**Table 2** Clinical scoring of organ-specific symptoms related to chronic GVHD in long-term survivors after NIMA-mismatched haploidentical transplantation[a]

| *Involved organ site*[b] | *No. of evaluable patients* | *Score 0* | *Score 1* | *Score 2* | *Score 3* |
|---|---|---|---|---|---|
| PS | 14[c] | 6 (43%) | 7 (50%) | 1 (7%) | 0 |
| Skin | 16 | 5 (31%) | 7 (44%) | 2 (13%) | 2 (13%) |
| Mouth | 16 | 11 (69%) | 5 (31%) | 0 | 0 |
| Eyes | 16 | 10 (63%) | 3 (19%) | 3 (19%) | 0 |
| GI tract | 16 | 14 (88%) | 2 (13%) | 0 | 0 |
| Liver | 16 | 10 (63%) | 3 (19%) | 3 (19%) | 0 |
| Lungs | 16 | 9 (56%) | 2 (13%) | 1 (6%) | 4 (25%) |
| Joints and fascia | 16 | 12 (75%) | 2 (13%) | 2 (13%) | 0 |

Abbreviations: GI = gastrointestinal; PS = performance status.
[a]Scoring was based on the worst symptoms associated with chronic GVHD.
[b]Symptoms involving female genital tract were not reported.
[c]Two patients succumbed to pulmonary complications were excluded.

older, whereas Lansky score was applied for those who were younger than 16 years of age. Organ-specific symptoms related to chronic GVHD and their severity were diagnosed and evaluated by consensus criteria proposed by the National Institutes of Health Chronic GVHD Diagnosis and Staging Working Group.[6]

At a median follow-up of 56 months (range, 38–74), 13 (81%) of 16 patients were alive and free of their primary disease. One patient was alive with relapsed disease, and two died from pulmonary complications at 51 and 52 months after transplantation in continuous remission. Fifteen (94%) patients developed classical chronic GVHD; type of onset was *de novo* in five, quiescent in six and progressive in four. According to the National Institutes of Health Clinical Scoring Systems the affected organs scored two or greater, which included lungs ($n=5$), skin ($n=4$), eyes ($n=3$), liver ($n=3$) and joints/fascia ($n=2$) (Table 2). Overall severity of chronic GVHD among these patients was classified as mild in three (20%) cases, moderate in seven (47%) and severe in five (33%). It is noted that immunosuppressive agents were successfully withdrawn from eight (50%) patients with *de novo* or quiescent onset of disease at a median of 19 months (range, 3–46) after transplantation, although two of four patients who experienced severe chronic GVHD (score 3) of lung eventually succumbed to bronchiolitis obliterans. Karnofsky or Lansky performance score at the time of last follow-up among the 14 surviving patients was 100% in 6 (43%), 80–90% in 5 (36%), 70% in 2 (14%) and less than 70% in 1 (7%).

In the present study, we found that substantial proportion of long-term survivors after NIMA-mismatched haploidentical SCT could discontinue administration of immunosuppressive agents despite the frequent occurrence of moderate-to-severe chronic GVHD. This paradoxical observation contrasts sharply with the conventional assumption that the establishment of robust tolerance across multiple HLA disparities is hardly possible in the setting of marrow or peripheral blood SCTs without employing T-cell depletion. However, clinical significance of NIMA-mismatched donor selection has been controversial because the mechanisms underlying the tolerogenic effect against NIMA or IPA have not been fully elucidated.[7] Using murine BM transplant models, Matsuoka *et al.*[8] showed that transplant from donors exposed to NIMA *in utero*, but not from those exposed to IPA, reduced the morbidity and mortality associated with GVHD in an antigen-specific and a $CD4^+CD25^+$ T cells-dependent manner. In contrast, Opiela *et al.*[9] showed that murine neonates exposed to low levels of NIMA can develop vigorous *in vivo* cytotoxic rather than tolerogenic responses against NIMA, which might explain the inconsistent severity of GVHD after NIMA-mismatched SCTs in humans. Intriguingly, Stern *et al.*[10] recently reported that recipients of T cell-depleted haploidentical SCT using mother as the donor had the better overall survival than those using father as the donor, implying a mechanism by which previous exposure to paternally derived antigens in maternal donors would positively affect transplant outcomes.

In conclusion, our observations in this study suggested that long-term survival without continuous immunosuppressive treatment is possible after T-cell-replete HLA-haploidentical SCT from a microchimeric NIMA-mismatched donor. Although the small number of patients limits the interpretation of our results, further studies are warranted to compare late sequelae after HLA-haploidentical SCTs with various protocols in larger cohorts.

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# Clinical Significance of Serum Hepcidin Levels on Early Infectious Complications in Allogeneic Hematopoietic Stem Cell Transplantation

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The association of iron overload with complications of allogeneic hematopoietic stem cell transplantation (HSCT) has been suggested in previous studies. Because hepcidin plays a central role in the regulation of iron homeostasis, we analyzed the association between pretransplant serum hepcidin-25 levels and early infectious complications after allogeneic HSCT. We studied 55 consecutive adult patients with a median age of 47 years (range: 20–64 years) who underwent allogeneic HSCT for hematologic malignancies at our institution. Thirty-two patients had myelogenous malignancies; the remaining 23 had lymphogenous malignancies. The median pretransplant serum hepcidin level of patients in the study was 21.6 ng/mL (range: 1.4–371 ng/mL), which was comparable to that of healthy volunteers (median: 19.1 ng/mL [range: 2.3–37 ng/mL]; n = 17). When cumulative incidences of documented bacterial and cytomegalovirus (CMV) infections at day 100 were compared according to pretransplant hepcidin-25 levels, the incidence of bacterial, but not CMV, infection, was significantly higher in the high-hepcidin group (≥50 ng/mL; n = 17) than in the low-hepcidin group (<50 ng/mL; n = 38) (65% [95% confidence interval, 38%–82%] versus 11% [3%–23%]; $P < .001$). This finding was confirmed by multivariate Cox analysis adjusted for confounders, including pretransplant ferritin and C-reactive protein (CRP) levels. No fungal infection was documented in either group. These results suggest that the pretransplant serum hepcidin-25 level may be a useful marker for predicting the risk of early bacterial complications after allogeneic HSCT. Larger prospective studies are, however, warranted to confirm our findings.

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**KEY WORDS:** Hepcidin, Bacterial infection, Allogeneic stem cell transplantation

## INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has been widely performed as a potentially curative treatment for intractable hematologic malignancies with conventional chemotherapy. However, despite recent advances in the treatment of infectious diseases and conditioning regimens for transplantation, treatment-related complications remain a major problem. Therefore, it is particularly important to identify a good biomarker that can predict treatment-related complications before transplantation. A recently accumulated body of evidence suggests that iron overload is associated with adverse clinical outcomes in HSCT [1-10]. Armand et al. [2] showed that a high pretransplant serum ferritin level was strongly associated with lower overall and disease-free survival (OS, DFS) in patients with allogeneic HSCT that was performed as a treatment for acute leukemia and myelodysplastic syndrome (MDS). Other studies have shown that pretransplant iron overload in autologous or allogeneic HSCT was a risk factor associated with posttransplant complications, such as mucositis, bacterial, and fungal infection, and hepatic veno-occlusive disease (VOD) [3-6,8-11].

Hepcidin, first identified in human blood and urine as an antimicrobial small peptide [12,13], is now considered to be a central molecule that regulates iron metabolism. Hepcidin decreases iron absorption from the intestine and blocks its release from iron stores by

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downregulating the expression of the cellular iron exporter, ferroportin [14,15]. Hepatic expression of hepcidin can be upregulated by iron loading [16,17] as well as by inflammatory stimuli such as interleukin-6 (IL-6) [18]. Therefore, we hypothesized that serum hepcidin level could be a useful predictor of iron overload and inflammatory condition prior to HSCT. Here, we performed a single-center retrospective study at our institution to evaluate the significance of serum hepcidin levels as a predictor of early treatment-related complications after allogeneic HSCT with special reference to infectious complications.

## PATIENTS AND METHODS

### Study Population

The study population comprised 66 consecutive adult patients who underwent allogeneic HSCT for the treatment of hematologic malignancies at Kyoto University Hospital from July 2006 to September 2008. A total of 55 patients, excluding those who had received prior transplantations within 1 year or who had any active infections before the current transplantation, were included in the analysis. This study was approved by the Ethics Committee of Kyoto University Graduate School and the Faculty of Medicine. Written informed consent was obtained from all patients.

### Serum Analysis

Before the administration of conditioning regimens, serum samples were obtained at around 8:00 am, allocated in tubes, and stored at –80°C until analysis. The levels of serum hepcidin-25 (the main form of active hepcidin peptide) were quantified using a liquid chromatography-tandem mass spectrometry-based assay system following the method described by Murao et al. [19]. Other serum parameters were measured using standard laboratory techniques.

### Prophylaxis, Monitoring, and Diagnosis of Infection

The patients were isolated in a single room equipped with a high-efficiency particulate air filter (HEPA) system from 1 day before transplantation until at least 4 weeks after transplantation. No bacterial prophylaxis was prescribed for the patients according to our institutional protocols [20]. Trimethoprim-sulfamethoxazole (160 mg/day [trimethoprim], 3 times a week) was administered as prophylactic therapy for *Pneumocystis jirovecii* pneumonia from the day of admission until the day of transplantation and restarted after the day of neutrophil engraftment. All patients received fluconazole (200 or 400 mg/day) and acyclovir (1000 mg/day) prophylaxis from the period of conditioning until 30 days after transplantation. After the first 30 days, the patients received fluconazole at a dose of 100 mg/day until at least 100 days after transplantation. The administration of acyclovir (400 mg/day) was continued when patients received steroid therapy for acute graft-versus-host disease (aGVHD). For each febrile episode, 1 or 2 sets of blood samples were cultured, and the cultures of specimens other than blood and imaging examinations were performed according to clinical judgment. The occurrence of cytomegalovirus (CMV) infection was closely monitored by CMV pp65 antigenemia testing with C10/C11 monoclonal antibodies (mAbs) from the day after neutrophil engraftment until at least 100 days after transplantation. Documented bacterial infection included any incidence of bloodstream infection or any other bacterial infection. Bloodstream infection was diagnosed if at least 1 of the following criteria was met: (1) blood culture obtained during a febrile episode was positive, at least once, for bacterial organisms not considered to be common skin contaminants; (2) blood culture obtained during a febrile episode was positive for the same common skin contaminant on separate occasions within 72 hours; (3) blood culture was positive, at least once, for a common skin contaminant, and the patient was diagnosed with septicemia, including hypotension (systolic blood pressure, <90 mmHg) and abnormal coagulopathy. Infections other than bloodstream infection were diagnosed if the following criteria were met: (1) bacterial organisms were observed from specimens such as sputum, urine, and stool at least on 2 occasions, and (2) the patient showed symptoms of infection corresponding to those specimens. *Clositridium difficile* enterocolitis was excluded from the analysis, because this disease is toxin-mediated, and cannot be prevented by administration of common bacterial prophylactic agents such as fluoroquinolones, even if patients with a high risk of bacterial infection can be identified by using a putative biomarker. CMV infection was defined as positive if either C10 or C11 antigenemia assay showed at least 2 positive cells per 150,000 leukocytes. Invasive fungal infection was diagnosed according to the criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [21].

### Statistical Analysis

Endpoints included cumulative incidences of documented bacterial infection, fungal infection, CMV infection, and infection-related mortality, and OS within 100 days post transplantation. Patient and transplant characteristics between 2 groups were compared using the Mann-Whitney *U*-test or $\chi^2$ analysis, as appropriate. The day of neutrophil

engraftment was defined as the first of 3 consecutive days when the absolute neutrophil count (ANC) exceeded 500/μL. The day of neutrophil engraftment between 2 groups was compared by using the Mann-Whitney *U*-test. To eliminate the effect of competing risk, the cumulative incidences were assessed using methods described elsewhere [22]. The competing event in the cumulative incidence analyses was defined as death without an event of interest within 100 days post transplantation. OS was estimated using Kaplan-Meier methods. Infection-related death was defined as death associated with any infection within 100 days after transplantation. Standard risk disease was defined as complete remission (CR) in cases of acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), adult T cell leukemia/lymphoma (ATL), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), and untreated or CR in MDS and myeloproliferative disorder (MPD). High-risk disease was defined as statuses other than CR in AML, ALL, ATL, HL, and NHL and in MDS and MPD after treatment. The Cox proportional-hazard model was applied to assess the effect of factors that potentially affected the study endpoints. The following items were added as confounders: recipient's sex (male or female), recipient's age (<50 or ≥50 years), diagnosis (myelogenous or lymphogenous malignancies), risk of disease (standard or high risk), conditioning regimen (reduced or myeloablative intensity [RIC, MA]), type of donor (related or unrelated donor), reticulocyte count ($<60 \times 10^9$ or $\geq 60 \times 10^9$/L), ferritin level (<1000 or ≥1000 mg/dL), and C-reactive protein (CRP) level (<0.3 or ≥0.3 μg/dL). The cutoff points for reticulocyte count and the ferritin and CRP levels were chosen such that we could make optimal use of the information with a proviso that the smaller group contained at least 30% of patients. *P* values of < .05 were considered statistically significant. All analyses were conducted using STATA software version 10 (STATA Corp., College Station, TX).

**Table 1. Characteristics of Patients and Transplants**

| Variables | Hepcidin, Low (<50 ng/mL) n = 38 | Hepcidin, High (≥50 ng/mL) n = 17 | *P* Value |
|---|---|---|---|
| Age at transplant | | | |
| Median age (range) | 47.5 (23–64) | 47 (20–63) | .750 |
| Sex | | | .171 |
| Male | 21 (55%) | 6 (35%) | |
| Female | 17 (45%) | 11 (65%) | |
| Disease | | | .612 |
| Myeloid malignancies | 23 (61%) | 9 (53%) | |
| AML | 15 | 8 | |
| MDS/MPD | 8 | 1 | |
| Lymphoid malignancies | 15 (39%) | 8 (47%) | |
| ALL | 4 | 4 | |
| ATL | 4 | 1 | |
| HL | 1 | 0 | |
| NHL | 6 | 3 | |
| Risk of disease | | | .051 |
| Standard | 22 (58%) | 5 (29%) | |
| High | 16 (42%) | 12 (71%) | |
| Conditioning regimen | | | .545 |
| Myeloablative intensity | 19 (50%) | 10 (59%) | |
| Reduced intensity | 19 (50%) | 7 (41%) | |
| Prophylaxis against GVHD | | | .663 |
| Cyclosporine-based | 5 (13%) | 3 (18%) | |
| Tacrolimus-based | 33 (87%) | 14 (82%) | |
| Type of donor | | | .181 |
| Related donor | | | |
| HLA*-matched | 10 (26%) | 3 (18%) | |
| HLA-mismatched | 3 (8%) | 1 (6%) | |
| Unrelated donor | | | |
| HLA-matched | 18 (47%) | 5 (29%) | |
| HLA-mismatched | 7 (18%) | 8 (47%) | |
| Source of stem cells | | | .259 |
| Bone marrow | 29 (76%) | 10 (59%) | |
| Peripheral blood | 1 (3%) | 0 (0%) | |
| Cord blood | 8 (21%) | 7 (41%) | |
| Serum ferritin (μg/dL) | | | |
| mean (±SD) | 664 (±796) | 1551 (±993) | <.001 |
| CRP (mg/dL) | | | |
| mean (±SD) | 0.36 (±0.68) | 0.70 (±1.63) | .176 |
| Reticulocyte ($\times 10^9$/L) | | | |
| mean (±SD) | 63.7 (±40.2) | 64.0 (±42.2) | .979 |

AML indicates acute myelogenous leukemia; MDS/MPD, myelodysplastic syndrome and myeloproliferative disorders; ALL, acute lymphoblastic leukemia; ATL, acute T cell leukemia/lymphoma; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; GVHD, graft-versus-host disease; Cyclosporine-based, cyclosporine with or without other agents; Tacrolimus-based, tacrolimus with or without other agents; HLA, human leukocyte antigen; CRP, C-reactive protein.
Data are counts of individuals unless specified otherwise.
*HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, -B, and -DR antigens.

## RESULTS

### Characteristics of Patients and Transplants

Characteristics of patients and transplants are shown in Table 1. The median age of patients was 47 years (range: 20–64 years). The primary disease in these patients was as follows: AML in 23, MDS/MPD in 9, ALL in 8, NHL in 9, HL in 1, and ATL in 5. The risk of diseases was standard in 27 and high in 28 patients. Nearly half of the patients (n = 26) received a RIC regimen. The stem cell sources used were bone marrow (BM) in 39, peripheral blood (PB) in 1, and cord blood (CB) in 15 patients. The median pretransplant serum hepcidin level was 21.6 ng/mL (range: 1.4–371 ng/mL), which was comparable to that of healthy volunteers (median: 19.1 ng/mL [range: 2.3–37 ng/mL]; n = 17) [23]. Because the lower hepcidin level of the third tertile among the patients in this study was 49.1 ng/mL, we set a cutoff hepcidin level of 50 ng/mL for practical use to divide the patients into low- and high-hepcidin groups (n = 17 and 38, respectively). There was no difference in patient and transplant characteristics between the low- and high-hepcidin groups, except for serum ferritin levels ($P < .001$).

## Documented Bacterial Infections

There was no significant difference between the days of neutrophil engraftment of the low- and high-hepcidin groups (median day: 21 [range: 14–99] and median day: 22.5 [range: 12–53], respectively, $P = .54$). A total of 16 episodes of bacterial infections were documented; these included 15 episodes of bloodstream infections and 1 episode of pneumonia. No patient experienced more than 1 episode of bacterial infection within 100 days after transplantation. The documented bacterial organisms are listed in Table 2. The main organisms were Gram-negative bacilli in both the low- and high-hepcidin groups. In the antimicrobial-susceptibility tests, 12 of the 13 Gram-negative isolates were sensitive to fluoroquinolone. We documented 2 bacterial infections in the late period of transplantation; 1 patient showed infection at day 89 after transplantation, which was attributed to delayed neutrophil engraftment, and another patient showed infection at day 68, when the neutrophil counts had temporarily decreased. The cumulative incidences of the documented bacterial infection in the low- and high-hepcidin groups were 11% (95% confidence interval [CI], 3%–23%) and 65% (95% CI, 38%–82%), respectively (Figure 1A). In the low-hepcidin group, the cumulative incidence of bacterial infection was lower in patients with a hepcidin level of <25 ng/mL than in those with a hepcidin level ranging from ≥25 to <50 ng/mL (10% [95% CI, 2%–23%] versus 17%, [95% CI, 1%–52%]). Univariate analysis of various potential confounders showed that high hepcidin level was the only factor that affected the cumulative incidence of documented bacterial infection (hazard ratio [HR], 8.98; 95% CI, 2.82–28.57; $P < .001$) (Table 3). To exclude the effect of other confounders, the significance of high hepcidin level was assessed in the stratified category of each confounder (eg, in either the high- or low-ferritin group); we noted consistently high HRs in the high-hepcidin group in each stratified category (data not shown). We also found that hepcidin had a significant impact on the patients, excluding the patients in other specific categories, such as those who received a CB transplant or those who underwent a transplant from an unrelated HLA-mismatched donor (data not shown). Furthermore, the significant effect of hepcidin persisted even after the adjustment for confounders in multivariate analysis (HR, 28.46; 95% CI, 2.51–323.34; $P = .007$) (Table 3). Even when the variables were treated as continuous instead of categoric, the significant effect of hepcidin persisted (HR, 1.01; 95% CI, 1.00-1.01; $P = .001$).

**Table 2. Documented Bacterial Organisms within 100 Days after Stem Cell Transplantations**

| Category | Hepcidin, Low (<50 ng/mL) n = 38 | Hepcidin, High (≥50 ng/mL) n = 17 |
|---|---|---|
| Gram-positive cocci (n) | *Staphylococcus epidermidis* (1) | *Enterococcus faecium* (2) |
| Gram-negative bacilli (n) | *Klebsiella pneumoniae* (2) | *Klebsiella pneumoniae* (3) |
| | *Enterobacter cloacae* (1) | *Escherichia coli* (3) |
| | *Prevotella intermedia* (1) | *Pseudomonas aeruginosa* (2) |
| | | *Klebsiella oxytoca* (1) |

*P. intermedia* was detected in the sputum of 1 patient with pneumonia. Other organisms were detected in blood culture bottles.

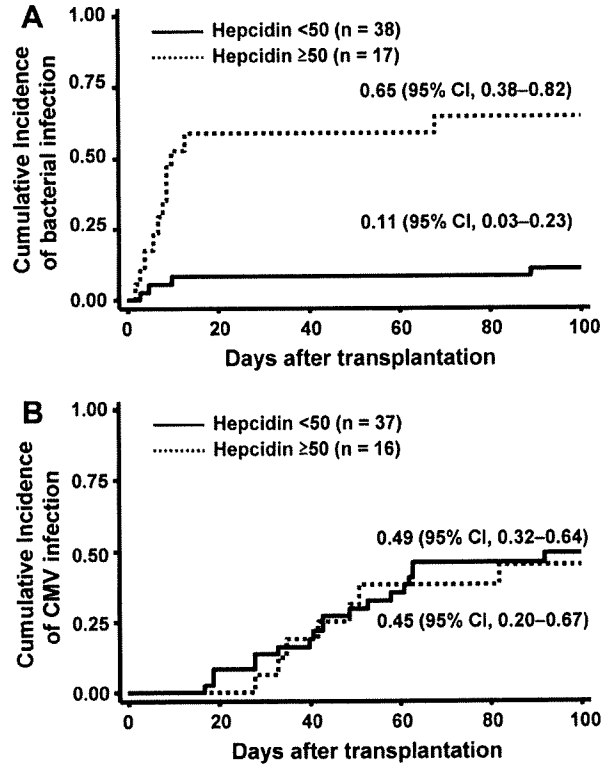


**Figure 1.** The cumulative incidences of documented bacterial infection (A) and cytomegalovirus (CMV) infection (B) at 100 days after stem cell transplantation. Solid black line, the low-hepcidin group (<50 ng/mL); solid gray line, the high-hepcidin group (≥50 ng/mL); CI, confidence interval. CMV infection was not assessable in 2 patients because of early death before neutrophil engraftment.

## Other Transplant-Related Complications and Mortality

The cumulative incidences of CMV infection in the low- and high-hepcidin group were 49% (95% CI, 32%–64%) and 45% (95% CI, 20%–67%), respectively (Figure 1B); univariate and multivariate analyses showed no significant difference between the 2 groups (Table 3). All CMV infections were well treated by the administration of ganciclovir or foscarnet. No fungal infection was documented. Therefore, all infection-related deaths were attributed to bacterial infection. The cumulative incidence of infection-related mortality in the low-hepcidin group was 3% (95% CI, 0.2%–12%), whereas that in the high-hepcidin group was 6% (95% CI, 0.4%–24%),

**Table 3. Univariate and Multivariate Analyses of Documented Bacterial Infection, CMV Infection, and Overall Survival at 100 Days after Stem Cell Transplantations**

| | | Univariate Analysis | | Multivariate Analysis | |
|---|---|---|---|---|---|
| | Number | HR (95% CI) | *P* Value | HR (95% CI) | *P* Value |
| 1) Documented bacterial infection | | | | | |
| Hepcidin, low (<50 ng/mL) | 5/38 | 1 | – | 1 | – |
| Hepcidin, high (≥50 ng/mL) | 11/17 | 8.98 (2.82–28.57) | <.001 | 28.46 (2.51–323.34) | .007 |
| 2) CMV antigenemia (C10 or C11 ≥2) | | | | | |
| Hepcidin, low (<50 ng/mL) | 18/37 | 1 | – | 1 | – |
| Hepcidin, high (≥50 ng/mL) | 7/16 | 0.97 (0.40–2.32) | .939 | 0.63 (0.16–2.49) | .511 |
| 3) Overall survival | | | | | |
| Hepcidin, low (<50 ng/mL) | 36/38 | 1 | – | – | – |
| Hepcidin, high (≥50 ng/mL) | 14/17 | 3.60 (0.60–21.56) | .161 | – | – |

CMV indicates cytomegalovirus; CI, confidence interval.
Hazard ratios (HRs) in multivariate analysis were adjusted for recipient's sex (male or female), recipient's age (<50 or ≥50 years), diagnosis (myelogenous or lymphoid malignancies), risk of disease (standard or high risk), conditioning regimen (reduced or myeloablative intensity), type of donor (related or unrelated donor), reticulocyte count (<60 × 109 or ≥60 × $10^9$/L), ferritin level (<1000 or ≥1000 mg/dL), and C-reactive protein (CRP) level (<0.3 or ≥0.3 μg/dL). Overall survival was not analyzed in the multivariate model because of the low incidence of death.

with no statistical difference between the 2 groups. OS at 100 days after transplantation in the low- and high-hepcidin groups was 95% (95% CI, 81%–99%) and 82% (95% CI, 55%–94%), respectively (Figure 2). No significant difference in OS was observed (Table 3).

## DISCUSSION

In our cohort of patients who underwent allogeneic HSCT for hematologic malignancies, we found a significant association between the pretransplant serum hepcidin levels and the cumulative incidence of documented bacterial infection. To our knowledge, this is the first study that has evaluated the clinical significance of serum hepcidin levels in predicting transplant-related complications; the findings suggest that the pretransplant serum hepcidin level can be used as a good pretransplant biomarker to predict bacterial infection in a patient scheduled for HSCT.

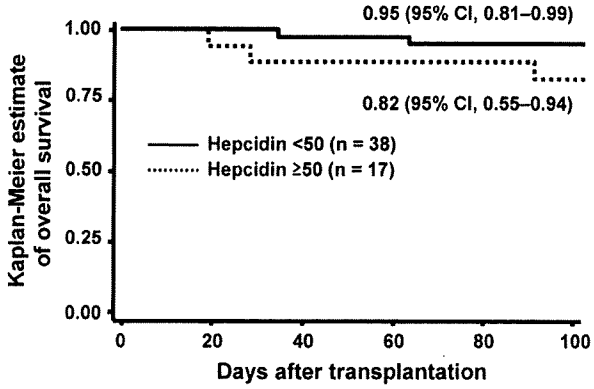


**Figure 2.** Kaplan-Meier estimate of OS at 100 days after stem cell transplantation. Solid black line, the low-hepcidin group (<50 ng/mL); solid gray line, the high-hepcidin group (≥50 ng/mL); CI, confidence interval.

Hepcidin production is regulated by at least 3 factors: iron load [16,17], inflammation [18], and unknown erythropoietic signals [23-25]. Therefore, the good predictive value of hepcidin with respect to the incidence of documented bacterial infection can be partly explained by the cumulative effect of at least these 3 factors on bacterial infection. Iron overload increases the level of circulating non-transferrin-bound iron, which is known to amplify free-radical reactions in inflammatory or ischemia-related conditions [7,26]. Such reactions could enhance tissue damage such as mucositis during the conditioning regimen, thereby allowing bacterial translocation through the damaged mucosa [27]. In addition, iron is a necessary nutrient for bacteria and fungus [28]. The association between hemochromatosis, 1 of the iron overload disorders, and infection with certain organisms has already been described [29]. Therefore, the high hepcidin levels might reflect iron overload status, which has an adverse effect on bacterial infections. Second, a high hepcidin level may indicate inflammation because of a latent bacterial infection that was undetectable before HSCT, but may surface in posttransplant neutropenic status. Last, a high hepcidin level could reflect suppressed erythropoiesis, probably because of the short duration from the last chemotherapy to the start of the conditioning regimen for transplantation. Repeated cytotoxic chemotherapy in a short period may exacerbate tissue damage and increase the risk of bacterial infection.

Although serum ferritin levels do not necessarily correlate with the amount of iron load in patients with inflammation or specific diseases [1,30,31], it is frequently used and regarded as a indicator of iron overloading, and several studies have demonstrated the association between high ferritin levels and treatment-related mortality (TRM) [3,11]. In this cohort, an elevation of serum ferritin level was not found to be a significant risk factor for bacterial infection,

whereas an elevated hepcidin level was a strong risk factor even after adjustment for other potential confounders. Furthermore, we observed consistent association of high hepcidin levels with high risk for developing bacterial infection when analyses were confined to either the low- or high-ferritin subgroups. These findings collectively suggest that hepcidin can be used as a better predictor of documented bacterial infections than serum ferritin levels. Moreover, various new techniques to quantify hepcidin-25, such as a competitive enzyme-linked immunoassay as well as mass spectrometry-based methods, have been recently developed [19,25,32,33]. Standardization of those methods will make it possible to use the serum hepcidin level as a biomarker in routine clinical practice.

Hepcidin was first isolated and characterized as an antimicrobial peptide in human blood [12]. In radial diffusion assays, synthetic hepcidin suppressed the growth of several strains of Gram-positive bacteria and some strains of Gram-negative bacteria, but not of *Escherichia coli* or *Pseudomonas fluorescens*. Our findings pertaining to the adverse association of high hepcidin levels with bacterial infection indicated that the bactericidal effect of hepcidin was either considerably limited in neutropenic settings such as HSCT or was ineffective on the bacterial organisms observed in our cohort. Moreover, we observed a significant adverse effect of hepcidin even after the adjustment for potential confounders, suggesting that hepcidin itself may play an unknown biologic role in susceptibility to bacterial infection, or it may represent an unknown surrogate marker for predicting bacterial infection. To answer this issue, the significance of pretransplant serum hepcidin levels needs to be evaluated in a more homogeneous group of patients having the same level of confounders.

We did not detect any adverse effect of high hepcidin levels on infection-related mortality or OS at 100 days after transplantation, although there was a marked difference in the incidence of bacterial infection. One possible explanation for this observation is that bacterial infection of the blood was well managed by prompt and appropriate treatment with antibiotics in our transplant centers. However, because the incidence of early death after HSCT is considerably low, the effect of bacterial infection on early mortality should be evaluated in larger cohort studies to gain enough statistical power for comparison. Alternatively, selective prophylactic administration of oral antibiotics such as fluoroquinolones to patients with a high risk of bacterial infection may be an effective approach; however, this approach will be effective only if most of the bacterial isolates at the transplant center are sufficiently sensitive to these prophylactic antibiotics. With regard to other endpoints, there was no association between high hepcidin levels and the incidence of CMV infection. The effect of hepcidin level on the incidence of fungal infection could not be evaluated because of the very low incidences of these conditions in our cohorts. These effects should also be evaluated in studies with a larger cohort in the future.

The present study, however, has some limitations. We cannot exclude the possibility of a pseudonegative result for bloodstream infection, because broad-spectrum antibiotics were administered to all neutropenic patients at the time of blood culture, regardless of the results of blood culture. In addition, the retrospective study design and heterogeneous background of diseases and transplantation procedures could also bias the results. Particularly, in the small cohort of 55 patients, the adjustment of HRs by confounders may be incomplete. In particular, the higher proportion of CB transplants and the high risk of diseases in the high-hepcidin group may cause bias, although we found consistently high HRs in the high-hepcidin group in various stratified categories. Therefore, larger studies are necessary to confirm our results.

In conclusion, our study revealed that the pretransplant serum hepcidin level was significantly associated with bacterial infection, particularly bloodstream infection, suggesting that quantification of serum hepcidin levels could be useful for predicting early bacterial complications. Prophylactic antibiotic therapy based on the local sensitivities of common bacterial isolates can be considered in the patients with high hepcidin levels who are undergoing allogeneic HSCT. Larger prospective studies are, however, warranted to confirm our findings.

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Conflict of interest: N.T. declares that he is the President of Medical Care Proteomics Biotechnology Co. Ltd. (Ishikawa-ken, Japan), a startup company, the stock of which is not publicly traded. The other authors declare that they have no conflicts of interest relevant to this paper.

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ORIGINAL ARTICLE

# Pretransplant serum ferritin and C-reactive protein as predictive factors for early bacterial infection after allogeneic hematopoietic cell transplantation

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**Although fluoroquinolones or other antibiotics are commonly used to prevent bacterial infections after hematopoietic cell transplantation (HCT), because of the growing presence of multidrug-resistant microorganisms, it is important to identify patients who are more likely to benefit from antibacterial prophylaxis. To evaluate risk factors for early bacterial infection after allogeneic HCT, we retrospectively analyzed clinical data for 112 consecutive adult patients with hematological malignancies who received transplants without any antibacterial prophylaxis. The cumulative incidence of bacterial infection at 30 days after transplantation was 16%. Among various pre-transplant factors, only high serum ferritin (>700 ng/mL, 47 patients) and high C-reactive protein (CRP) (>0.3 mg/dL, 28 patients) levels were significantly associated with the development of bacterial infection in a multivariate analysis (hazard ratio (95% confidence interval): ferritin, 4.00 (1.32–12.17); CRP, 3.64 (1.44–9.20)). In addition, septic shock and sepsis with organ failure were exclusively observed in patients who had high ferritin and/or high CRP levels. These results suggest that pretransplant serum ferritin and CRP levels can be useful markers for predicting the risk of early bacterial infection after allogeneic HCT. It may be prudent to limit antibacterial prophylaxis to patients with predefined risk factors to ensure the safety of HCT with the use of fewer antibiotics.**

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**Keywords:** antibacterial prophylaxis; fluoroquinolone; bacterial infection; allogeneic hematopoietic cell transplantation

## Introduction

Bacterial infection is an important cause of mortality and morbidity after autologous or allogeneic hematopoietic cell transplantation (HCT).[1,2] When neutropenic patients who receive cytotoxic chemotherapy are compared with HCT recipients, the risk of severe bacterial infection appears to be considerably higher in the latter because high-dose chemotherapy and/or TBI may cause severe mucosal damage that facilitates massive bacterial translocation under profound post-transplant immunosuppression. Therefore, the use of oral fluoroquinolones (FQs) or other antibiotics as antibacterial prophylaxis is strongly considered for HCT recipients, although significant variations have been reported among transplant centers and countries.[3–5]

Recently, the widespread emergence of FQ-resistant or multidrug-resistant microorganisms in hematology–oncology units has been suggested to compromise the effectiveness of routine antibacterial prophylaxis with FQs in patients undergoing cytotoxic chemotherapy or HCT.[6–14] In our center, the isolation rate of FQ-resistant Gram-negative bacilli was high (57.1%) during a period when FQs were routinely administered as antibacterial prophylactic agents; in particular, among isolated *Enterobacteriaceae* strains, 66.7, 33.3 and 22.2% were resistant to levofloxacin, piperacillin and ceftazidime, respectively.[8] In an attempt to reduce the emergence of antibiotic-resistant microorganisms, we stopped using any antibacterial prophylaxis in both autologous and allogeneic HCT recipients in 2004,[8] and found that this discontinuation of FQ prophylaxis, even in the setting of myeloablative allogeneic HCT did not significantly affect early mortality after transplantation.[15]

Another approach to balance the safety of HCT with judicious antibiotic use would be to limit the use of antibacterial prophylaxis to HCT recipients who are at high risk of bacterial infection, because a delay in antibiotic treatment may lead to serious complications after infectious episodes in such patients if prophylactic antibiotics are not administered. To identify the pretransplant characteristics of patients who are more likely to be susceptible to bacterial infection after allogeneic HCT, we conducted a single-center retrospective study with the clinical data

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of 112 consecutive allogeneic transplants for hematologic malignancies that were performed without antibacterial prophylaxis. As a potential biomarker for predicting bacterial infection, high levels of pretransplant ferritin levels have recently been shown to be associated with an increased incidence of bloodstream infection, as well as decreased overall survival and increased early mortality.[16] In addition, elevated pretransplant serum C-reactive protein (CRP) levels have been shown to be associated with an increased incidence of bacterial infection in the setting of chemotherapy for acute myeloid leukemia[17] and allogeneic transplantation.[18] Sato *et al.*[17] reported that pretreatment serum CRP levels of greater than 0.26 mg/dL were useful for predicting the incidence of documented infection in patients who received their first consolidation chemotherapy for acute myeloid leukemia. As these biomarkers are easy to measure and may be useful in clinical practice, in this study we explored the association between bacterial infection and these biomarkers as well as various patient characteristics.

## Subjects and methods

*Study population*

The medical records of 137 consecutive adult patients with hematological malignancies who underwent T-cell replete allogeneic HCT from September 2004 to March 2009 at Kyoto University Hospital were reviewed. Patients who had active infections before the transplantation procedure ($n=23$) and those who had a recent history of autologous or allogeneic HCT within 1 year ($n=2$) were excluded; thus, a total of 112 patients were included in the analysis, without any duplication of subjects. Patients were considered to have standard-risk disease if they received a transplant without prior chemotherapy or in CR, whereas those who received a transplant in any other status were considered to have high-risk disease. This study was approved by the Ethics Committee of Kyoto University Graduate School and the Faculty of Medicine. Written informed consent for the transplantation protocol was obtained from all of the patients.

*Prophylaxis monitoring and diagnosis of infection*

A central venous catheter was inserted in the subclavian vein before the beginning of the conditioning regimens for all patients. Each patient was isolated in a single room equipped with a HEPA system from a day before the transplantation until at least 4 weeks after transplantation. Each patient was then maintained on a low-microbial diet and asked to take strict control measures under the supervision of the ward staff to prevent the acquisition of nosocomial pathogens. No bacterial prophylaxis was prescribed for these patients according to our institutional protocols.[8] Intravenous antibiotics with anti-pseudomonal activity were promptly administered in response to episodes of febrile neutropenia or suspected bacterial infections. Trimethoprim-sulfamethoxazole (trimethoprim; 160 mg/day, three times a week) was administered as prophylactic therapy for *Pneumocystis jiroveci* pneumonia from the day of admission until the day of transplantation, and this prophylaxis was reinitiated after the day of neutrophil engraftment. All patients received 200 or 400 mg of fluconazole and 400–1000 mg of acyclovir per day as prophylactic agents from the conditioning period until 30 days after transplantation. Prophylactic fluconazole and acyclovir were usually continued when patients were receiving steroid therapy for acute or chronic GVHD. For each febrile episode, 1 or 2 sets of blood samples were cultured, and cultures of specimens other than blood specimens and imaging examinations to search for the focus of infection were performed according to the clinician's judgment. Microbiologically documented infections included the presence of bloodstream bacterial infection or any other bacterial infection. Bloodstream bacterial infection was diagnosed when at least 1 of the following criteria was met: (1) the culture of blood obtained during a febrile episode tested positive at least once for bacterial organisms other than common skin contaminants, (2) the culture of blood obtained during a febrile episode tested positive for the same common skin contaminant in independent analysis conducted within an interval of 72 h, and (3) the blood culture tested positive at least once for a common skin contaminant, and the patient was diagnosed with septicemia with hypotension (systolic blood pressure, $<90$ mm Hg) and disseminated intravascular coagulation. Infections other than bloodstream infection were diagnosed when the following criteria were met: (1) sputum, urine or stool samples were found to contain pathogenic bacteria on at least two occasions, and (2) the patient showed symptoms of infection corresponding to those specimens. Septic shock and sepsis with organ failure were defined as severe infection.

*Measurement of serum biomarkers (serum ferritin and C-reactive proteins)*

Both serum ferritin and CRP levels were measured using peripheral blood samples obtained just before the start of the conditioning regimen. The serum ferritin concentration was measured by an immunoenzymometric assay (AIA-PACK FER, Tosoh Corporation, Tokyo, Japan) (normal range; $\leqslant 150$ ng/mL), and the serum CRP concentration was measured by a latex agglutination assay (N-Assay LA CRP-S, Nittobo, Tokyo, Japan) (normal range; $\leqslant 0.2$ mg/dL), according to the respective manufacturer's instructions.

*Statistical analysis*

The primary endpoint was the cumulative incidence of documented bacterial infections during the first 30 days after transplantation. Overall survival and treatment-related mortality were also analyzed as secondary endpoints. To eliminate the effect of a competing risk, the cumulative incidence was assessed using methods described elsewhere.[19] In the analysis of the cumulative incidence of bacterial infections and treatment-related mortality, a competing event was defined as death without an event of interest. The overall survival was estimated using the Kaplan–Meier method. We applied Fine and Gray's proportional hazards model for the sub-distribution of a competing risk to analyze the cumulative incidence of

bacterial infection and treatment-related mortality, and the Cox proportional hazards model for that of overall survival.[20] Factors with *P*-values of less than 0.10 in the univariate analysis were included in the multivariate analysis. Factors evaluated in the analysis included the recipient's age (≤50 or >50), recipient's sex (female or male), diagnosis (myeloid or lymphoid malignancies), disease status at transplant (standard risk or high risk), duration from diagnosis to transplant (≤1 or >1 year), duration from the last pretransplant cytotoxic chemotherapy to conditioning of transplant (no history of prior chemotherapy or >2, or ≤2 months), number of courses of prior cytotoxic chemotherapy (≤5 or >5), source of stem cells (related BM or peripheral blood, unrelated BM, or unrelated cord blood), conditioning regimen (conventional or reduced-intensity regimen), use of granulocyte CSF (G-CSF) (yes or no), serum ferritin levels (≤700 ng/mL, >700 ng/mL, or unknown), and serum CRP levels (≤0.3 or >0.3 mg/dL). We assessed the interaction between ferritin and CRP levels, using interaction terms between a ferritin category with scores of 0 (ferritin ≤700 ng/mL) and 1 (ferritin >700 ng/mL) and a CRP category with scores of 0 (CRP ≤0.3 mg/dL) and 1 (CRP >0.3 mg/dL). The cutoff point for the ferritin levels was the median value and that for the CRP levels was the higher tertile value. The correlation between ferritin and CRP levels was also tested by Pearson's correlation coefficient. *P*-values of less than 0.05 were considered statistically significant. All analyses were conducted using Stata software version 11 (StataCorp., College Station, TX, USA).

## Results

### *Patient characteristics*

The patient characteristics are shown in Table 1. The median age of the patients was 47 years (range, 18–66 years). The primary diseases in these patients were as follows: acute myeloid leukemia in 46 patients, acute lymphoblastic leukemia in 11, myelodysplastic syndrome in 16, chronic myelogenous leukemia in four, non-Hodgkin's lymphoma in 19, adult T-cell leukemia/lymphoma in 10, myeloproliferative disorder in four, and plasma-cell myeloma in two. Sixty-six patients (58.9%) had standard-risk disease. The source of stem cells used for HCT was related BM or peripheral blood in 40 patients (35.7%), unrelated BM in 52 (46.4%), and unrelated cord blood in 20 (17.9%). A conventional myeloablative regimen was used in 54 patients (48.2%), and G-CSF was used after HCT in 57 patients (50.9%). The number of patients with pretransplant serum ferritin levels of ≤700, >700 ng/mL, and unavailable were 49, 47 and 16, respectively, and the number of those with pretransplant serum CRP levels of ≤0.3 and >0.3 mg/dL were 84 and 28, respectively.

**Table 1** Patient characteristics

| *Category* | *Patients (n = 112)* |
|---|---|
| *Age, years* | |
| Median (range) | 47 (18–66) |
| *Sex, n (%)* | |
| Male | 49 (43.8) |
| Female | 63 (56.3) |
| *Diagnosis, n (%)* | |
| Acute myeloid leukemia | 46 (41.1) |
| Acute lymphoblastic leukemia | 11 (9.8) |
| Myelodysplastic syndrome | 16 (14.3) |
| Chronic myelogenous leukemia | 4 (3.6) |
| Non-Hodgkin's lymphoma | 19 (17.0) |
| Adult T-cell leukemia/lymphoma | 10 (8.9) |
| Myeloproliferative disorder | 4 (3.6) |
| Plasma-cell myeloma | 2 (1.8) |
| *Disease status at transplant, n (%)* | |
| Standard risk | 66 (58.9) |
| High risk | 46 (41.1) |
| *Source of stem cells, n (%)* | |
| Related bone marrow or peripheral blood | 40 (35.7) |
| Unrelated bone marrow | 52 (46.4) |
| Unrelated cord blood | 20 (17.9) |
| *Conditioning regimen, n (%)* | |
| Conventional-intensity regimen | 54 (48.2) |
| BU/CY | 10 |
| TBI/CY-based regimen | 44 |
| Reduced-intensity regimen | 58 (51.8) |
| Flu/BU ± TBI | 23 |
| Flu/Mel ± TBI | 34 |
| Flu/TT | 1 |
| *Use of G-CSF, n (%)* | |
| Yes | 57 (50.9) |
| No | 55 (49.1) |
| *Duration from diagnosis to transplant, n (%)* | |
| ≤1 year | 57 (50.9) |
| >1 year | 55 (49.1) |
| *Duration from the last pretransplant cytotoxic chemotherapy to conditioning of transplant, n (%)* | |
| No history of previous cytotoxic chemotherapy or >2 months | 68 (60.7) |
| ≤2 months | 44 (39.3) |
| *Number of courses of previous cytotoxic chemotherapy, n (%)* | |
| ≤5 courses | 60 (53.6) |
| >5 courses | 52 (46.4) |
| *Pretransplant serum ferritin level (ng/mL)* | |
| Median (range) | 694.6 (34.7–12079.1) |
| *Pretransplant serum CRP level (mg/dL)* | |
| Median (range) | 0.1 (0.0–4.6) |

Abbreviations: Flu = fludarabine; Mel = melphalan; TT = thiotepa, G-CSF = granulocyte CSF.

### *Documented bacterial infections*

A total of 19 episodes of bacterial infections were documented during the first 30 days after HCT; these included 18 episodes of bloodstream infections and 1 of pneumonia. No patient had more than 1 episode of bacterial infection within 30 days after HCT. The bacterial organisms associated with the documented infections are listed in Table 2. The detected bacterial organisms were mainly Gram-negative bacilli ($n = 16$, 84.2%), 15 of which (93.6%) were sensitive to FQs.

**Table 2** Documented bacterial organisms within 30 days after transplantation

| *Category* | *Bacterial isolates* |
|---|---|
| Gram-positive cocci (*n*) | *Enterococcus faecium* (2)<br>*Streptococcus epidermidis* (1) |
| Gram-negative bacilli (*n*) | *Klebsiella pneumoniae* (5)<br>*Escherichia coli* (4)<br>*Pseudomonas aeruginosa* (2)<br>*Klebsiella oxytoca* (1)<br>*Enterobacter cloacae* (1)<br>*Capnocytophaga species* (1)<br>*Prevotella intermedia* (1)<br>*Bacteroides thetaiotaomicron* (1) |

*P. aeruginosa* was detected in the sputum of one patient with pneumonia. Other organisms were detected in blood culture bottles.

The cumulative incidence of bacterial infections was 16% (95% confidence interval (CI), 10–24%). Among confounding factors that were potentially associated with bacterial infection, only high pretransplant serum ferritin (>700 vs ⩽700 ng/mL) and high CRP (>0.3 vs ⩽0.3 mg/dL) levels were significantly associated with the development of bacterial infection in the multivariate analysis (hazard ratio (95% CI): ferritin, 3.97 (1.35–11.69), $P=0.012$; CRP, 3.63 (1.45–9.10), $P=0.006$) (Table 3). Even when serum ferritin and CRP levels were treated as continuous variables, their impact remained significant. Although there was no correlation between ferritin and CRP levels ($P=0.062$), we analyzed the impact of high ferritin levels in subgroups of patients with either high (>0.3 mg/dL) or low CRP levels (⩽0.3 mg/dL), to exclude the effect of inflammation on ferritin levels. We obtained almost consistent results in both groups (hazard ratio (95% CI): CRP >0.3 mg/dL, 3.67 (0.87–15.63), $P=0.078$; CRP ⩽0.3 mg/dL, 4.12 (0.86–19.64), $P=0.076$). Furthermore, no interaction was observed between the ferritin and CRP categories ($P=0.949$). Next, we re-evaluated the risk of bacterial infection with the combination of these two risk factors (ferritin and CRP levels). Figure 1 shows the cumulative incidence of bacterial infection for patients divided into three risk groups according to this model. The cumulative incidences of bacterial infections were 5.3% (95% CI; 1.0–15.7%) in patients without any risk factors ($n=39$), 20.5% (95% CI; 10.1–33.3%) in those with 1 factor ($n=44$), and 53.8% (95% CI; 24.8–76.0%) in those with two factors ($n=13$). The hazard ratios for 1 and 2 risk factors relative to no risk factors in the multivariate analysis were 4.04 (95% CI, 0.88–18.62) and 14.68 (3.02–71.30), respectively. Among patients with bacterial infections, septic shock or organ failure was observed in one patient with two risk factors and four patients with one risk factor, but not in any patients with no risk factors.

*Overall survival and treatment-related mortality*

Next, we evaluated the impact of the ferritin and CRP levels on other endpoints in 96 patients for whom data on ferritin levels were available (Figures 2 and 3). The median duration of follow-up was 23 months (range, 2.2–54.9). With regard to overall survival, only a high ferritin level (hazard ratio (95% CI): 2.47 (1.19–5.11), $P=0.015$) and a duration of less than 2 months from the last cytotoxic chemotherapy to the conditioning for transplant (hazard ratio (95% CI): 2.16 (1.10–4.26), $P=0.026$) were significant variables in the multivariate analysis. The causes of death are shown in Table 4. Interestingly, seven patients among those with high ferritin levels died within 100 days (causes of death: acute GVHD, $n=2$; infection, $n=3$; hepatic veno-occlusive disease, $n=1$; organ failure, $n=1$), whereas none of the patients with low ferritin levels died. With regard to treatment-related mortality, only ferritin and CRP levels were adversely associated with higher treatment-related mortality in the multivariate analysis (hazard ratio (95% CI): ferritin, 5.21 (1.41–19.30), $P=0.013$; CRP, 5.76 (1.70–19.48), $P=0.005$).

## Discussion

In our cohort of 112 patients with hematologic malignancies who underwent allogeneic HCT without antibacterial prophylaxis, we found that only high serum ferritin and high CRP levels before transplantation were significant risk factors for the post-transplant development of bacterial infection; patients with high ferritin levels and those with high CRP levels had an almost 4-fold higher risk of bacterial infection than those with low ferritin levels or those with low CRP levels. In addition, although severe complications associated with bacterial infection were observed in five patients with high ferritin levels and/or high CRP levels, none were seen in patients with low ferritin and low CRP levels. These results suggest that pretransplant serum ferritin and CRP levels may be useful markers for predicting the risk of early bacterial complications after allogeneic HCT.

An association between iron overload and bacterial or fungal infection has been shown in hereditary and secondary hemochromatosis.[21,22] With regard to HCT, Pullarkat *et al.*[16] reported that ferritin levels of ⩾1000 ng/mL were associated with a 2-fold higher risk of bloodstream infection compared with patients with ferritin levels of <1000 ng/mL in myeloablative HCT. In agreement with their finding, in this study, ferritin levels of >700 ng/mL were associated with a 4-fold increased risk compared with the risk in patients with levels of ⩽700 ng/mL. An increase in plasma non-transferrin-bound iron (NTBI) is considered to have an important role in the adverse effect of iron overload on bacterial infection. Under normal conditions, toxic reactions due to the production of NTBI are prevented by circulating transferrin, which forms a compound with $Fe^{3+}$.[23] However, plasma NTBI increases to a measurable level in patients with iron overload because transferrin is almost saturated with $Fe^{3+}$.[24] The inhibition of iron utilization in erythrocytes by chemotherapeutic agents and irradiation further increases NTBI levels.[25] Hydroxyl radical reactions by NTBI exacerbate mucosal damage caused by chemotherapeutic agents and irradiation, which allows bacterial organisms to enter through circulation.[26] In addition, iron is an important nutrient for the proliferation of bacteria and fungi.[27] In the HCT

**Table 3** Univariate and multivariate analyses of factors that are potentially associated with documented bacterial infection

| *Category* | *Number* | *Univariate analysis* | | *Multivariate analysis* | |
|---|---|---|---|---|---|
| | | *Hazard ratio (95% CI)* | P-*value* | *Hazard ratio (95% CI)* | P-*value* |
| Age, years | | | | | |
| ⩽50 | 10/64 | 1.00 | Reference | | |
| >50 | 9/48 | 1.27 (0.51–3.17) | 0.608 | | |
| *Sex* | | | | | |
| Female | 9/49 | 1.00 | Reference | | |
| Male | 10/63 | 0.80 (0.32–2.00) | 0.632 | | |
| *Diagnosis* | | | | | |
| Myeloid malignancies | 13/72 | 1.00 | Reference | | |
| Lymphoid malignancies | 6/40 | 0.86 (0.33–2.26) | 0.766 | | |
| *Disease status at transplant* | | | | | |
| Standard risk | 11/66 | 1.00 | Reference | | |
| High risk | 8/46 | 1.13 (0.45–2.84) | 0.795 | | |
| *Source of stem cells* | | | | | |
| Related bone marrow or peripheral blood | 6/40 | 1.00 | Reference | | |
| Unrelated bone marrow | 8/52 | 0.88 (0.30–2.59) | 0.816 | | |
| Unrelated cord blood | 5/20 | 1.84 (0.56–6.05) | 0.318 | | |
| *Conditioning regimen* | | | | | |
| Conventional-intensity regimen | 8/54 | 1.00 | Reference | | |
| Reduced-intensity regimen | 11/58 | 1.48 (0.57–3.79) | 0.419 | | |
| *Use of G-CSF* | | | | | |
| No | 10/55 | 1.00 | Reference | | |
| Yes | 9/57 | 0.98 (0.39–2.45) | 0.966 | | |
| *Duration from diagnosis to transplant* | | | | | |
| ⩽1 year | 9/57 | 1.00 | Reference | | |
| >1 year | 10/55 | 1.33 (0.53–3.34) | 0.550 | | |
| *Duration from the last pretransplant cytotoxic chemotherapy to conditioning of transplant* | | | | | |
| No history of previous cytotoxic chemotherapy or >2 months | 13/68 | 1.00 | Reference | | |
| ⩽2 months | 6/44 | 0.76 (0.29–2.03) | 0.589 | | |
| *Number of courses of previous cytotoxic chemotherapy* | | | | | |
| ⩽5 courses | 9/60 | 1.00 | Reference | | |
| >5 courses | 10/52 | 1.44 (0.57–3.64) | 0.441 | | |
| *Serum ferritin level* | | | | | |
| ⩽700 ng/mL | 5/49 | 1.00 | Reference | 1.00 | Reference |
| >700 ng/mL | 14/47 | 4.04 (1.35–12.05) | 0.012 | 3.97 (1.35–11.69) | 0.012 |
| Not available | 0/16 | — | — | — | — |
| *Serum CRP level* | | | | | |
| ⩽0.3 mg/dL | 10/84 | 1.00 | Reference | 1.00 | Reference |
| >0.3 mg/dL | 9/28 | 3.38 (1.36–8.39) | 0.009 | 3.63 (1.45–9.10) | 0.006 |

Abbreviations: CI = confidence intervals; G-CSF = granulocyte CSF.

setting, the ability of NTBI to induce the proliferation of *Staphylococcus epidermidis* has been shown in an *in vitro* study using the serum of patients undergoing HCT.[28]

In addition to the adverse impact of iron overload on early infection-related complications, several studies have suggested that high ferritin levels are adversely associated with overall survival and treatment-related mortality.[16,29–31] In agreement with these studies, our results showed that high ferritin levels are associated with a 2.5-fold increased risk of overall mortality and a 5-fold increased risk of higher treatment-related mortality, compared with low ferritin levels. These studies collectively suggest that iron overload is an important and strong prognostic factor in various clinical outcomes of allogeneic HCT.

Recently, an association between iron chelation therapy and longer overall survival was shown in patients with MDS or severe anemia requiring multiple blood transfusions,[32,33] and adequate iron chelation therapy is recommended for such patients.[34] The administration of oral iron-chelating agents such as deferasirox may be an attractive treatment for iron-overloaded patients compared with deferoxamine, which requires s.c. or i.v.

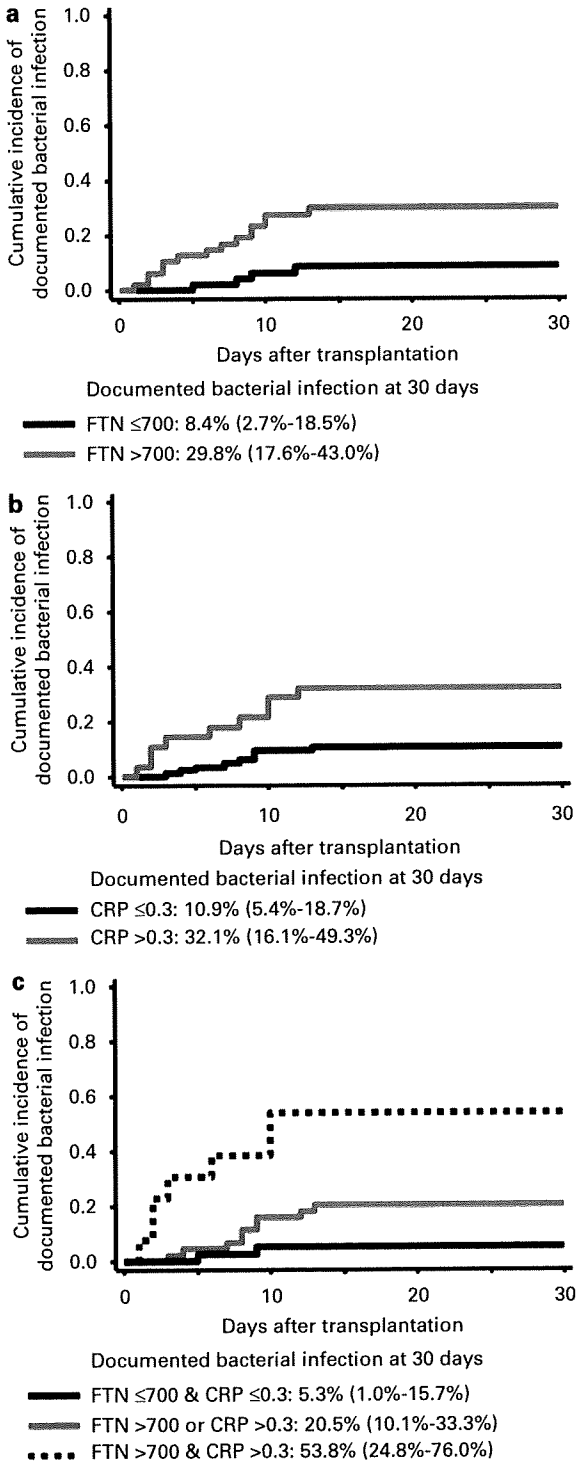


**Figure 1** Cumulative incidence of documented bacterial infection within 30 days after transplantation. (**a**) Solid black line, patients with low ferritin levels ($\leqslant$700 ng/mL) ($n=49$); gray line, patients with high ferritin levels ($>$700 ng/mL) ($n=47$), (**b**) Solid black line, patients with low CRP levels ($\leqslant$0.3 mg/dL) ($n=84$); gray line, patients with high CRP levels ($>$0.3 mg/dL) ($n=28$), (**c**) Solid black line, patients with low ferritin ($\leqslant$700 ng/mL) and low CRP levels ($\leqslant$0.3 mg/dL) ($n=39$); gray line, patients with low ferritin and high CRP levels ($>$0.3 mg/dL) or high ferritin ($>$700 ng/mL) and low CRP levels ($n=44$); dotted black line, patients with high ferritin and high CRP levels ($n=13$).

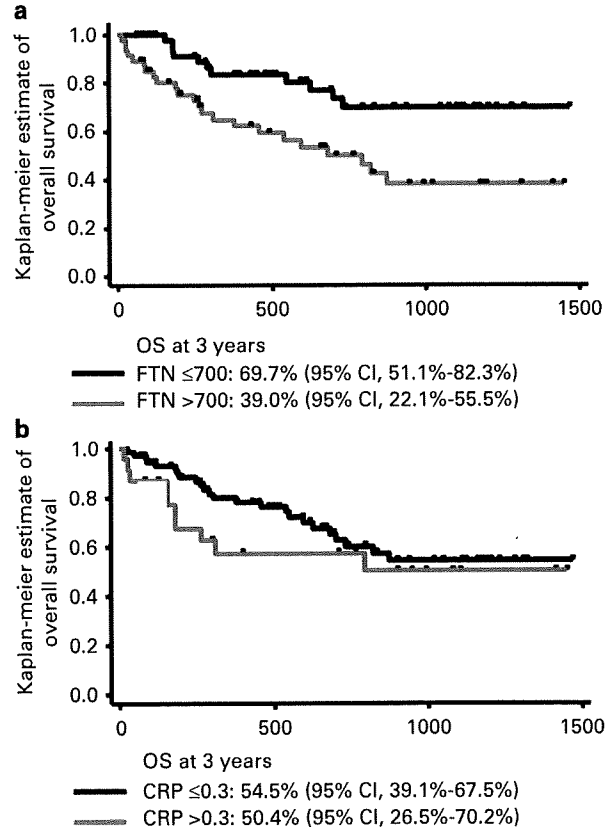


**Figure 2** Kaplan–Meier estimate of overall survival after transplantation. (**a**) Solid black line, patients with low ferritin levels ($\leqslant$700 ng/mL) ($n=49$); gray line, patients with high ferritin levels ($>$700 ng/mL) ($n=47$), (**b**) Solid black line, patients with low CRP levels ($\leqslant$0.3 mg/dL) ($n=73$); gray line, patients with high CRP levels ($>$0.3 mg/dL) ($n=23$).

administration. However, the optimal dosage and timing for the administration of deferasirox in allogeneic HCT should be carefully determined in future studies because its renal and gastrointestinal side effects may exacerbate complications of HCT.

At present, only one report has referred to the association between pretransplant CRP levels and transplant outcomes.[18] In that report, pretransplant CRP levels had a marginally significant association with infection within 100 days after reduced-intensity HCT, whereas other confounding factors, including age, disease status, hematopoietic cell transplantation-specific comorbidity index (HCT-CI), and performance status, had no association; this result is consistent with our present findings. One possible explanation of these findings is that the slightly elevated CRP levels might have reflected minute inflammation, which may represent the presence of latent bacterial infection with negative clinical signs and negative results in pretransplant screening tests, such as X-ray or CT scans. Undetectable bacterial organisms colonized under bacteriostatic conditions before transplant might have rapidly proliferated in the post-transplant neutropenic and immunosuppressive state. Therefore, even if no bacterial infection is detected before transplant in screening tests, latent bacterial infection should be considered in patients with high CRP levels. With regard to treatment-related

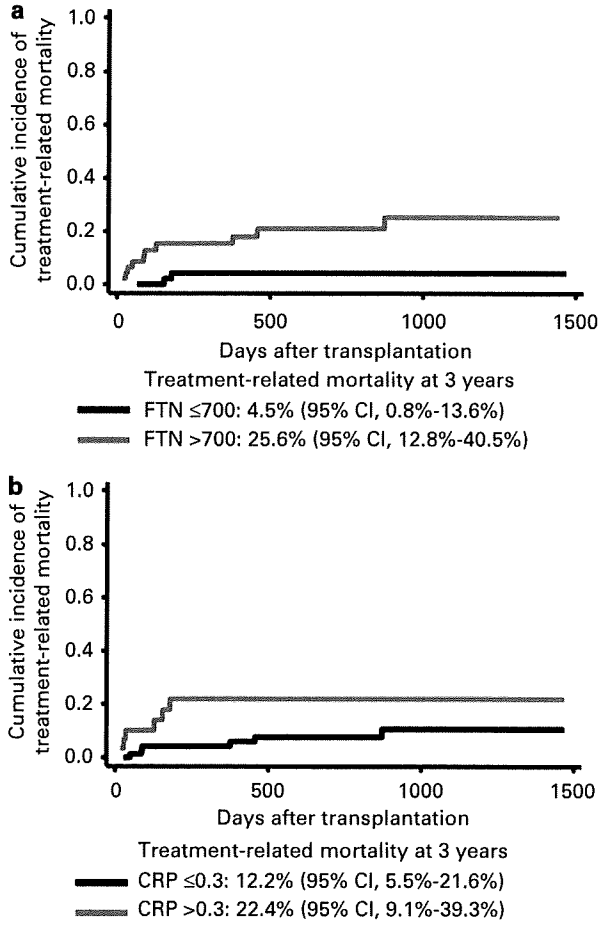


**Figure 3** Cumulative incidence of treatment-related mortality after transplantation. (**a**) Solid black line, patients with low ferritin levels (≤700 ng/mL) ($n=49$); gray line, patients with high ferritin levels (>700 ng/mL) ($n=47$), (**b**) Solid black line, patients with low CRP levels (≤0.3 mg/dL) ($n=73$); gray line, patients with high CRP levels (>0.3 mg/dL) ($n=23$).

**Table 4** Causes of death

| *Category* | *Low ferritin group (≤700 ng/mL) (n=49)* | *High ferritin group (>700 ng/mL) (n=47)* |
|---|---|---|
| *Within 100 days after transplant* | | |
| Infection | 0 | 2 (29%) |
| Organ failure | 0 | 2 (29%) |
| Graft-versus-host disease | 0 | 2 (29%) |
| Hepatic veno-occlusive disease | 0 | 1 (14%) |
| Total | 0 | 7 |
| *More than 100 days after transplant* | | |
| Relapse | 9 (82%) | 12 (75%) |
| Infection | 1 (9%) | 2 (13%) |
| Organ failure | 1 (9%) | 0 |
| Idiopathic pneumonia syndrome | 0 | 1 (6%) |
| Bleeding | 0 | 1 (6%) |
| Total | 11 | 16 |

mortality, an elevated pretransplant CRP level was found to be a significant risk factor in our study, consistent with a previous report.[18] The reason for the worse treatment-related mortality in patients with elevated pretransplant CRP levels remains unclear and needs to be clarified in future studies.

To ensure the safety of allogeneic HCT with the limited use of antibacterial agents, the selective prophylactic administration of antibacterial agents such as FQs only to patients at high risk of bacterial infection may be effective. In this study, Gram-negative bacilli that were highly sensitive to FQs (93.6%) were the main bacterial organisms isolated, which suggests that these infections may have been prevented by the prophylactic administration of FQs in our center. However, this approach may be effective only if most of the bacterial isolates at the transplant center were sufficiently sensitive to these prophylactic antibiotics. In future studies, it would be worthwhile evaluating whether the incidence of early bacterial infection can be reduced by the prophylactic administration of antibiotics in patients with predefined risk factors such as high ferritin levels or high CRP levels. Iron chelation therapy before HCT is another intriguing strategy that is worthy of future evaluation.

This study had several limitations. The retrospective study design, small sample size and heterogeneous background of diseases and transplantation procedures may have biased the results. In addition, HCT-CI, including the performance status, was not evaluated in this cohort due to a lack of adequate information. Furthermore, the impact of serum ferritin levels on the outcomes should be interpreted with caution. Although we consistently determined that high ferritin levels have an adverse impact on early bacterial infection regardless of CRP levels, serum ferritin levels can be affected by conditions associated with other diseases.[35] In a future study, it may be worthwhile to quantify iron overload by other methods, such as magnetic resonance imaging of the liver,[36] and to re-analyze the effect of iron content on the outcome.

In conclusion, these results suggest that pretransplant serum ferritin and CRP levels, which can be easily measured in various centers, may be useful markers for predicting the risk of early bacterial complications after allogeneic HCT. However, larger prospective studies are warranted to validate our findings and further research is needed to identify other biomarkers that may be associated with the development of post-transplant bacterial complications.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

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Bone Marrow Transplantation (2009), 1–3

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## LETTER TO THE EDITOR

# Impact of discontinuing fluoroquinolone prophylaxis on early mortality after allogeneic marrow or peripheral blood SCT with myeloablative conditioning

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Oral fluoroquinolones (FQs) or other antibiotics are commonly used as antibacterial prophylaxis after cytotoxic chemotherapy for malignant neoplasms, although significant practice variations have been reported among centers and countries.[1] Despite such practical variations, the efficacy of oral FQ as a prophylactic agent has not been fully evaluated in the SCT setting.[2,3] Further, the widespread emergence of multidrug-resistant microorganisms in hematology–oncology units has also increased the need for re-evaluating the role of antibacterial prophylaxis administered to patients undergoing cytotoxic chemotherapy or SCT.[4–9] In attempts to reduce the emergence of antibiotic-resistant bacterial organisms, since 2003, we have discontinued the administration of oral FQs as prophylactic agents for patients undergoing standard-dose chemotherapy other than SCT; further, since 2004, we have withdrawn the use of any antibacterial prophylaxis in auto- and allo-SCT recipients as well.[6] In allogeneic SCT after myeloablative conditioning regimens, the risk of bacterial infection is considerably high because high-dose chemotherapy and/or TBI may cause severe mucosal damage that facilitates bacterial translocation under profound post transplant immunosuppression. Therefore, we conducted a single-center retrospective study to evaluate the effect of such restriction of FQ prophylaxis on the incidence of bacterial infection and early mortality rate among patients receiving allo-SCT after myeloablative conditioning for hematologic malignancies.

The medical records were reviewed with respect to data on 145 consecutive adult patients with hematologic malignancies who underwent allogeneic myeloablative SCT with the use of T-cell-replete marrow or peripheral blood graft between January 2000 and December 2008 at our institution. Patients who had repeated episodes of bacterial infections, and those who had active infections before transplantation procedure were excluded; a total of 128 patients with the median age of 41.5 years (range, 17–61 years) were included in the analysis. Between January 2000 and August 2004, the patients received oral FQs (levofloxacin, tosufloxacin or ciprofloxacin) as antibacterial prophylactic agents (prophylaxis group). Patients who could not ingest oral drugs were temporarily administered anti-pseudomonal ß-lactams i.v. instead. From September 2004 to December 2008, the use of any prophylactic antibacterial agents, including oral FQs, was discontinued (non-prophylaxis group). In both groups, i.v. antibiotics with anti-pseudomonal activity were promptly administered in the episodes of febrile neutropenia or suspected bacterial infections.

There were no differences in patient characteristics between the prophylaxis and non-prophylaxis groups (Table 1), except with regard to the preferential use of G-CSF in the prophylaxis group, which conferred significantly earlier neutrophil engraftment as compared with the non-prophylaxis group (median day, 15 (range, 10–67) in the prophylaxis group and 19.5 (12–30), in the non-prophylaxis group, $P<0.001$). During the entire study period, a total of 12 episodes of bacterial infections were documented; these included 11 cases of bloodstream infections and a single case of pneumonia. All organisms detected in the prophylaxis group were resistant to FQs; most of these organisms were gram-positive cocci ($n=4$, 67%). On the other hand, four of the six organisms detected in the non-prophylaxis group were FQ-sensitive gram-negative bacilli. Organ failure and septic shock because of bacterial infection were not observed, except in one patient in the prophylaxis group who succumbed to infection with metallo-β-lactamase-producing multidrug-resistant *Pseudomonas aeruginosa*. In both groups, microbiologically documented infections developed during the early period after SCT (median day, 5.5 (range, 2–11) in the prophylaxis group and median day, 6.5 (1–13) in the non-prophylaxis group), and there was no statistically significant difference between the two groups in this regard ($P=0.750$). Although the cumulative incidence of microbiologically documented infections was slightly higher in the non-prophylaxis group (15%; 95% confidence interval (CI), 6–27%) than in the prophylaxis group (7%; 95% CI, 3%–13%), multivariate Cox analysis revealed this difference was not statistically significant (hazard ratio for the non-prophylaxis group vs prophylaxis group, 1.69; 95% CI, 0.40–7.08; $P=0.473$). The overall survival rates at 100 days after transplantation in the prophylaxis group and non-prophylaxis group were 89% (95% CI, 80–94%) and 90% (95% CI, 76–96%), respectively, with no significant difference between the groups in multivariate Cox analysis ($P=0.682$) (Figure 1).

The emergence of multidrug-resistant microorganisms is becoming a serious problem in clinical settings in which SCT and cytotoxic chemotherapy are performed. Frère *et al.*[5] studied the drug susceptibility of bacterial organisms isolated from 492 patients who underwent auto- or allo-SCT between 1982 and 1999. They reported that the susceptibility to ciprofloxacin among gram-positive and

**Table 1** Characteristics of patients

| *Characteristics* | *Prophylaxis group* (n = 87) | *Non-prophylaxis group* (n = 41) | P-*value* |
|---|---|---|---|
| *Age, years* | | | |
| Median (range) | 39 (17–61) | 43 (19–58) | 0.505 |
| *Sex* | | | |
| Male | 48 (55%) | 21 (51%) | 0.675 |
| Female | 39 (45%) | 20 (49%) | |
| *Diagnosis*[a] | | | |
| Myeloid neoplasms | 53 (61%) | 26 (63%) | 0.786 |
| Lymphoid neoplasms | 34 (39%) | 15 (37%) | |
| *Disease status at transplant*[b] | | | |
| Standard risk | 43 (49%) | 24 (59%) | 0.336 |
| High risk | 44 (51%) | 17 (42%) | |
| *Type of donor* | | | |
| HLA-identical sibling donor | 29 (33%) | 16 (39%) | 0.162 |
| Other related donor | 15 (17%) | 2 (5%) | |
| Unrelated donor | 43 (49%) | 23 (56%) | |
| *Stem cell source* | | | |
| BM | 72 (83%) | 39 (95%) | 0.054 |
| Peripheral blood | 15 (17%) | 2 (5%) | |
| *Conditioning regimen* | | | |
| TBI-based regimen | 76 (87%) | 34 (83%) | 0.134 |
| Busulfan-based regimen | 7 (8%) | 7 (17%) | |
| Other non-TBI regimen | 4 (5%) | 0 (0%) | |
| *Courses of chemotherapy before transplant* | | | |
| <5 | 42 (48%) | 21 (51%) | 0.756 |
| ⩾5 | 45 (52%) | 20 (49%) | |
| *Interval days between the last chemotherapy and transplant* | | | |
| <60 | 34 (39%) | 17 (42%) | 0.797 |
| ⩾60 | 53 (61%) | 24 (59%) | |
| *Hospitalized months before transplant* | | | |
| <3 | 18 (21%) | 10 (24%) | 0.417 |
| ⩾3, <6 | 33 (38%) | 19 (46%) | |
| ⩾6 | 36 (41%) | 12 (29%) | |
| *Use of G-CSF* | | | |
| Yes | 76 (87%) | 20 (49%) | <0.001 |
| No | 11 (13%) | 21 (51%) | |

Abbreviations: Busulfan-based regimen = conditioning regimen including 16 mg/kg of busulfan; Other non-TBI regimen = myeloablative conditioning regimen including ranimustine and melphalan without TBI nor busulfan; TBI-based regimen = conditioning regimen including more than 8 Gy of TBI.
Data are no. of patients (%) except for age.
[a]Myeloid neoplasms include AML, MDS, CML, and other myeloproliferative neoplasms, while lymphoid neoplasms include ALL, adult T-cell leukemia/lymphoma, and other mature B-cell or T-cell neoplasms.
[b]Patients were considered to have standard-risk disease if they received transplant without previous chemotherapy or in CR, while those who received transplant in all the other status were considered to have high-risk disease.

gram-negative bacterial isolates was more than 70% in 1990, while it was <30% in 1997–1998; this observation suggested that FQ prophylaxis might no longer promise its

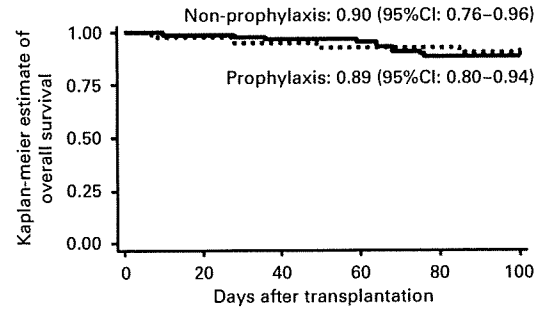


**Figure 1** Kaplan–Meier estimate of overall survival within 100 days after SCT. Solid black line, the prophylaxis group ($n = 87$); dotted black line, the non-prophylaxis group ($n = 41$); CI, confidence interval.

clinical benefit at least in SCT settings. Given the increasing prevalence of FQ-resistant microorganisms, trials involving neutropenic patients with hematologic malignancies have been conducted to evaluate the effects of withdrawal of FQs as prophylactic agents. It is noteworthy that these studies showed that the susceptibility of enterobacterial isolates to FQ was significantly restored after FQ prophylaxis was discontinued.[6,9] In addition, routine administration of FQs can induce cross-resistance to other antibiotics, such as β-lactams, through various mechanisms.[10] Therefore, the routine use of antibacterial prophylaxis in patients undergoing SCT should be carefully re-evaluated to reduce the prevalence of multidrug-resistant microorganisms.

Our present findings suggested that withdrawal of FQ as antibacterial prophylaxis is feasible without significant increase in the early mortality rate in allogeneic T-cell-replete BM or peripheral blood SCT after myeloablative conditioning, provided appropriate antibiotic treatment is promptly initiated in the event of febrile neutropenia. However, the retrospective study design, the heterogeneous underlying diseases in the small number of patients involved, and the variability in transplantation procedures used may have caused a bias in the results. Therefore, larger well-controlled prospective studies are needed to evaluate the role of antibacterial prophylaxis in BM or peripheral blood SCT after myeloablative conditioning. In addition, the significance of antibacterial prophylaxis in other SCT settings such as cord blood transplantation or SCT after reduced-intensity conditioning is also worth evaluating in the future study.

## Conflict of interest

The authors declare no conflict of interest.

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