., Ltd., Tokyo, Japan). The primers used for each bacterial species are shown in **Table 2**. PCR reactions were carried out in a DNA thermal cycler (GeneAmp PCR System 9600; Applied Biosystems, Foster City, CA, USA) with preliminary denaturation at 95°C for 10 min, followed by 45 cycles of amplification consisting of denaturation at 94°C, primer annealing at 62°C, and elongation at 72°C, each lasting for 1 min. The positive control for bacterial PCR was 100 CFU/mL of *Staphylococcus aureus* ATCC29213. Nuclease-free water was used as a negative control. When these positive and negative controls did not work as expected, we considered the assay invalid. We validated the bacterial PCR using 18 strains of nine bacteria (data not shown). The sensitivity of detecting bacteria was 50 CFU/mL.

Results

Oral management

Periodontitis was found in all 10 patients prior to transplantation: mild grade in three patients and middle grade in seven patients (**Table 3**). Basic periodontal treatment (scaling and root planing) was administered to these patients. The dental procedure was conducted and completed in five patients (cases 2, 4, 7, 9, and 10) before the start of the transplantation. In case 2, the extraction fossa of the lower left 6^{th} tooth was cleaned because of healing failure after extraction by another clinic. In case 4, the lower left 8^{th} tooth was extracted. In case 7, the lower left 6th tooth was extracted, and caries of the lower left 7^{th} tooth was treated. In case 9, the radicular cyst of the upper left first tooth was removed, and root canal treatment of the upper left 6^{th} tooth was performed. In case 10, caries treatment was performed on the lower left 7^{th} tooth. Oral mucositis occurred in all patients after transplantation: grade 1 in three patients, grade 2 in two patients, and grade 3 in five patients (**Table 3**). Oral mucositis worsened during days 710 in all the patients. Oral hygiene instruction and oral care (brushing and dental cleaning) were performed for these patients every other day (**Figure 1**). By day 21, acute GVHD and GVHD-related oral lesions were not observed in any patient.

PCR detection of oral species and the results of blood culture

PCR results of blood and oral samples are shown in **Table 4**. Three to six species were detected in the oral samples from nine patients. *Streptococcus* species were identified in the nine patients. In case 4, no positive oral samples were observed during the observation, although the patient had mild grade periodontitis and grade 3 oral mucositis. In contrast, no species was identified in PB from any of the 10 patients at any point of evaluation. On the other hand, blood culture was positive in three patients (cases 5, 6, and 8) (**Table 1**). *Roseomonas gilardii* was identified on day 8 in case 5, *Enterococcus faecalis* on days 7 and 8 in case 6, and *Stenotrophomonas maltophilia* on day 12 in case 8. Although these bacteria were isolated during febrile neutropenia, they disappeared with empirical antibacterial therapies.

Discussion

Bloodstream infections due to orally derived microorganisms have been increasingly reported in ASCT recipients, highlighting the importance of oral health care to reduce such systemic infections [1,2,6]. Although several studies have indicated that intensive oral management before and during ASCT reduces the incidence of severe oral mucositis, little data are available on the relationship between oral management and the occurrence of bloodstream infections caused by oral pathogens [9-13]. To the best of our knowledge, this is the first report to describe the clinical efficacy of oral management in ASCT recipients by microbiological evidence based on the molecular analysis of oral bacteria.

In this study, we focused on seven species of oral bacteria and analyzed the presence of DNA in oral samples and PB using a PCR assay. As blood culture studies have drawbacks in terms of sensitivity and specificity, we applied molecular analysis to confirm sensitively and accurately whether these oral bacteria translocate into the bloodstream. Oral assessment revealed periodontitis before ASCT and oral mucositis during ASCT. Three to six species were identified from oral samples in nine of 10 patients. Importantly, *Streptococcus* species were detected in all nine patients. Fluoroquinolone prophylaxis, which mainly targets gram-negative rods derived from the gut, has been associated with an increased incidence of systemic infections due to gram-positive bacteria, and oral *Streptococci* are among the most common pathogens. Fluoroquinolone was used as a prophylactic medication in all patients. Previously, we reported the frequent occurrence of bacteremia due to *Streptococcus* species in ASCT recipients who had not received oral management [14,22]. Periodontal infections appear to affect the oral tissues, and bacteria may translocate into the bloodstream via the ulcerated crevice, pocket epithelium, and adjacent gingival microcirculation. However, seven species, including *Streptococcus* species, were not identified from the PB in all patients, even in patients with medium grade periodontitis or grade 3 oral mucositis. These results seem to indicate that oral management successfully prevented oral pathogens from causing bloodstream infections despite the existence of periodontitis or oral mucositis in ASCT recipients. On the other hand, blood culture revealed bacteremia with *Roseomonas gilardii*, *Enterococcus faecalis*, and *Stenotrophomonas maltophilia* in three patients (cases 5, 6, and 8). However, these microorganisms were not considered likely to have originated from the oral cavity. Although blood culture was performed during the febrile period in all patients, only these three species of bacteria were identified, and no bacteria usually related to oral origin were isolated in our study [15]. These results may also support the utility of oral management.

However, our study had three limitations. First, the number of cases examined was small. Larger-scale studies are required to validate our observations in more detail. The second issue concerns the selection of the bacterial species evaluated. There is a possibility that orally derived bacteria, except the seven species, could have caused bloodstream infections during the febrile period in patients other than cases 5, 6, and 8, who showed positive blood culture results. More comprehensive molecular analyses using broad-range primers for bacteria are needed. The third is the standardization of oral management [16-18]. In this study, we conducted and completed various dental procedures for patients needed before the start of ASCT, and performed oral hygiene instruction and

oral care every other day after ASCT. This method is not standardized and is often left at individual discretion. These three issues have not been addressed in this research, and this is a future problem.

In conclusion, our study showed that ASCT could be performed safely without causing any bloodstream infections from orally derived bacteria, even in patients with periodontitis and oral mucositis. In the future, with a rapidly aging population, there could be an increase in the number of patients with periodontitis requiring ASCT [19-21]. This research provides the rationale for future studies to confirm the clinical importance of oral management in reducing systemic infections due to oral microorganisms in ASCT recipients with oral lesions.

Declarations

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Tables

Table1 Clinical characteristics of patients

Case	Age& SEX	Disease	Method	preconditioning Treatment	Immunosuppressant	Prophylactic Medication	Fever(37.5℃～)	Empirical antibacterial therapy	Leukocyte Count (After 1 Month)	Blood Culture
1	37/M	AML	BMT	TBI(12Gy),CA,CY	Tacrolimus,sMTX	LVFX	day9-11,17-19	DRPM+ABK→ CFPM+TEIC→ DRPM+TEIC→ PIPC/TA+TEIC→ DRPM+ABK	0.01	-
2	58/F	ALL	PBSCT	TBI(12Gy),VP-16,CY	Ciclosporin,sMTX	CPFX	day5-7	CFPM+ABK→ CFPM+TEIC	0.05	-
3	41/F	AML	BMT	TBI(12Gy),CY	Tacrolimus,sMTX	CPFX	day-5-11	DRPM+TEIC	0.05	-
4	40/F	AML	CBT	TBI(12Gy),CY,CA	Ciclosporin,sMTX	CPFX	day3-20	CFPM→MEPM+TEIC	0.01	-
5	37/M	CML	PBSCT	TBI(12Gy),VP-16,CY	Ciclosporin,sMTX	CPFX	day3-13,15-19	MEPM+TEIC	0.03	Roseomonas giardii(day8)
6	45/M	ALL	CBT	TBI(12Gy),CA,CY	Ciclosporin,sMTX	CPFX	day6-9	CFPM+ABK→ DRPM+TEIC	0.03	Enterococcus faecalis(day7-8)
7	40/M	ATLL	CBT	TBI(4Gy),Flu,Mel	Tacrolimus,sMTX	CPFX	day10-11,19-22,26-28	CFPM→MEPM+TEIC	0.01	-
8	28/M	T-ALL	CBT	TBI(12Gy),CA,CY	Ciclosporin,sMTX	CPFX	day2,3,5,7,8,11-16	MEPM+TEIC→ MEPM+TEIC+LVFX	0.01	Stenotrophomonas maltophilia(day12)
9	20/M	ML	BMT	TBI(12Gy),CY	Tacrolimus,sMTX	CPFX	day5-12,14-19,21-24	MEPM+TEIC	0.02	-
10	40/F	AML	PBSCT	Flu,Bu	Ciclosporin,sMTX	LVFX	day9	DRPM+TEIC→TEIC	0.06	-

M Male, F Female, ATLL adult T-cell leukemia/lymphoma, AML acute myeloid leukemia, ALL acute lymphoblastic leukemia, LBL lymphoblastic lymphoma, MDS myelodys- plastic syndrome, CML Chronic Myelogenous Leukemia, T-ALL T-cell acute lymphoblastic leukemia , ML Malignant Lymphoma, BMT bone marrow engraftment, PBSCT Peripheral Blood Stem Cell Transplantation, ALCL anaplastic large cell lymphoma, CR complete remission, PR patial remission, TBI total body irradiation, CY cyclophosphamide, flu fludarabine, CA cytarabine, Mel melphalan, sMTX short methotrexate, LVFX levofloxacin, CPFX ciprofloxacin. MEPM meropenem, ABK arbekacin, CFPM cefepime, DRPM doripenem, TEIC teicoplanin, AMK Amikacin, PIPC/TA piperacillin/tazobactam.

Table2 Primers for PCR of 7 species

Bacterium	Primer
Porphyromonas gingivalis	F-GCG AGA GCC TGA ACC AGC CA
	R-ACT CGT ATC GCC CGT TAT TCC CGT A
Treponema denticola	F-TAA GGG ACA GCT TGC TCA CCC CTA
	R-CAC CCA CGC GTT ACT CAC CAG TC
Tannerella forsythia	F-GCG TAT GTA ACC TGC CCG CA
	R-TGC TTC AGT GTC AGT TAT ACC T
Prevotella intermedia	F-GCG TGC AGA TTG ACG GCC CTA T
	R-GGC ACA CGT GCC CGC TTT ACT
Fusobacterium nucleatum	F-CGC CCG TCA CAC GAG A
	R-ACA CCC TCG GAA CAT CCC TCC TTA C
Streptococcus species	F-TCG GAT CGT AAA GCT CTG TTG TA
	R-GGA CAA CGC TCG GGA CCT AC
Streptococcus Lactbacillus	F-CGA AAC TTT CTT ACA CCG AAT GC
	R-GTC CAT TGT GGA AGA TTC CC

Table3 Periodontal disease and oral mucosal condition

Case	Periodontal disease	Oral mucositis
1	Mild	G1
2	Middle	G3
3	Middle	G3
4	Mild	G3
5	Middle	G3
6	Middle	G1
7	Middle	G1
8	Middle	G3
9	Middle	G2
10	Mild	G2

Table4 PCR results of blood and oral samples

		Porphyromonas gingivalis		Treponema denticola		Tannerella forsythia		Prevotella intermedia		Fusobacterium nucleatum		Streptococcus species		Streptococ Lactbacillu	
Case	Day	Blood	Dental samples	Blood	Dental samples	Blood	Dental samples	Blood	Dental samples	Blood	Dental samples	Blood	Dental samples	Blood	D sa
1	Day-21	/	+	/	-	/	+	/	+	/	-	/	+	/	-
	Day-1	/	+	/	-	/	+	/	+	/	-	/	+	/	-
	Day7	-	+	-	-	-	+	-	+	-	-	-	+	-	-
	Day14	-	+	-	-	-	+	-	+	-	-	-	+	-	-
	Day21	-	+	-	-	-	+	-	+	-	-	-	+	-	-
2	Day-21	/	-	/	+	/	+	/	-	/	+	/	+	/	-
	Day-1	/	-	/	+	/	+	/	-	/	+	/	+	/	-
	Day7	-	-	-	+	-	-	-	-	-	+	-	+	-	-
	Day14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Day21	-	-	-	+	-	+	-	-	-	+	-	+	-	-
3	Day-21	/	+	/	+	/	-	/	+	/	-	/	+	/	+
	Day-1	/	-	/	-	/	+	/	-	/	+	/	+	/	-
	Day7	-	-	-	-	-	+	-	+	-	-	-	+	-	+
	Day14	-	+	-	-	-	-	-	-	-	+	-	+	-	-
	Day21	-	-	-	-	-	+	-	+	-	+	-	+	-	+
4	Day-21	/	-	/	-	/	-	/	-	/	-	/	-	/	-
	Day-1	/	-	/	-	/	-	/	-	/	-	/	-	/	-
	Day7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Day14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Day21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	Day-21	/	+	/	-	/	-	/	+	/	+	/	+	/	-
	Day-1	/	+	/	+	/	-	/	-	/	+	/	+	/	-
	Day7	-	+	-	+	-	-	-	+	-	+	-	+	-	-
	Day14	-	+	-	+	-	-	-	+	-	+	-	+	-	-
	Day21	-	+	-	+	-	-	-	-	-	+	-	+	-	-
6	Day-21	/	+	/	-	/	+	/	+	/	+	/	+	/	+
	Day-1	/	-	/	-	/	-	/	+	/	+	/	+	/	+
	Day7	-	-	-	-	-	+	-	+	-	+	-	+	-	+
	Day14	-	+	-	-	-	+	-	-	-	-	-	+	-	-
	Day21	-	+	-	-	-	-	-	+	-	-	-	+	-	+
7	Day-21	/	-	/	+	/	-	/	-	/	+	/	+	/	-
	Day-1	/	+	/	+	/	-	/	-	/	+	/	+	/	-
	Day7	-	-	-	+	-	-	-	-	-	+	-	+	-	-
	Day14	-	-	-	+	-	+	-	-	-	+	-	+	-	-
	Day21	-	+	-	+	-	-	-	-	-	+	-	+	-	-
8	Day-21	/	-	/	+	/	+	/	-	/	-	/	+	/	+
	Day-1	/	-	/	-	/	-	/	-	/	-	/	-	/	-

	Day7	-	+	-	-	-	-	-	-	-	+	-	+	-	-
	Day14	-	-	-	-	-	-	-	+	-	-	-	+	-	-
	Day21	-	+	-	-	-	-	-	-	-	-	-	+	-	-
9	Day-21	/	+	/	-	/	-	/	+	/	+	/	+	/	+
	Day-1	/	-	/	-	/	-	/	-	/	+	/	+	/	-
	Day7	-	-	-	-	-	+	-	+	-	+	-	+	-	-
	Day14	-	+	-	-	-	+	-	+	-	+	-	+	-	-
	Day21	-	+	-	-	-	+	-	+	-	+	-	+	-	-
10	Day-21	/	-	/	-	/	-	/	+	/	+	/	+	/	-
	Day-1	/	+	/	-	/	+	/	-	/	+	/	+	/	-
	Day7	-	-	-	-	-	+	-	+	-	+	-	+	-	-
	Day14	-	-	-	-	-	-	-	+	-	+	-	-	-	-
	Day21	-	+	-	-	-	-	-	-	-	+	-	+	-	-

/:Not performed, -:Negative, +:Positive

Figures

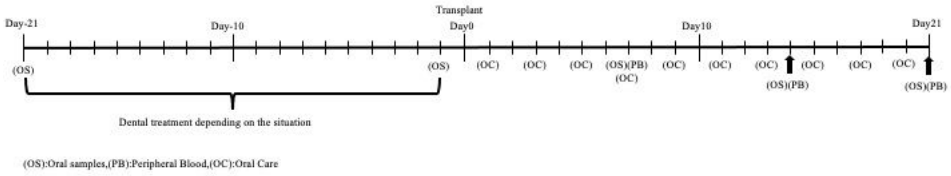

Figure 1

Oral management schedule and PCR date (oral samples and peripheral blood)