Hyperglycemia During the Neutropenic Period Is Associated With a Poor Outcome in Patients Undergoing Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation

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Background. Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) frequently require support with parenteral nutrition and immunosuppressive drugs, which introduce the risk of hyperglycemia. Van den Berghe et al. showed that the strict glucose control improved the outcome of patients treated in the intensive care unit, and this point was evaluated in this study in a HSCT setting.

Methods. A cohort of 112 consecutive adult patients treated by myeloablative allogeneic HSCT between January 2002 and June 2006 was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, preexisting infectious diseases, preexisting neutropenia or previous allogeneic HSCT. The remaining 91 patients were categorized according to mean fasting blood glucose (BG) level in the neutropenic period after conditioning: normoglycemia (BG <110 mg/dL, n=28), mild hyperglycemia (110 to 150 mg/dL, n=49), and moderate/severe (>150 mg/dL, n=14). The primary endpoint was the occurrence of febrile neutropenia (FN) and documented infection during neutropenia, and the secondary endpoints included organ dysfunction according to the definition used by van den Berghe, acute graft-versus-host disease (GVHD), overall survival, and nonrelapse mortality (NRM).

Results. Although the incidence of FN or documented infections was similar between the three groups, hyperglycemia was significantly associated with an increased risk of organ dysfunction, grade II–IV acute GVHD, and NRM.

Conclusions. While the results suggested an association between the degree of hyperglycemia during neutropenia and an increased risk of posttransplant complications and NRM, the possibility that intensive glucose control improves the outcome after HSCT can only be confirmed in a prospective randomized trial.

Keywords: Allogeneic transplantation, Hyperglycemia, Nonrelapse mortality, Acute graft-versus-host disease.

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Van den Berghe et al. showed with patients nursed in the intensive care unit (ICU) that the rigid control of hyperglycemia with intensive insulin therapy to keep the blood glucose level at 80–110 mg/dL reduced morbidity, including infections, and mortality compared to patients who received standard care maneuvers that maintained the level at <200 mg/dL (*1–3*). Although these results have been confirmed in several subsequent studies (*4–7*), the precise mechanism that underlies this association is unclear. In animal models, it has been shown that insulin itself has a direct inhibitory effect on the inflammation process (*8, 9*). However in human studies, it has been suggested that these benefits could be directly attributed to intense glucose control rather than to any pharmacological activity of administered insulin per se (*3, 4*).

Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) suffer from serious complications including infection, graft-versus-host disease (GVHD) and organ dysfunction. They are also at higher risk of hyperglycemia due to the use of steroids for the treatment of graft-versus-host disease (GVHD), prolonged total parenteral nutrition (TPN), immunosuppressive drugs, and infectious complications (*10, 11*). This makes them susceptible to numerous serious complications, including multiple organ failure (*12–14*). In this study, we evaluated whether hyperglycemia during the cytopenic pe-

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riod after conditioning for HSCT could be a significant risk factor for the subsequent clinical course.

PATIENTS AND METHODS

Patient Characteristics

A cohort of 112 consecutive adult patients who received myeloablative allogeneic HSCT between January 2002 and June 2006 at the National Cancer Center Hospital (Tokyo, Japan) was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, pre-existing infectious diseases or neutropenia before HSCT, and previous allogeneic HSCT. The remaining 91 patients were subjected to further analysis, and their characteristics are listed in Table 1. Their median age was 36 years (range, 18–57 years), and their diagnosis included acute myeloid leukemia (AML, n=41), acute lymphoblastic leukemia (ALL, n=21), non-Hodgkin lymphoma (NHL, n=13), myelodysplastic syndrome (MDS, n=10), and chronic myelogenous leukemia (n=6). Standard-risk patients included those with acute leukemia in first complete remission, chronic leukemia in first chronic phase, MDS in refractory anemia, and NHL in complete remission, and the remaining patients were categorized as high-risk. Forty-six and 45 patients received a graft from a related donor and an unrelated donor, respectively. Stem cell sources included bone marrow (n=46), peripheral blood (n=41), and cord blood cells (n=4). In this study, only two patients were diagnosed as type 2 diabetes mellitus before HSCT, which reflects the low prevalence of this condition in Japan, especially in younger patients who can be the target of allogeneic HSCT with a myeloablative conditioning regimen. These two diabetic patients were included in the moderate and severe hyperglycemia group. None of the patients, including these two patients, had major organ dysfunction or diabetic complications before HSCT. For the transplantation procedure, signed informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

All patients received a myeloablative conditioning regimen that included oral busulfan (BU) plus cyclophosphamide (CY, n=45), CY plus 12 Gy total body irradiation (TBI, n=43) or cytarabine (CA) plus CY plus TBI (n=3: Table 1). GVHD prophylaxis included cyclosporine- (n=62) and tacrolimus-based regimens (n=29), with an additional short course of methotrexate (MTX) in 89 patients. Granulocyte

TABLE 1. Patient characteristics

Variable	Normoglycemia (<110 mg/dl)	Mild hyperglycemia (110–150 mg/dl)	Moderate and severe hyperglycemia (>150 mg/dl)
N	28	49	14
Blood glucose, median mg/dl (range)	104 (81–109)	120 (110–150)	168 (150–211)
Age, median years (range)	31 (21–52)	36 (18–57)	45 (30–57)
<40	20 (71)	32 (65)	4 (29)
≥40	8 (29)	17 (35)	10 (71)
Sex			
Male	9 (32)	34 (69)	8 (57)
Female	19 (68)	15 (31)	6 (43)
Disease risk			
Standard	16 (57)	18 (37)	6 (43)
High	12 (43)	31 (63)	8 (57)
Conditioning			
TBI-containing	11 (39)	26 (53)	9 (64)
Non-TBI-containing	17 (61)	23 (47)	5 (36)
GVHD prophylaxis			
Cyclosporine-based	24 (86)	33 (67)	5 (36)
Tacrolimus-based	4 (14)	16 (33)	9 (74)
Relation to donor			
Related	19 (68)	24 (49)	3 (21)
Unrelated	9 (32)	25 (51)	11 (79)
Stem cell source			
Bone marrow	11 (39)	24 (49)	11 (79)
PBSC	16 (57)	22 (45)	3 (21)
Cord blood	1 (4)	3 (6)	0 (0)
HLA match			
Match	25 (89)	34 (69)	10 (71)
Mismatch	3 (11)	15 (31)	4 (29)

Data are n (%) unless noted.
TBI, total body irradiation; GVHD, graft-versus-host disease; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen.

colony-stimulating factor (G-CSF) was administered in all patients from day +6 after transplantation until engraftment. Most patients received ciprofloxacin (200 mg orally three times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and varicella zoster virus until the cessation of immunosuppressive agents. Prophylaxis against *Pneumocystis jiroveci* infection consisted of trimethoprim-sulfamethoxazole (400 mg of sulfamethoxazole once daily) from the first day of conditioning to day −3 of transplantation, and from day +28 until day +180 or the cessation of immunosuppressive agents. Patients who developed fever during the neutropenic period were treated with cefepime, and additional agents including vancomycin, aminoglycosides and amphotericin B were given as clinically indicated. Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count exceeded 0.5×10^9/L.

Grouping of Patients

Patients were categorized according to the mean blood glucose (BG) level in the preengraftment neutropenic period: normoglycemia BG maintained at <110 mg/dL (group 1, n=28), mild hyperglycemia at 110–150 mg/dL (group 2, n=49), and moderate/severe hyperglycemia at >150 mg/dL (group 3, n=14). Blood glucose level was routinely tested in the morning at least three times a week. Daily caloric intake was calculated by dietitian following the chart record.

Outcome Measures

The primary outcome measure was the occurrence of febrile neutropenia (FN) and documented infection including bacteremia, pneumonia and central venous catheter infection in the neutropenic period. Secondary outcome measurements were organ dysfunction in the neutropenic period, acute GVHD, overall survival (OS) and nonrelapse mortality (NRM). Organ dysfunction was defined with reference to van den Berghe (*5–7*) as follows: 1) hypercreatininemia: serum creatinine level ≥2.0 mg/dL or more than twice the baseline; 2) hyperbilirubinemia: serum total bilirubin level ≥2.0 mg/dL; and 3) increased inflammatory markers: serum C-reactive protein (CRP) level ≥15 mg/dL. Acute GVHD was graded by the Consensus Criteria (*15*).

Statistical Analyses

Standard descriptive statistics were used. The Student's *t*-test, chi-square, and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. Multiple logistic regression analysis was conducted to ascertain odds ratios (ORs) and 95% confidence intervals (CIs). OS was estimated using Kaplan-Meier curves. The cumulative incidences of NRM were estimated based on a Cox regression model for the cause-specific hazards by treating progressive disease or relapse as a competing event. Cox proportional hazard models were used for multivariate analysis of variables on NRM and OS after HCT. Clinical factors that were assessed for their association with NRM and OS included patient age, sex, conditioning regimen (TBI-based vs. non-TBI-based), donor [human leukocyte antigen (HLA)-matched vs. HLA-mismatched, related vs. unrelated], GVHD prophylaxis (cyclosporine-based vs. tacrolimus-based) and disease risk (standard vs. high). Factors with $P<0.10$ in the univariate analyses were subjected to a multivariate analysis. A level of $P<0.05$ was defined as statistically significant. All P values are two-sided. All analyses were performed using SPSS 10.0 statistical software (Chicago, IL).

RESULTS

Patients and Transplantation Characteristics

The median ages of the patients in the normoglycemia, mild hyperglycemia, and moderate/severe hyperglycemia groups were, respectively, 31, 36, and 45 years. The percentages of patients who received graft from an unrelated donor were 32%, 51%, and 79%, and the percentages of patients who received GVHD prophylaxis with tacrolimus were 14%, 33%, and 74%. To clarify the risk factor to be included in moderate and severe hyperglycemia group, logistic analysis was performed, which showed older age and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.04$, OR 3.9 (1.1–14.0), and $P=0.01$, OR 5.5 (1.5–20.3), respectively], and there was a trend that patients who received stem cell from unrelated donor were associated with moderate and severe hyperglycemia [$P=0.07$, OR 3.6 (0.9–14.2)]. Multiple logistic analysis showed age more than 40 years old and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.042$, OR 4.1 (1.1–15.7), and $P=0.01$, OR 5.8 (1.5–22.1), respectively].

Although in practice we generally keep the parenteral glucose dose relatively low to avoid severe metabolic complications including hyperglycemia and hyperlipidemia during the acute phase of allogeneic HSCT, the possibility that the dose of parenteral nutrition affects the blood glucose level should be explored. We calculated the total caloric intake by combining both oral and parenteral nutrition. Although the mild hyperglycemia group received significantly more parenteral nutrition than the normoglycemia group (group 1 694+322 kcal/day vs. group 2 969+383 kcal/day), overall there was no essential difference in caloric intake between the three groups (1070+303 kcal/day, 1190+393 kcal/day, 1045+530 kcal/day, respectively). The median duration of the follow-up time in surviving patients was 809 days (range, 132-1530 days) in group 1, 369 days (105–1550 days) in group 2, and 587 days (170–774 days) in group 3. Described as hydrocortisone-equivalent dose, the median dose of corticosteroid used during neutropenia was 0 mg (0–1610 mg) in group 1, 100 mg (0–9700 mg) in group 2, and 375 mg (0–2468 mg) in group 3. Statistically more dose of corticosteroid was used in group 2 and group 3, compared with group 1.

Primary Endpoints

The incidence of FN and documented infections is summarized in Table 2. The incidences of FN and documented infections including bacteremia, pneumonia, and central venous catheter infection in groups 1, 2 and 3 were, respectively, 89% and 32% (25%, 4% and 11%), 88% and 20% (16%, 6% and 6%), and 98% and 43% (36%, 14% and 14%). Overall, no statistically significant difference was observed between the three groups in the incidence of infectious episodes, including FN and documented infections.

TABLE 2. Endpoints

Variable	Normoglycemia (<110 mg/dl)	Mild hyperglycemia (110–150 mg/dl)	Moderate and severe hyperglycemia (>150 mg/dl)
N	28	49	14
Febrile neutropenia	23 (89)	43 (88)	13 (98)
Documented infection	9 (32)	10 (20)	6 (43)
Bacteremia	7 (25)	8 (16)	5 (36)
Pneumonia	1 (4)	3 (6)	2 (14)
Central-venous catheter infection	3 (11)	3 (6)	2 (14)
Organ dysfunction			
Hypercreatininemia	1 (4)	4 (8)	4 (29)
Hyperbilirubinemia	3 (11)	11 (22)	6 (43)
Increased inflammatory markers	4 (14)	15 (31)	9 (64)

Data are n (%).
Hypercreatininemia, serum creatinine level ≥2.0 mg/dl or more than twice of baseline; hyperbilirubinemia, serum bilirubin level ≥2.0 mg/dl; increased inflammatory markers, serum C-reactive protein level ≥15 mg/dl.

Secondary Endpoints

The incidence of hypercreatininemia was 4% in group 1, 8% in group 2 and 29% in group 3, as summarized in Table 2, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 1.1–108.6; P=0.018) and group 2 (OR 4.5, 95% CI 1.0–21.1; P=0.043). The incidence of hyperbilirubinemia was, respectively, 11%, 22% and 43%, in the three groups, and that in group 3 was significantly higher than that in group 1 (OR 6.3, 95% CI 1.3–30.9; P=0.017). The incidence of increased inflammatory markers was, respectively, 14%, 31% and 64%, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 2.4–49.5; P<0.001) and group 2 (OR 4.1, 95% CI 1.2–14.3; P=0.022). Multiple logistic regression analysis showed that the degree of hyperglycemia was associated with hypercreatininemia, hyperbilirubinemia, and increased inflammatory markers (Table 3).

TABLE 3. Multiple logistic regression analysis for organ dysfunction and multiple variate analysis for acute GVHD, nonrelapse mortality, and overall survival

Outcomes and variables	Odds/hazard ratio	95% CI	P value
Multiple logistic regression analysis			
Hypercreatininemia			
Hyperglycemia	5.2	1.1–24.6	0.039
Hyperbilirubinemia			
Hyperglycemia	4.9	1.6–14.9	0.005
Increased inflammatory markers			
Hyperglycemia	6.7	2.2–20.3	0.001
Tacrolimus-based	6.9	1.6–30.5	0.011
Multivariate analysis (Cox-proportional hazard model)			
Acute GVHD			
Hyperglycemia	2.3	1.2–4.3	0.013
Disease risk (high)	2.3	1.0–5.1	0.047
HLA mismatch	2.8	1.3–5.9	0.009
Nonrelapse mortality			
Hyperglycemia	2.9	1.2–6.6	0.013
Disease risk (high)	2.7	0.9–8.7	0.091
Overall survival			
Hyperglycemia	2.0	1.1–3.6	0.019
TBI-containing	2.3	1.1–5.0	0.035
Disease risk (high)	1.9	0.9–4.1	0.10

Odds ratios are presented for multiple logistic regression analysis; hazard ratios are presented for multivariate analysis.
GVHD, graft versus host disease; TBI, total body irradiation.

The cumulative incidence of grade II–IV acute GVHD is shown in Figure 1. The degree of hyperglycemia was associated with a higher incidence of grade II–IV acute GVHD

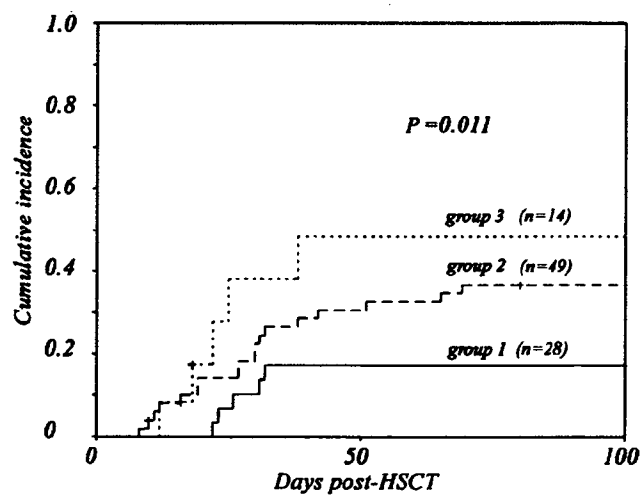

FIGURE 1. Cumulative incidence of acute GVHD grade II–IV stratified according to the mean glucose level during neutropenia. Group 1 included patients with normoglycemia, group 2 included patients with mild hyperglycemia, and group 3 included patients with moderate and severe hyperglycemia.

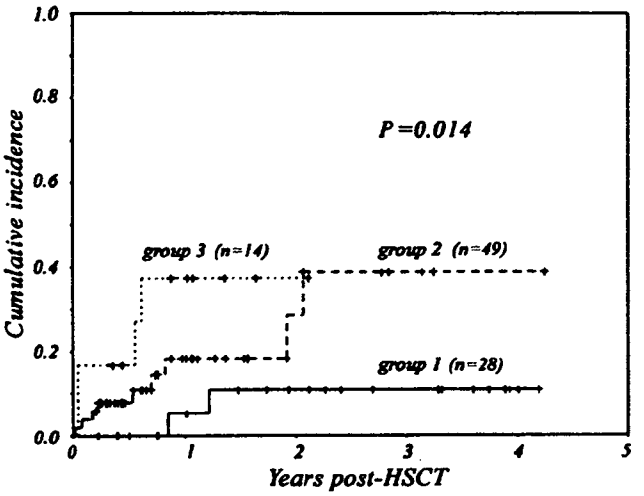

FIGURE 2. Cumulative incidence of treatment-related mortality stratified according to the mean glucose level during neutropenia.

(P=0.002). A Cox proportional hazard model showed that hyperglycemia, high-risk underlying disease, and HLA mismatch were risk factors for grade II-IV acute GVHD (Table 3).

The cumulative incidence of NRM was, respectively, 5%, 17%, and 35% at 1 year, and was significantly related to the degree of hyperglycemia (P=0.014; Fig. 2). The probability of OS was, respectively, 88%, 70%, and 56%, and was significantly associated with hyperglycemia (P=0.008; Fig. 3). A Cox proportional hazard model showed that the degree of hyperglycemia was associated with NRM and OS (Table 3).

DISCUSSION

In this study, we evaluated whether hyperglycemia during the cytopenic period after conditioning for HSCT could be a significant risk factor for the subsequent clinical course. Infectious diseases remain a major cause of morbidity and mortality in patients who receive HSCT, and we speculated that this might be exaggerated in the presence of hyperglycemia. Alternatively, hyperglycemia can be caused by infectious diseases and also aggravates infectious diseases to lead to a vicious cycle, with resultant morbidities that include organ dysfunction and mortality. Theoretically, strict glucose control should prevent this vicious cycle and help to reduce morbidity and mortality in patients after HSCT, as shown previously in ICU settings (*1, 2*). However, in this study the incidences of FN and documented infections were not different among the three groups. On the other hand, we found that hyperglycemia was associated with organ dysfunction and increased inflammatory markers, which was consistent with previous reports that demonstrated the impact of hyperglycemia on clinical outcomes of patients suffering from nonhematological diseases (*1–3, 12–14*). Additionally, a multivariate analysis showed that hyperglycemia was a risk factor for acute GVHD.

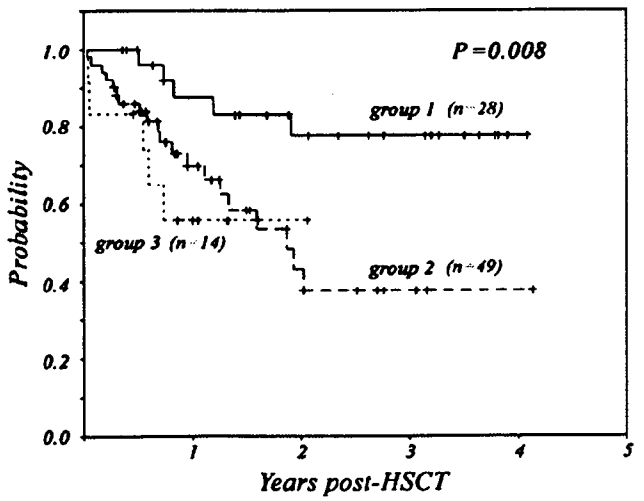

FIGURE 3. Overall survival stratified according to the mean glucose level during neutropenia.

The reason for the association between early hyperglycemia and late complications needs to be clarified. The increase in the levels of circulating cytokines due to hyperglycemia may further aggravate hyperglycemia itself (*16–21*). Therefore, this condition which occurs during the critical period of neutropenia before engraftment may influence the afferent phase of acute GVHD, as suggested by Ferrara et al. Elevated cytokine levels during the afferent phase then lead to subsequent acute GVHD in the effector phase (*22, 23*). Teshima et al. reported that the effector phase of acute GVHD is not antigen-specific and inflammatory cytokines mediate target destruction (*24*), and other reports have shown that inflammatory cytokines were required in acute GVHD and these molecules can cause tissue damage (*25–27*). With these reports in mind, it is reasonable to speculate that the aggravated production of inflammatory cytokines by hyperglycemia may be a risk factor in the pathogenesis of acute GVHD and organ dysfunction.

This study has several limitations, including heterogeneous patient populations and a retrospective nature. First, hyperglycemia can be caused by infection itself and it has been previously shown that the level of hyperglycemia was correlated with the severity of illness (*4*). In this retrospective study, we could not confirm whether hyperglycemia directly influenced organ dysfunction or increased inflammatory markers. Furthermore, statistically more corticosteroid was used in the group of moderate and severe hyperglycemia, and statistically more parenteral nutrition was used in the group of mild hyperglycemia. However, the observation that hyperglycemia and the severity of illness were independently associated with a worse prognosis has been well confirmed in the ICU setting (*4*), and several prospective studies have shown that intensive glucose control reduced both morbidity and mortality (*1, 2*). Considering these findings, we suggest that our data still support the possibility that the degree of hyperglycemia was associated with morbidity and mortality in the allogeneic HSCT setting. Second, we must consider that the patients who developed moderate and severe hyperglycemia included older patients, those who received more unrelated grafts, and those who received tacrolimus compared to other groups. In terms of immunosuppressive drugs, tacrolimus has recently become a preferred immunosuppressive drug for GVHD prophylaxis in unrelated or HLA-mismatched HSCT, based on the results of two Japanese studies, which showed that, compared to cyclosporine, tacrolimus was associated with a lower incidence of acute GVHD and better overall survival, which were similar to those in related HSCT, even

after HSCT with alternative donors, including unrelated donors (*28, 29*). Therefore, the effect of unrelated graft and tacrolimus on the incidence of acute GVHD and NRM might not be significant in this study.

The effects of tacrolimus on hyperglycemia, hyperbilirubinemia, and hypercreatininemia need to be clarified. It is well known that hyperglycemia occurs more often in patients receiving tacrolimus than in those receiving cyclosporine (*30–32*). In the present study, patients receiving tacrolimus were more likely to have moderate to severe hyperglycemia. However, the association of hyperbilirubinemia with tacrolimus has not been previously reported and two other studies (*33, 34*) showed that cyclosporine was more likely to cause hyperbilirubinemia than tacrolimus after allogeneic HSCT or kidney transplantation. Although the relative nephrotoxicity attributed to tacrolimus compared to cyclosporine has been controversial (*30, 33, 35*), studies that have reported such nephrotoxicity used a higher target tacrolimus level (>20 ng/ml) (*30, 35*). On the other hand, it has been reported that the use of lower levels of tacrolimus (10–15 ng/ml in our hospital) was associated with reduced complications in allogeneic HSCT (*36, 37*), with no difference in the incidence of hypercreatininemia compared to cyclosporine (*33*). Based on a consideration of all of these results, we think that tacrolimus might not be the direct cause of hypercreatininemia in this study. Finally, due to the nature of this retrospective study, during the period evaluated we did not apply any consistent protocol for glucose control and nutritional support, although we tried to avoid severe hyperglycemia (BG ≥200 mg/dl), which certainly biases the interpretation of the data, although it has been reported that the overall glucose level, rather than the dose of insulin administered, directly influenced the outcome of patients (*3*).

Even with these limitations, we believe that our observation is still of value in considering the clinical impact of the strict control of hyperglycemia during the early phase of HSCT. To confirm our preliminary observation, a prospective pilot study is underway to assess the effect of intensive glucose control after HSCT. If this pilot study shows a beneficial effect of intensive glucose control, a prospective randomized trial would be warranted to confirm the possibility that intensive glucose control improves the outcome after HSCT. Additionally, in this ongoing pilot study, we evaluate the diurnal blood glucose and insulin levels, including postprandial levels, to detect hyperglycemia more precisely before transplantation since the level of HgA1c is affected by both the blood glucose level and the turnover rate of red blood cells, and would not precisely correlate with the true mean blood glucose level in patients who received courses of blood transfusion for anemia.

In conclusion, the association of the degree of hyperglycemia during neutropenia and an increased risk of post-transplant complications and NRM was suggested, but the possibility that intensive glucose control improves the outcome after HSCT would only be confirmed in a prospective randomized trial.

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REFERENCES

1. Van den Berghe G, Wouters P, Weekwrs F, et al. Intensive insulin therapy in the critically ill patients. *N Engl J Med* 2001; 345: 1359.
2. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006; 354: 449.
3. Van den Berghe G, Wouters PJ, Bouillon R, et al. Outcome benefit of intensive insulin therapy in the critically ill: Insulin dose versus glycemic control. *Crit Care Med* 2003; 31: 359.
4. Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc* 2003; 78: 1471.
5. Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. *Mayo Clin Proc* 2004; 79: 992.
6. Vogelzang M, Nijboer JM, van der Horst IC, et al. Hyperglycemia has a stronger relation with outcome in trauma patients than in other critically ill patients. *J Trauma* 2006; 60: 873.
7. Ingels C, Debaveye Y, Milants I, et al. Strict blood glucose control with insulin during intensive care after cardiac surgery: Impact on 4-years survival, dependency on medical care, and quality-of-life. *Eur Heart J* 2006; 27: 2716.
8. Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin attenuates the systemic inflammatory response in endotoxemic rats. *Endocrinology* 2004; 145: 4084.
9. Brix-Christensen V, Andersen SK, Andersen R, et al. Acute hyperinsulinemia restrains endotoxin-induced systemic inflammatory response: An experimental study in a porcine model. *Anesthesiology* 2004; 100: 861.
10. Sheean PM, Freels SA, Helton WS, Braunschweig CA. Adverse clinical consequences of hyperglycemia from total parenteral nutrition exposure during hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; 12: 656.
11. Sheean PM, Braunschweig C, Rich E. The incidence of hyperglycemia in hematopoietic stem cell transplant recipients receiving total parenteral nutrition: A pilot study. *J Am Diet Assoc* 2004; 104: 1352.
12. Fietsam R Jr., Bassett J, Glover JL. Complications of coronary artery surgery in diabetic patients. *Am Surg* 1991; 57: 551.
13. Ortiz A, Ziyadeh FN, Neilson EG. Expression of apoptosis-regulatory genes in renal proximal tubular epithelial cells exposed to high ambient glucose and in diabetic kidney. *J Invest Med* 1997; 45: 50.
14. Vanhorebeek I, De Vos R, Mesotten D, et al. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 2005; 365: 53.
15. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; 15: 825.
16. Cavallo MG, Pozzilli P, Bird C, et al. Cytokines in sera from insulin-dependent diabetic patients at diagnosis. *Clin Exp Immunol* 1991; 86: 256.
17. Morohoshi M, Fujisawa K, Uchimura I, Numano F. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996; 45: 954.
18. Borst SE. The role of TNF-alpha in insulin resistance. *Endocrine* 2004; 23: 177.
19. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* 2002; 106: 2067.
20. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor α inhibits signaling from the insulin receptor. *Proc Natl Acad Sci USA* 1994; 91: 4854.
21. Tsigos C, Papanicolaou DA, Kyrou I, et al. Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. *J Clin Endocrinol Metab* 1997; 82: 4167.
22. Ferrara JL, Reddy P. Pathophysiology of graft-versus-host disease. *Semin Hematol* 2006; 43: 3.
23. Ferrara JL, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. *Int J Hematol* 2003; 78: 181.
24. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med* 2002; 8: 575.
25. Laster SM, Wood JG, Gooding LR. Tumor necrosis factor can induce both apoptotic and necrotic forms of cell lysis. *J Immunol* 1988; 141: 2629.
26. Schmaltz C, Alpdogan O, Muriglan SJ, et al. Donor T cell-derived TNF

is required for graft-versus-host disease and graft-versus-tumor activity after bone marrow transplantation. *Blood* 2003; 101: 2440.
27. Krenger W, Ferrara JL. Dysregulation of cytokines during graft-versus-host disease. *J Hematother* 1996; 5: 3.
28. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2001; 28: 181.
29. Yanada M, Emi N, Naoe T, et al. Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: A nationwide survey conducted in Japan. *Bone Marrow Transplant* 2004; 34: 331.
30. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood* 1998; 92: 2303.
31. Webster AC, Woodroffe RC, Taylor RS, et al. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: Meta-analysis and meta-regression of randomised trial data. *BMJ* 2005; 331: 810.
32. McAlister VC, Haddad E, Renouf E, et al. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. *Am J Transplant* 2006; 6: 1578.
33. Woo M, Przepiorka D, Ippoliti C, et al. Toxicities of tacrolimus and cyclosporin A after allogeneic blood stem cell transplantation. *Bone Marrow Transplant* 1997; 20: 1095.
34. Margreiter R; European Tacrolimus vs Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. *Lancet* 2002; 359: 741.
35. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 2000; 96: 2062.
36. Wingard JR, Nash RA, Przepiorka D, et al. Relationship of tacrolimus (FK506) whole blood concentrations and efficacy and safety after HLA-identical sibling bone marrow transplantation. *Biol Blood Marrow Transplant* 1998; 4: 157.
37. Przepiorka D, Nash RA, Wingard JR, et al. Relationship of tacrolimus whole blood levels to efficacy and safety outcomes after unrelated donor marrow transplantation. *Biol Blood Marrow Transplant* 1999; 5: 94.

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Invasive Fungal Infection Following Reduced-Intensity Cord Blood Transplantation for Adult Patients with Hematologic Diseases

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ABSTRACT

Invasive fungal infection (IFI) is a significant complication after allogeneic hematopoietic stem cell transplantation (HSCT); however, we have little information on its clinical features after reduced intensity cord blood transplantation (RICBT) for adults. We reviewed medical records of 128 patients who underwent RICBT at Toranomon Hospital between March 2002 and November 2005. Most of the patients received purine-analogbased preparative regimens. Graft-versus-host disease (GVHD) prophylaxis was a continuous infusion of either tacrolimus 0.03 mg/kg or cyclosporine 3 mg/kg. IFI was diagnosed according to the established EORTC/NIH-MSG criteria. IFI was diagnosed in 14 patients. Thirteen of the 14 had probable invasive pulmonary aspergillosis and the other had fungemia resulting from *Trichosporon* spp. Median onset of IFI was day 20 (range: 1-82), and no patients developed IFI after day 100. Three-year cumulative incidence of IA was 10.2%. Four of the 13 patients with invasive aspergillosis (IA) developed grade II-IV acute GVHD, and their IA was diagnosed before the onset of acute GVHD. The mortality rate of IFI was 86%. Multivariate analysis revealed that the use of prednisolone >0.2 mg/kg (relative risk 7.97, 95% confidence interval 2.24-28.4, P = .0014) was a significant risk factor for IA. This study suggests that IFI is an important cause of deaths after RICBT, and effective strategies are warranted to prevent IFI.

KEY WORDS

Invasive aspergillosis • Graft-versus-host disease • Corticosteroid

INTRODUCTION

Cord blood transplantation (CBT) is an attractive alternative for patients with hematologic diseases who lack a matched related or unrelated donor. The usefulness of CBT using myeloablative preparative regimens has been confirmed for pediatric patients [1,2]. Myeloablative CBT for adult patients achieves engraftment in 90% of the patients, but carries 50% risk of transplant-related mortality (TRM), mostly resulting from infection [3,4]. We and other groups have reported the feasibility of CBT using reduced-intensity regimens (RICBT) for adult patients with advanced hematologic diseases [5,6].

Because of delayed immune recovery and graft-versus-host disease (GVHD), infection is the leading cause of TRM after CBT using myeloablative preparative regimens [2-4,7]. However, studies on immune recovery following RICBT gave us hope that RICBT recipients may less frequently experience GVHD and infectious complications. Invasive fungal infection (IFI) has been 1 of the most feared infectious complications in conventional allogeneic marrow or peripheral blood stem cell (PBSC) transplantation [8,9], whereas we have little information on IFI following RICBT. We investigated its incidence and clinical features in patients who underwent RICBT for advanced hematologic diseases.

PATIENTS AND METHODS

Data Collection

We reviewed medical records of 128 recipients who underwent first reduced-intensity allogeneic hematopoietic stem cell transplantation (HSCT) using cord blood (CB) between March 2002 and November 2005 at Toranomon Hospital. Their characteristics are shown in Table 1. Of the 128 patients, 101 had high-risk diseases including acute myelogenous leukemia (AML) in relapse or the second and higher complete remission (CR; n = 42), acute lymphoid leukemia (ALL) except those in the first CR (n = 12), chronic myelogenous leukemia (CML) in blastic phase (n = 4), myelodysplasia except refractory anemia (n = 10), refractory lymphoma (n = 30), idiopathic myelofibrosis (n = 1), plasma cell leukemia in relapse (n = 1), and chronic myelomonocytic leukemia (n = 1).

Table 1. *Patients' Characteristics and Transplantation Procedures*

Variables	Number
Patients Characteristics	
Age, median (range)	56 (17-71)
Sex, male/female	80/48
Primary diseases	
AML/MDS	63
Malignant lymphoma	33
Acute lymphoblastic leukemia	17
Severe aplastic anemia	6
Chronic myelogenous leukemia	6
Chronic myelomonocytic leukemia	1
Plasmacytic leukemia	1
Idiopathic myelofibrosis	1
Risk of underlying diseases,*1 high/low	101/27
Prior autologous stem cell transplant, yes/no	9/119
Transplantation procedures	
Conditioning regimen	
Flu + Mel + TBI 2 Gy or 4 Gy/8 Gy	112/2
Flu + BU + TBI 4 Gy/8 Gy	8/1
Others	5
GVHD prophylaxis, cyclosporine/tacrolimus	64/64
Number of infused nucleated cell, median (range) $\times 10^7$/kg	2.7 (1.6-4.8)
HLA disparity (antigen), 2/1/0	108/17/3
Transplantation outcomes	
Neutrophil engraftment	99/128
Complete donor chimerism*2	90/99
Grade II-IV acute GVHD*2	45/99
Chronic GVHD*3*4	11/40
CMV antigenemia*3	48/93
CMV disease	10
Relapse*2	24/98

*1 We divided the risk of transplantation into two groups. The low-risk group was as follows: acute myelogenous or lymphoid leukemia in first and second remission, chronic myelogenous leukemia in chronic phase, and myelodysplastic syndrome refractory anemia. The other patients were defined as having high-risk diseases.

*2 Percentage was calculated based on 99 patients who achieved primary engraftment.

*3 Percentage was calculated based on the number of patients who achieved engraftment and evaluated.

*4 No patients received systemic corticosteroids for the treatment of chronic GVHD.

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndromes; ULN, upper limit of normal; GVHD, graft-versus-host disease; CMV, cytomegalovirus.

Transplantation Procedures and Supportive Cares

Transplantation procedures were shown in Table 1, and we previously reported details of the procedures [6]. GVHD prophylaxis was either tacrolimus 0.03 mg/kg or cyclosporine 3 mg/kg continuous infusion starting on day −1. Trough blood levels of these drugs were monitored 2-3 times a week and the dosage were modified to maintain the target level of 10-15 ng/mL for tacrolimus and 200-400 ng/mL for cyclosporine [10-12]. Immunosuppressants were tapered off from day 100 until day 150. If grade II-IV acute GVHD (aGVHD) developed, 1-2 mg/kg/day of methylprednisolone was added to cyclosporine or tacrolimus, and tapered from the beginning of clinical response.

The diagnosis and management of preengraftment immune reactions were reported previously [13].

Management of Infections

Patients were managed in reverse isolation laminar airflow-equipped rooms. All patients received tosufloxacin 450 mg/day from the start of conditioning until neutrophil engraftment. Fluconazole 200 mg/day or micafungin 150 mg/day, and acyclovir 600 mg/day were given from the start of conditioning until the discontinuation of GVHD prophylaxis, which were restarted when patients developed GVHD and were treated with steroids and immunosuppressants. They received prophylaxis with trimethoprim-sulfamethoxazole against *Pneumocystis jirovecii* infection from the start of conditioning until the discontinuation of immunosuppressants or disappearance of chronic GVHD (cGVHD). When patients develop neutropenic fever, tosufloxacin was changed to broad-spectrum antibiotics [14]. Intravenous administration of amphotericin B at a dose of 0.5 mg/kg/day was added when the fever persisted for more than 5 to 7 days. If the diagnosis of aspergillus infection was confirmed, the dosage of amphotericin B was increased to 1.0 mg/kg/day. We used blood tests, enzyme-linked immunosorbent assay for galactomannan antigen, (1-3)-beta-D glucan assay, and chest computed tomography for the early diagnosis of invasive aspergillosis (IA), as previously reported [15]. Because most patients had been heavily treated and received multiple transfusions prior to transplantation, anti-CMV antibodies were not examined before transplantation. All patients were monitored for cytomegalovirus pp65 antigenemia once a week. When CMV antigenemia exceeded 10/50,000, patients preemptively received foscarnet 30 mg/kg intravenously twice daily.

Diagnostic Criteria for IFI

Invasive fungal infection was diagnosed according to the established EORTC/NIH-MSG criteria [16]. Briefly, we diagnosed patients as having proved IFI when any 1 of the following examinations was positive: histopathologic or cytopathologic examinations for hyphae or yeasts in needle aspiration or biopsy specimens, fungal cultures obtained from normally sterile sites by sterile procedures, and *Cryptococcus* antigen in cerebrospinal fluid. Probable IFI was diagnosed when a patient satisfied at least 1 host factor, microbiologic criteria, and clinical criteria. Possible IFI was not included in this study. The day of diagnosis of IFI was defined as the day when the first diagnostic test was performed.

Endpoints and Statistical Analysis

The cumulative incidence of IA was evaluated using Gray's method, considering death without IA as a competing risk [17].

Potential confounding factors considered in the analysis of risk factors of IA were age, sex, disease status, previous stem cell transplantation, conditioning regimens, HLA mismatch, stem-cell dose (all nucleated cells, and CD 34-positive cells), GVHD prophylaxis, grade II-IV aGVHD, and use of prednisolone. Proportional hazard modeling was used to evaluate the influence of these factors on the incidences of IA treating the development of aGVHD and the use of prednisolone as time-dependent covariates. Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. *P*-values of <.05 were considered statistically significant. Survival was estimated by the Kaplan-Meier method. Median follow-up of surviving patients was 628 days (range: 26-1347 days).

RESULTS

Clinical Outcomes after RICBT

Ninety-nine (77%) patients achieved primary engraftment at a median of day 20 (range: 9-53 days). Of the remaining 29 patients who failed to achieve primary engraftment, 4 patients received second RICBT, and the other 25 patients died before engraftment. Their causes of death included bacteremia (n = 22), invasive pulmonary aspergillosis (IPA) (n = 1), and progression of primary disease (n = 2). Of the 99 engrafted patients, 45 and 22 patients developed grade II and grade III-IV aGVHD, respectively. The median onset of grade II-IV aGVHD was day 28 (range, 11-92). Eleven of 40 patients (28%) who survived longer than 100 days without disease progression developed cGVHD. Estimated 3-year overall survival was 33% (95% confidence interval (95% CI), 24%-42%). Causes of deaths comprised nonrelapse mortality (n = 31) and disease progression (n = 23). Infection was the leading cause of nonrelapse mortality (n = 20). Autopsy was performed in 5 patients (3.9%) in this series of patients.

Incidence and Clinical Features of IFI

Invasive fungal infection was diagnosed in 14 patients. Their clinical features are shown in Table 2. Thirteen of the 14 patients had probable IA, and the other had fungemia from *Trichosporon* spp. Three-year cumulative incidence of probable IA was 10.2% (Figure 1). Median onset of IFI was day 20 (range: 1-82), and no patients developed IFI after day 100. IFI was diagnosed after day 30 in 1 patient. Prophylactic uses of antifungal agents included fluconazole (n = 12) and micafungin (n = 1) among the 13 patients with IA. Of the 63 patients who survived 100 days or longer, none developed IFI after day 100. Four of the 13 patients with IFI developed grade II-IV aGVHD, and their diagnosis of IFI was before the onset of aGVHD (Table 2).

Seven patients were given prednisolone >0.2 mg/kg/day for the treatment of preengraftment immune reactions, of whom 5 developed grade II-IV aGVHD. Twelve of the 14 patients with IFI died, and the mortality rate was 86%. IFI was the primary cause of deaths in 4 patients.

Risk Factors of IPA

Table 3 shows the results of univariate and multivariate analyses. Reactivation of cytomegalovirus (CMV) is a well-known risk factor of IA [18,19]. However, it was not included in the analysis of this study, because the onset of IFI was earlier than the onset of CMV antigenemia. Use of prednisolone >0.2 mg/kg (relative risk [RR], 7.97; 95% CI, 2.24-28.4; $P = .0014$) was a significant risk factor in multivariate analysis.

DISCUSSION

The present study demonstrated that IFI early after RICBT is a significant complication. Among IFI, the incidence of IA was high, which was consistent with the studies on reduced intensity stem cell transplantation (RIST) using other stem cell sources [9,19]. Our results contrasted with the previous reports that the incidence of infection because of non-*Candidia albicans* species was high in myeloablative allogeneic stem cell transplantation [20]. The observations may be associated with the milder gastrointestinal mucosal toxicity by conditioning regimens in RIST than in myeloablative transplantation [21] and the less fre-

Table 2. *Clinical Characteristics of Patients with Invasive Aspergillosis*

UPN	Age	Sex	Primary Disease	Disease Status at Transplant	No. of Prior Regimens	Neutrophil Engraftment Day	Grade II-IV Acute GVHD Onset Day	PSL Started	Invasive Fungal Infection (IFI)*1	Onset (Day)	Other Infectious Complication	Overall Survival (Day)	Outcomes of IFI	Causes of Death
286	57	M	AML	PIF	3				Probable IA	4		4	dead	IFI
365	69	M	AML	RL I	3				Probable IA	3		14	dead	IFI
411	56	F	ML	PD	1			11	Probable IA	19	Bacteremia	24	dead	Bacteremia complicated with IA
196	61	M	AML	PIF	2	11	22		*T. cutaneum* fungemia	1		28	dead	IFI
202	62	M	AML	RL I	4	21	21	8	Probable IA	21	Bacteremia	28	dead	Bacteremia complicated with IA
344	55	F	ML	PD	1	19			Probable IA	4	Bacteremia	30	dead	IFI
262	59	M	AML	PIF	1			10	Probable IA	20		31	dead	PD
151	52	F	MDS	RAEB	3	20		7	Probable IA	12		33	improved	GI bleeding
114	52	F	ML	PD	4	13		0	Probable IA	23		39	dead	PD
153	70	M	AML	1st CR	2	14	30		Probable IA	29	Bacteremia	46	dead	IP
197	33	M	MDS	RA	0				Probable IA	28	Bacteremia	47	dead	Bacteremia complicated with IA
160	66	M	ML	PD	2	14	29	9	Probable IA	25		75	dead	MOF
120	70	F	SAA		0	13			Probable IA	82		1308+	improved	
127	20	M	SAA		3	31	55	12	Probable IA	3		1347+	improved	

AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndromes; CR, complete remission; GI, gastrointestinal; IA, invasive aspergillosis; IFI, invasive fungal infection; SAA, severe aplastic anemia; RA, refractory anemia; PD, progressive disease; PIF, primary induction failure; RARB, refractory anemia with excess of blasts; MOF, multiple organ failure.

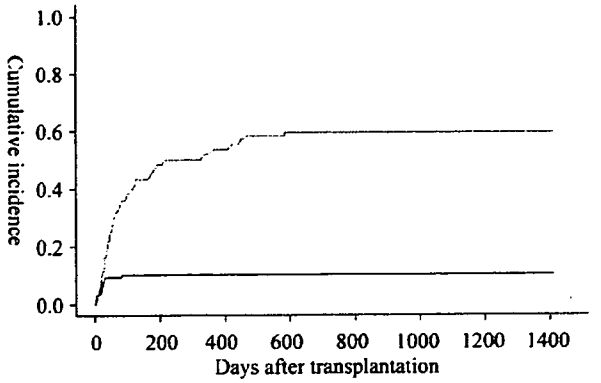

Figure 1. Cumulative incidence of invasive aspergillosis (IA) considering death without IA as a competing risk. Black line indicates incidence of death with IA, and gray line indicates incidence of death without IA.

quent and milder GVHD following CBT. Because gastrointestinal mucosal toxicity is milder in RICBT than in myeloablative transplantation, the incidence of infection from non-*Candida albicans* species as part of the gastrointestinal normal flora might be low, and hence aspergillus infection might become the majority of IFI.

The median onset of IFI was day 20 (range: 1-82) in the present study; the majority developed IFI early after RICBT. Majority of the patients who developed IFI died of causes other than fungal infection (Table 2), as reported previously by Saavedra et al. [7]. Our results were consistent with a previous report on CBT [18], and contrasted to reports on RIST using marrow or peripheral blood [19,22,23], in which IA develops late after transplantation. The low incidence of IA after day 100 would be related to the low incidence of cGVHD. The short duration from RICBT to IFI development suggests aggravation of latent infection, which would have existed before transplantation. These findings were consistent with a recent report by Martino et al. [23]. Given the possibility, several issues need to be addressed in the management of IFI following RICBT. First, selection of RICBT candidates would have to include accurate evaluation for the risk of fungal infection [24] and high-risk patients might need to be excluded from the indication of RICBT. Pretransplant CT scan of the chest and sinus would be useful in the screening of IA following RICBT, and bronchoalveolar lavage should be performed in patients with abnormal findings. Second, the importance needs to be stressed in prophylactic antifungal agents with antiaspergillus activity and attempts for early diagnosis of aspergillosis such as methods using molecular techniques [25], antigen tests [26], and imaging tests [15,22]. Third, the way of steroid use after RICBT requires further investigations. The present study showed that the administration of steroids 0.2 mg/kg and more was a strong risk factor of IA (RR, 7.97; 95% CI, 2.24-28.4; $p = .0014$). Our observation that the use of small-dose steroids was a risk factor of IFI after RICBT supports the previous results of severe immunosuppression early after RICBT [27]. In RICBT using our regimens, immunologic reactions such as a preengraftment immune reaction frequently develops in addition to GVHD, requiring steroid administration early after RICBT [13]. Because steroids suppress phagocyte activities and cellular immunity [28], the risk of fungal infection early after RICBT may be increased.

The incidence of late IFI was not high in the present study. Of 102 patients who survived longer

Table 3. *Univariate and Multivariate Analyses for the Incidence of IA*

	Incidence of IPA (95% CI)	P Value
Univariate analysis		
Pretransplantation factors		
Age		.30
<55	7%	
≥55	13%	
Sex		.94
Female	11%	
Male	10%	
Disease risk		.63
Standard	16%	
High	9%	
Previous ASCT		.55
No	11%	
Yes	0%	
Regimen		.72
FM-based	11%	
FB-based	10%	
HLA mismatch		.076
0 or 1 antigen	0%	
2 antigens	12%	
Cell dose		.42
ANC <2.5 × 10^7/kg	8%	
ANC ≥2.5 × 10^7/kg	12%	
Cell dose		.03
CD34 <0.8 × 10^6/kg	5%	
CD34 ≥0.8 × 10^6/kg	16%	
GVHD prophylaxis		.42
Cyclosporine	13%	
Tacrolimus	8%	
Fungal prophylaxis		.99
Fluconazole	10%	
Micafungin	10%	
Posttransplantation factors (time-dependent covariates)		
Acute GVHD		.96
Grade 0-I	1.00	
Grade II-IV	1.06 (0.12-9.40)	
Prednisolone		.001
<0.2 mg/kg/day	1.00	
≥0.2 mg/kg/day	7.97 (2.24-28.4)	
Multivariate analysis		
Prednisolone		.0014
<0.2 mg/kg/day	1.00	
≥0.2 mg/kg/day	7.97 (2.24-28.4)	

GVHD indicates graft-versus-host disease; ASCT, autologous stem cell transplantation.

than 30 days, 1 patients developed IFI after day 30. None of 63 patients who survived longer than 100 days developed IFI after day 100. Our results contrast with the previous reports on BMT and PBSC transplantation where improvement in fungal management decreased early IFI and late IFI became the majority of IFI [8,19,22,29]. In myeloablative CBT, late infection is considered a significant complication [4], whereas study results focused on fungal infection have not been published. Some hypotheses can explain the low incidence of late IFI after RICBT. First, cGVHD after RICBT is uncommon and mild. There is minimal effect of cGVHD on delay in immune recovery following RICBT. Second, steroids are not frequently administered late after RICBT for the treatment of complications such as GVHD. The incidence of cGVHD was 28% in the present study, and none of them required steroid treatments. Further studies are awaited for the clinical features of late IFI after RICBT.

The present study demonstrated clinical features of fungal infections after RICBT, leaving several issues to be investigated. First, the present study is a small-sized retrospective 1 . Unrecognized bias might affect the study results, and we obtained little information on rare fungal infections such as Fusarium and Zygomycetes. Large-sized prospective studies are awaited. Second, the diagnostic yields of IFI need to be addressed. Most of the diagnoses in our study were made based on EORTC/MSG criteria [16] using clinical, laboratory, and imaging findings. Although the clinical usefulness of the diagnostic criteria has been established, pathologic diagnosis of IFI was not confirmed in many patients and the diagnostic yields remain unclear. Underestimation of IFI incidence also remains possible, because postmortem examinations were not obtained in most patients who died without diagnosis of IFI. Because such limitations cannot be avoided in studying deep fungal infections [30], clinicians need to be aware of the limitations.

REFERENCES

1. Gluckman E. Hematopoietic stem-cell transplants using umbilical-cord blood. *N Engl J Med.* 2001;344:1860-1861.
2. Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. *N Engl J Med.* 1998;339:1565-1577.
3. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. *N Engl J Med.* 2004;351:2276-2285.
4. Laughlin MJ, Eapen M, Rubinstein P, et al. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. *N Engl J Med.* 2004;351:2265-2275.
5. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. *Blood.* 2003;102:1915-1919.
6. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. *Clin Cancer Res.* 2004;10:3586-3592.
7. Saavedra S, Sanz GF, Jarque I, et al. Early infections in adult patients undergoing unrelated donor cord blood transplantation. *Bone Marrow Transplant.* 2002;30:937-943.
8. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood.* 2002;100:4358-4366.
9. Kojima R, Kami M, Nannya Y, et al. Incidence of invasive aspergillosis after allogeneic hematopoietic stem cell transplantation with a reduced-intensity regimen compared with transplantation with a conventional regimen. *Biol Blood Marrow Transplant.* 2004;10:645-652.
10. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood.* 2000;96:2062-2068.
11. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 2001;28:181-185.
12. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood.* 1998;92:2303-2314.
13. Kishi Y, Kami M, Miyakoshi S, et al. Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. *Transplantation.* 2005;80:34-40.
14. Hughes WT, Armstrong D, Bodey GP, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis.* 2002;34:730-751.
15. Kami M, Tanaka Y, Kanda Y, et al. Computed tomographic scan of the chest, latex agglutination test and plasma (1-3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. *Haematologica.* 2000;85:745-752.
16. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis.* 2002;34:7-14.
17. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med.* 1999;18:695-706.
18. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis.* 2002;34:909-917.
19. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. *Blood.* 2003;102:827-833.
20. Wingard JR. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. *Clin Infect Dis.* 1995;20:115-125.

21. Sakiyama M, Kami M, Hori A, et al. Regimen-related toxicity following reduced-intensity stem-cell transplantation (RIST): comparison between Seattle criteria and National Cancer Center Common Toxicity Criteria (NCI-CTC) version 2.0. *Bone Marrow Transplant.* 2004;34:787-794.
22. Kojima R, Tateishi U, Kami M, et al. Chest computed tomography of late invasive aspergillosis after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant.* 2005;11:506-511.
23. Martino R, Parody R, Fukuda T, et al. Impact of the intensity of the pretransplantation conditioning regimen in patients with prior invasive aspergillosis undergoing allogeneic hematopoietic stem cell transplantation: a retrospective survey of the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Blood.* 2006;108:2928-2936.
24. Einsele H, Quabeck K, Muller KD, et al. Prediction of invasive pulmonary aspergillosis from colonisation of lower respiratory tract before marrow transplantation [letter]. *Lancet.* 1998;352:1443.
25. Kami M, Fukui T, Ogawa S, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis.* 2001;33:1504-1512.
26. Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood.* 2001;97:1604-1610.
27. Narimatsu H, Matsumura T, Kami M, et al. Bloodstream infection after umbilical cord blood transplantation using reduced-intensity stem cell transplantation for adult patients. *Biol Blood Marrow Transplant.* 2005;11:429-436.
28. Hebart H, Bollinger C, Fisch P, et al. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individuals and patients with hematologic malignancies. *Blood.* 2002;100:4521-4528.
29. Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of aspergillus infections in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis.* 1997;175:1459-1466.
30. Kami M, Machida U, Okuzumi K, et al. Effect of fluconazole prophylaxis on fungal blood cultures: an autopsy-based study involving 720 patients with haematological malignancy. *Br J Haematol.* 2002;117:40-46.

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Value of pretransplant screening for colonization of *Pseudomonas aeruginosa* in reduced-intensity umbilical cord blood transplantation for adult patients

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Dear Editor,

Bloodstream infection (BSI) is a serious complication after reduced-intensity cord blood transplantation (RI-CBT) [1]. Although BSI caused by gram-negative bacteria is less frequent than BSI of gram-positive organisms, it leads to considerable toxicities in RI-CBT recipients [1]. Most gram-negative organisms colonize in the alimentary tract, and mucosal damages due to preparative regimens allow their transition from colonization to BSI [2]. Surveillance cultures as pretransplant screening might be helpful for predicting the development of BSI; however, the high rates of false-positive results limit the clinical application of surveillance cultures even in patients at high-risk of chemotherapy-induced mucositis [2–5]. Some researchers reported that colonization of *P. aeruginosa*, which frequently causes fatal BSI after RI-CBT [1], might be a useful marker for predicting the development of BSI in patients who receive cytotoxic chemotherapy [2, 3]. We investigated whether the isolation of *P. aeruginosa* by pretransplant screening culture could predict BSI after RI-CBT.

Between January 2002 and March 2004, we obtained pretransplant screening cultures from 46 patients who underwent RI-CBT at Toranomon Hospital. Transplantation procedures and supportive cares were described previously [1], and patients' characteristics are shown in Table 1. Tosufloxacin 450 mg/day was given for prophylaxis against bacterial infections. All the patients provided written informed consent in accordance with the requirements of the Institutional Review Board. Definition of BSI was described previously [1]. Multidrug-resistance of *P. aeruginosa* was defined as resistance to fluoroquinolones, β-lactams, and aminoglycosides [6]. Cumulative incidence of BSI was evaluated by Gray's method, and death without BSI and relapse or progression of underlying diseases were considered as competing risks.

Pretransplant screening cultures were positive for *P. aeruginosa* in 6 of the 46 patients (13%; Table 1). Three of the six patients with positive *P. aeruginosa* cultures and 3 of the 40 patients with negative *P. aeruginosa* cultures developed BSI of *P. aeruginosa* within 30 days of RI-CBT (Table 1). The frequencies of developing BSI of *P. aeruginosa* in the two groups were significantly different (50% vs 7.5%, $p=0.022$). The positive predictive value was 50%, and the negative predictive value was 92.5%. The

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Table 1 Characteristics of the RI-CBT patients who underwent pretransplant screening culture

Variable		Patients with *P. aeruginosa* colonization (n=6)	Patients without *P. aeruginosa* colonization (n=40)
Patient backgrounds			
Age	Median (range)	54.5 (41–70)	55 (17–79)
Risk of underlying diseases[a]	High/low	5/1	28/12
Primary diseases	Acute lymphoblastic leukemia	1	3
	Acute myeloid leukemia	1	13
	Adult T cell leukemia	0	6
	Myelodysplastic syndrome	0	4
	Malignant lymphoma	2	11
	Multiple myeloma	1	1
	Aplastic anemia	1	2
Preparative regimens	Fludarabine, melphalan, TBI	6	38
	Fludarabine, busulfan, TBI	0	1
	Fludarabine, melphalan	0	1
Number of infused nuclear cells	Median (range), X10E7/kg	3.2 (2.3–4.3)	2.8 (1.7–5.2)
Number of infused CD34+ cells	Median (range), X10E5/kg	0.71 (0.4–1.23)	0.67 (0.27–3.28)
HLA matching	6/5/4	0/0/6	1/6/33
Transplantation-related mortality within 30 days		2(33%)	8(20%)
Pretransplant screening			
Cultured sites of *P. aeruginosa*	Throat/stool/urine	4/2/1	NA
Number of patients colonized with multidrug-resistant *P. aeruginosa*		2	NA
BSI due to *P. aeruginosa*			
Number of patients who developed BSI due to *P. aeruginosa* within 30 days of RI-CBT		3(50%)	3(7.5%)
	Onset day [median (range)]	4 (1–11)	18 (1–19)
Median neutrophil count at onset day (/L)		0	0
Antibiotic use for bacterial infection at onset day[b]			
	Fluoroquinolones	1	2
	β-Lactams	0	1
	Carbapenems	2	0
	Aminoglycosides	1	0
	Vancomycin	1	1
Response to antibiotics	(responded/not responded)	0/3	0/3
Mortality within 30 days in the patients with BSI due to *P. aeruginosa*		2(66%)	3(100%)

RI-CBT indicates reduced-intensity cord blood transplantation; *TBI* total body irradiation; *BSI* blood stream infection; *NA* not applicable

[a] We defined acute leukemia in complete remission, chronic myelogenous leukemia in chronic phase, malignant lymphoma in complete remission, multiple myeloma in complete remission, myelodysplastic syndrome in refractory anemia (RA) and aplastic anemia as low/high risk and the others as high risk.

[b] All the six patients received antibiotics at onset day.

BSI was fatal in five patients. Multidrug-resistant *P. aeruginosa* was cultured at pretransplant screening in two patients; one developed fatal BSI of multidrug-resistant *P. aeruginosa*, and the other died of pneumonia.

The present study suggested that pretransplant screening for *P. aeruginosa* colonization can predict its BSI and might be helpful for reducing transplant-related mortality after RI-CBT. Our results are in contrast with the previous studies that did not recommend routine surveillance cultures before cytotoxic chemotherapy and conventional allogeneic stem-cell transplantation (allo-SCT) due to its poor predictive value [2, 4]. Some hypotheses can be postulated on these observations. First, most patients in our study had received antibiotics during previous chemotherapies followed by prophylactic fluoroquinolones after RI-CBT, and they were at high risk of colonization of antibiotic-resistant organisms. Appropriate use of antibiotics is warranted to avoid colonization of such organisms.

Second, the preparative regimens including melphalan and total body irradiation, which have a significant mucosal toxicity, might have been associated with the development of BSI due to *P. aeruginosa*. As three of the six patients colonized with *P. aeruginosa* developed its BSI early after transplantation, damages to the gastrointestinal mucosa by preparative regimens might have allowed *P. aeruginosa* to enter the bloodstream. Use of preparative regimens with minimal mucosal toxicity might be beneficial to reduce the risk of BSI after RI-CBT. Alternatively, keratinocyte growth factor, which promotes the regeneration of damaged mucosa [7], is also worth investigating.

Optimal management of patients with colonization by *P. aeruginosa* has to be established. Prophylactic antimicrobial therapies directed against *P. aeruginosa* may only lead to the selection of multidrug-resistant isolates and will not improve the patients' outcomes. Rapid treatments including antibiotics against isolated *P. aeruginosa* might be important. Physicians should be alert to the clinical manifestations related to *P. aeruginosa* infection in these patients.

Infection of multidrug-resistant bacteria is a significant concern in allo-SCT. When allo-SCT recipients are colonized with methicillin-resistant *Staphylococcus aureus*, use of mupirocin calcium ointment is recommended to eliminate the bacteria [8]. Meanwhile, optimal management of multidrug-resistant *P. aeruginosa* has not been established in allo-SCT. BSI of multidrug-resistant *P. aeruginosa* causes high mortality [6], whereas the elimination of multidrug-resistant *P. aeruginosa* is difficult [9]. In the present study, BSI due to multidrug-resistant *P. aeruginosa* was fatal despite intensive antibiotic therapy. While all the patients enrolled in this study received fluoroquinolone-based prophylaxis, polymyxin might be worth investigating. It is the most consistently effective agent against *P. aeruginosa* in vitro [10]. Other options include novel antibiotic combinations, such as macrolides, tobramycin, trimethoprim, and rifampin [10]. Alternatively, RI-CBT might better be avoided in patients with colonization of multidrug-resistant *P. aeruginosa*.

We demonstrated that pretransplant screening of *P. aeruginosa* colonization could be predictive of the development of its BSI after RI-CBT. This study provided novel information to establish the risk stratification in the management of BSI after RI-CBT, although it was a small-sized, retrospective study. We plan to further investigate whether the risk stratification based on the results of pretransplant screening cultures is useful to reduce the risk of BSI after RI-CBT.

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References

1. Narimatsu H, Matsumura T, Kami M et al (2005) Bloodstream infection after umbilical cord blood transplantation using reduced-intensity stem cell transplantation for adult patients. Biol Blood Marrow Transplant 11:429–436
2. Feld R (1997) The role of surveillance cultures in patients likely to develop chemotherapy-induced mucositis. Support Care Cancer 5:371–375
3. Daw MA, Munnelly P, McCann SR, Daly PA, Falkiner FR, Keane CT (1988) Value of surveillance cultures in the management of neutropenic patients. Eur J Clin Microbiol Infect Dis 7:742–747
4. Riley DK, Pavia AT, Beatty PG, Denton D, Carroll KC (1995) Surveillance cultures in bone marrow transplant recipients: worthwhile or wasteful? Bone Marrow Transplant 15:469–473
5. LaRocco MT, Burgert SJ (1997) Infection in the bone marrow transplant recipient and role of the microbiology laboratory in clinical transplantation. Clin Microbiol Rev 10:277–297
6. Tacconelli E, Tumbarello M, Bertagnolio S et al (2002) Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: analysis of trends in prevalence and epidemiology. Emerg Infect Dis 8:220–221
7. Spielberger R, Stiff P, Bensinger W et al (2004) Palifermin for oral mucositis after intensive therapy for hematologic cancers. N Engl J Med 351:2590–2598
8. CDC (2000) Guidelines for preventing opportunistic infections among stem cell transplant recipients. Biol Blood Marrow Transplant 6:659–713, 715, 717–627, quiz 729–633
9. Hsueh PR, Teng LJ, Yang PC, Chen YC, Ho SW, Luh KT (1998) Persistence of a multidrug-resistant *Pseudomonas aeruginosa* clone in an intensive care burn unit. J Clin Microbiol 36:1347–1351
10. Rahal JJ (2006) Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and Acinetobacter species. Clin Infect Dis 43(Suppl 2):S95–S99

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Case Report

Living-Donor Lobar Lung Transplantation for Broncho-Bronchiolitis Obliterans after Allogeneic Hematopoietic Stem Cell Transplantation: Does Bronchiolitis Obliterans Recur in Transplanted Lungs?

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Abstract

We report a successful case of living-donor lobar lung transplantation (LDLLT) for therapy-resistant broncho-bronchiolitis obliterans (BBO) after allogeneic hematopoietic stem cell transplantation (HSCT). Bronchiolitis obliterans (BO) is one of the late-onset noninfectious pulmonary complications that occur after allogeneic HSCT and is usually resistant to immunosuppressive therapy. A 17-year-old girl with acute lymphoblastic leukemia (ALL) had undergone allogeneic bone marrow transplantation (BMT) from an HLA-matched sibling in 1997. Five years later, she relapsed with ALL and was treated with chemotherapy following stem cell rescue and donor lymphocyte infusion from the original BMT donor. Eight months later, BBO resistant to immunosuppressive therapies, including rituximab, developed in combination with chronic graft-versus-host disease (GVHD). In February 2004, the patient underwent LDLLT from 2 other family members who were mismatched at 3 HLA loci. The patient has been in good health for more than 30 months following LDLLT and shows no sign of BBO in the transplanted lungs, just as with other patients who have undergone lung transplantation for BO associated with chronic GVHD. LDLLT may therefore be considered a viable therapeutic option for the treatment of BO after allogeneic HSCT.
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Key words: Living-donor lung transplantation; Bronchiolitis obliterans (BO); Allogeneic hematopoietic stem cell transplantation; Graft-versus-host disease (GVHD)

1. Introduction

Pulmonary complications develop in 40% to 60% of recipients of allogeneic hematopoietic stem cell transplantation (HSCT) [1-3]. In 1998, Palmas et al [4] defined noninfectious pulmonary complications that occur later than 3 months after allogeneic SCT as late-onset noninfectious pulmonary complications (LONIPCs). Once LONIPCs occur, the recipient's quality of life is markedly impaired; therefore, LONIPCs are recognized as a major cause of morbidity and mortality after allogeneic HSCT [4,5]. LONIPCs include bronchiolitis obliterans (BO), bronchiolitis obliterans with organizing pneumonia (BOOP), diffuse alveolar damage, lymphocytic interstitial pneumonia (LIP), and nonclassifiable interstitial pneumonia (NCIP) [4]. Although the pathogenesis of LONIPCs remains unclear, LONIPCs are strongly associated with chronic graft-versus-host disease (GVHD) [4,6,7]. Immunosuppressive therapies have been considered to be the standard treatments for LONIPCs. In fact, LIP, NCIP, and BOOP have all been shown to successfully respond to these treatments [4,7]. BO, however, is usually resistant to such treatments [4,7,8]. The mortality rate for BO following allogeneic HSCT therefore may be as high as 100% [1,9,10].

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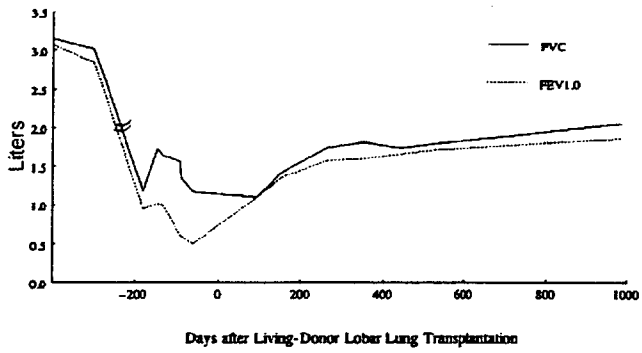

Figure 1. The pulmonary function test before and after living-donor lobar lung transplantation. FVC indicates forced vital capacity; FEV1.0, forced expiratory volume in 1 second.

Lung transplantation (LT) is an alternative therapeutic option for BO in some selected patients; however, the long-term efficacy of LT for BO and the recurrence rate of BO in transplanted lungs are unknown. We describe a patient who underwent living-donor lobar LT (LDLLT) for therapy-resistant BO after allogeneic HSCT and who has demonstrated no disease recurrence for more than 30 months after LDLLT.

2. Case Report

A 17-year-old girl received a diagnosis of acute lymphoblastic leukemia (ALL) in February 1997. She was treated with combination chemotherapy [11] and obtained complete remission. The patient underwent allogeneic bone marrow transplantation from her HLA-matched sibling in July 1997. The preconditioning regimen consisted of 3 Gy total body irradiation once daily for 4 consecutive days (total dose, 12 Gy), 2 g/m^2 cytarabine administered intravenously twice daily for 2 consecutive days (total, 4 doses), and 60 mg/kg cyclophosphamide administered intravenously once daily for 2 consecutive days (total dose, 120 mg/kg). GVHD prophylaxis consisted of cyclosporine (CsA) and short-term methotrexate. No GVHD was observed, and CsA was tapered off until February 1998. The patient relapsed with ALL in October 2002. An anthracycline-containing regimen [11] induced a second complete remission. Eight days after consolidation therapy consisting of 2 g/m^2 cytarabine administered intravenously twice daily for 5 consecutive days (total, 10 doses), the patient received donor buffy coat containing 5.4×10^6/kg CD34$^+$ cells and 0.7×10^8/kg CD3$^+$ cells, which were collected after administration of granulocyte colony-stimulating factor. No GVHD prophylaxis was given. Because GVHD did not develop until day 70 after the buffy coat infusion, the patient received donor leukocyte infusions at a dose of 0.7×10^8/kg of CD3$^+$ cells on day 34 and 1.4×10^8/kg on day 70 after the buffy coat infusion. In March 2003, 10 days after receiving the second donor lymphocyte infusion, the patient developed lichenoid lesions and ulcers on the buccal mucosa and eruptions on the skin. A lip biopsy revealed pathologic changes compatible with chronic GVHD. Oral administration of CsA was initiated in April 2003. The lichenoid lesions of the buccal mucosa gradually improved, but the symptoms of dry eyes and skin eruptions did not improve. In April 2003, the patient began complaining of dry cough, which gradually worsened. She was hospitalized in August 2003 because of an exacerbation of dry cough and dyspnea. A computed tomography examination of the chest showed atelectases of the right lower lobes, diffuse parenchymal hypoattenuation, and proximal bronchiectases. Her forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV1.0) had also decreased markedly (Figure 1). A bronchofiberscopy examination showed obstructions at the level of the broncho-bronchiols, predominantly in the lower lobes on both sides. The patient then received a diagnosis of broncho-bronchiolitis obliterans (BBO), a variant of BO. The CsA dose was increased, and oral administration of prednisolone was initiated; however, no response was observed. As the patient's symptoms worsened, CsA was replaced with tacrolimus. In addition, rituximab was administered at 375 mg/m^2 once a week for 4 weeks to treat scleroderma caused by the chronic GVHD [12]. We concluded that scleroderma had partially contributed to her constrictive respiratory failure by limiting chest wall compliance; however, the patient's respiratory failure associated with hypercapnia became exacerbated to such a degree that she became completely dependent on oxygen support. The patient became totally bedridden and had to undergo a tracheostomy in January 2004 to receive mechanical ventilation. At this time, her family requested that LT be performed. The patient was considered a candidate for LDLLT because she had end-stage BO (which is listed as a clinical status meeting the indication criteria for LT), because of the impossibility of waiting for a cadaveric lung graft owing to her rapid exacerbation of respiratory failure, and because there were no contraindications for LT except for the coexistence of malignant disease [13,14]. The patient's ALL had been in the second complete remission for more than a year, however, and the level of Wilms tumor gene in the bone marrow was 120 copies/μg RNA, thus indicating a low potential for an ALL relapse [15]. The ethics committee of the lung-transplantation center approved LDLLT for this patient because she was considered to have neither newly treated malignant disease nor widespread malignancy. LDLLT was performed on February 16, 2004, with a right lower lobe from her older brother (who was mismatched at 3 HLA loci in the direction of graft rejection) and a left lower lobe from her mother, who was mismatched at 3 antigens in the direction of graft rejection (Table 1). The patient's own lungs were removed completely. The ABO antigens of the recipient and donors were compatible

Table 1.
HLA of the Patient and Donors*

	A	B	DR
Recipient	11/24	52/–	9/15
HSCT donor (younger brother)	11/24	52/–	9/15
LDLLT donor (elder brother)	2/11	46/52	8/15
LDLLT donor (mother)	2/11	13/52	12/15

*HSCT indicates hematopoietic stem cell transplantation; LDLLT, living-donor lobar lung transplantation.

Table 2.
Reported Cases of Lung Transplantation (LT) for Bronchiolitis Obliterans (BO) after Hematopoietic Stem Cell Transplantation (HSCT)*

Case No.	Hematologic Disease	Age at LT, y	Time from HSCT to LT	cGVHD	Diagnosis of Lung Complication	LT Donor Type	Prophylaxis for Rejection	Survival Time from LT	Outcome	Reference
1	ALL	34	2 y	NE	Interstitial fibrosis with lymphoid infiltrates	Cadaver	CsA + AZP + PDN	9 mo	Alive	[18]
2	AA	14	8 y	+	Interstitial and focal parenchymal fibrosis, BO	Cadaver	FK506 + AZP + PDN	15 mo	Alive	[19]
3	ALL	27	NE	+	BO	Cadaver	NE	271 d	Died of BO	[20]
4	Immunodeficiency	11	6 mo	NE	Pulmonary fibrosis	Living	mPDN	14 mo	Alive	[21]
5	CML	38	15 mo	+	BO	Cadaver	CsA + MMF + mPDN	23 mo	Alive	[22]
6	AML	30	14 y	–	Radiation pneumonia	Cadaver	CsA + PDN	3 y	Died of pulmonary infections	[23]
7	AA	9	3 y	+	Diffuse interstitial and focal parenchymal fibrosis with compensatory emphysema and BO with cGVHD	Cadaver	FK506 + AZP + PDN	6 y	Died of lung rejection	[23]
8	Wiskott-Aldrich syndrome	6	3 y	+	Acute and chronic inflammatory change, bronchiectasis, BO, and extensive peribronchial fibrosis	Cadaver	FK506 + AZP + PDN	6 y	Alive	[23]
9	ALL	14.5	5.5 y	+	BO	Cadaver	FK506 + AZP + PDN	24 mo	Alive	[23]
10	AA	34	5 y	+	BO with interstitial pneumonia	Living	FK506 + MMF + PDN	38 mo	Alive	[24]
11	CML	17	14 mo	+	BO	Living	CsA + AZP + PDN	3 wk	Died of pulmonary hemorrhage	[25]
12	ALL	NR	7 y	+	BO	NR	CsA + AZP + PDN	NR	NR	[25]
13	ALL	NR	1 y	–	Pulmonary fibrosis	NR	CsA + AZP + PDN	NR	NR	[25]
14	ALL	NR	5 y	+	BO	NR	CsA + AZP + PDN	NR	NR	[25]
15	AML	NR	6 y	+	Pulmonary fibrosis	NR	CsA + AZP + PDN	NR	NR	[25]
Present case	ALL	24	23 mo	+	BBO	Living	FK506 + AZP + PDN	30 mo	Alive	—

*cGVHD indicates chronic graft-versus-host disease; ALL, acute lymphoblastic leukemia; NE, not evaluated; CsA, cyclosporine; AZP, azathioprine; PDN, prednisone or prednisolone; AA, aplastic anemia; FK506, tacrolimus; mPDN, methylprednisolone; CML, chronic myeloid leukemia; MMF, mycophenolate mofetil; AML, acute myeloid leukemia; NR, not reported; BBO, broncho-bronchiolitis obliterans.