4 Discussion

This report reviews the current experience of unrelated BMT with RIC regimens in Japan, with particular focus on the risk factors for engraftment, GVHD, NRM, survival and PD. Although the engraftment rate has been reported to be lower when RIC unrelated transplantation was performed with bone marrow compared to peripheral blood cells [9, 10], we observed that sustained engraftment was achieved in 99% of evaluable patients, with complete donor chimerism confirmed in 85%. The incidence of graft failure was not different from that in RIC transplantation from related donors in Japan; 3.7% in recipients with an HLA-matched donor and 5.7% in those with a 1-locus-mismatched donor [21]. Complete donor chimerism in our study was comparable with that reported from the National Marrow Donor Program (85 vs. 84%) [22]. In our study, two-thirds of patients successfully received 2–4 Gy TBI-containing regimens, which were aimed at the enhancement of engraftment, as suggested in a previous report with patients with aplastic anemia [23], while 2 of the 12 patients who received an ATG-containing regimen had late graft failure, similar to a previous report which noted an incidence of 19% [5]. It has been reported that the Japanese population is more homogenous than others in terms of the distribution of HLA. Thus, it would be possible that the impact of minor HLA disparities on engraftment may become prominent after RIC transplantation.

Despite the observed satisfactory engraftment rate, we confirmed a high NRM rate (47%) after unrelated BMT with variable RIC regimens, due mostly to GVHD-related complications, including infections under steroid therapy, as previously designated by Wong et al. [10]. On the other hand, the incidence of death due to relapse or progression of primary disease was low (14%). Hence, successful prophylaxis and treatment of GVHD is particularly important in this procedure, and studies with ATG [5, 24] or alemtuzumab [25–27] have reported encouraging results. Although the number of patients was still small, in our study an ATG-containing regimen resulted in a decreased incidence of acute and chronic GVHD, despite the use of a lower dose (ATG-Fresenius 10 mg/kg or Thymoglobulin 5 mg/kg) than reported elsewhere. This study showed that age older than 60 years tended to be associated with a higher risk of NRM after unrelated HSCT with RIC regimens, though this relation was not statistically significant in a multivariate analysis. This finding, however, is limited by the small sample size. Additional use of ATG may reduce the incidence of GVHD-related NRM even in older patients but ATG should be carefully incorporated since about 20% of patients who received an ATG-containing regimen developed late graft failure in our study.

This study suggested that the onset of grades II–IV acute GVHD was associated with a lower incidence of PD, although this was not statistically significant in a multivariate analysis, possibly due to the small sample size. However, GVHD in turn resulted in a higher incidence of NRM, and a desirable graft-versus-leukemia or lymphoma effect would be offset, particularly in older patients [10, 28]. Hence, our observation echoes the warning that the intentional induction of GVHD should be avoided.

Compared to the long-term follow-up data after unrelated HSCT with RIC from the NMDP reported by Giralt et al. [22], our NRM at 1 year was worse (43 vs. 30%), but OS was likely to be better (50% at 1 year and 46% at 2 years vs. 44% at 1 year, 28% at 3 years and 23% at 5 years). In their report, disease stage, performance status, stem cell source, HLA matching, and timing of transplant were the most important prognostic factors for survival after RIC unrelated donor transplantation. This study suggested that high risk and HLA-mismatched patients were associated with worse OS, although this was not statistically significant in the multivariate analysis. Interpretation of these results, however, should be careful because of relatively short period of follow-up and the small sample size in our study. Although high risk patients was 82%, rate of relapse were unexpectedly low in our study. This might be due to earlier mortality, which precludes estimate of relapse rate. Alternately, more patients (60%) received more intense conditioning composed of 8 mg/kg of busulfan or 80–140 mg/m^2 of melphalan and 4 Gy TBI in our study.

In conclusion, we confirmed that unrelated BMT with RIC regimens can be a curative therapeutic option in a subset of patients with advanced hematologic malignancy, but at the expense of a high risk of severe complications and NRM. The incorporation of low-dose TBI may be advantageous for enhancing engraftment, and a suitable prophylaxis for GVHD still remains a primary target of clinical research. Based on the observed data, a prospective trial is currently underway to determine the value of a lower dose of ATG (ATG-Fresenius 5 mg/kg) to be added to the combination of fludarabine and busulfan.

Acknowledgments We would like to thank Michihiro Hidaka, Katsuji Shinagawa, Tomomi Toubai, Yuichiro Nawa, Koichiro Yuji, Akinobu Takami, Nobuharu Fujii, Yoshinobu Takemoto, and Yoshihiro Yamasaki for their aid in collecting data and responding to the queries. This work was supported by grants from the Ministry of Health, Labor and Welfare, Japan (T.T., Y.T.)

References

1. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning

for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood. 2001;97:631–7. doi: 10.1182/blood.V97.3.631.
2. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood. 1998;91:756–63.
3. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97:3390–400. doi:10.1182/blood.V97.11.3390.
4. Izutsu K, Kanda Y, Ohno H, et al. Unrelated bone marrow transplantation for non-Hodgkin lymphoma: a study from the Japan Marrow Donor Program. Blood. 2004;103:1955–60. doi: 10.1182/blood-2003-03-0937.
5. Bornhäuser M, Thiede C, Platzbecker U, et al. Dose-reduced conditioning and allogeneic hematopoietic stem cell transplantation from unrelated donors in 42 patients. Clin Cancer Res. 2001;7:2254–62.
6. Nagler A, Aker M, Or R, et al. Low-intensity conditioning is sufficient to ensure engraftment in matched unrelated bone marrow transplantation. Exp Hematol. 2001;29:362–70. doi: 10.1016/S0301-472X(00)00655-X.
7. Chakraverty R, Peggs K, Chopra R, et al. Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen. Blood. 2002;99:1071–8. doi:10.1182/blood.V99.3.1071.
8. Niederwieser D, Maris M, Shizuru JA, et al. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases. Blood. 2003;101:1620–9. doi: 10.1182/blood-2002-05-1340.
9. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003;102:2021–30. doi:10.1182/blood-2003-02-0482.
10. Wong R, Giralt SA, Martin T, et al. Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years. Blood. 2003;102:3052–9. doi:10.1182/blood-2003-03-0855.
11. Kusumi E, Kami M, Yuji K, et al. Feasibility of reduced intensity hematopoietic stem cell transplantation from an HLA-matched unrelated donor. Bone Marrow Transplant. 2004;33:697–702. doi:10.1038/sj.bmt.1704425.
12. Rodriguez R, Parker P, Nademanee A, et al. Cyclosporine and mycophenolate mofetil prophylaxis with fludarabine and melphalan conditioning for unrelated donor transplantation: a prospective study of 22 patients with hematologic malignancies. Bone Marrow Transplant. 2004;33:1123–9. doi:10.1038/sj.bmt.1704493.
13. Inamoto Y, Oba T, Miyamura K, et al. Stable engraftment after a conditioning regimen with fludarabine and melphalan for bone marrow transplantation from an unrelated donor. Int J Hematol. 2006;83:356–62. doi:10.1532/IJH97.05168.
14. Baron F, Maris MB, Storer BE, et al. Unrelated donor status and high donor age independently affect immunologic recovery after nonmyeloablative conditioning. Biol Blood Marrow Transplant. 2006;12:1176–87. doi:10.1016/j.bbmt.2006.07.004.
15. Kim SW, Tanimoto TE, Hirabayashi N, et al. Myeloablative allogeneic hematopoietic stem cell transplantation for non-Hodgkin lymphoma: a nationwide survey in Japan. Blood. 2006;108:382–9. doi:10.1182/blood-2005-02-0596.
16. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. N Engl J Med. 1998;339:1177–85. doi:10.1056/NEJM199810223391701.
17. Petersdorf EW, Hansen JA, Martin PJ, et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med. 2001;345:1794–800. doi: 10.1056/NEJMoa011826.
18. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825–8.
19. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. Semin Hematol. 1991;28:250–9.
20. 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. doi:10.1002/(SICI)1097-0258(19990330)18:6<695::AID-SIM60>3.0.CO;2-O.
21. Teshima T, Matsuo K, Matsue K, et al. Impact of human leucocyte antigen mismatch on graft-versus-host disease and graft failure after reduced intensity conditioning allogeneic haematopoietic stem cell transplantation from related donors. Br J Haematol. 2005;130:575–87. doi:10.1111/j.1365-2141.2005.05632.x.
22. Giralt S, Logran B, Rizzo D, et al. Reduced-intensity conditioning for unrelated donor progenitor cell transplantation: long-term follow-up of the first 285 reported to the national marrow donor program. Biol Blood Marrow Transplant. 2007;13:844–52. doi:10.1016/j.bbmt.2007.03.011.
23. Deeg HJ, Amylon ID, Harris RE, et al. Marrow transplants from unrelated donors for patients with aplastic anemia: minimum effective dose of total body irradiation. Biol Blood Marrow Transplant. 2001;7:208–15. doi:10.1053/bbmt.2001.v7.pm11349807.
24. Kröger N, Sayer HG, Schwerdtfeger R, et al. Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality. Blood. 2002;100:3919–24. doi:10.1182/blood-2002-04-1150.
25. Ho AY, Pagliuca A, Kenyon M, et al. Reduced-intensity allogeneic hematopoietic stem cell transplantation for myelodysplastic syndrome and acute myeloid leukemia with multilineage dysplasia using fludarabine, busulphan, and alemtuzumab (FBC) conditioning. Blood. 2004;104:1616–23. doi: 10.1182/blood-2003-12-4207.
26. van Besien K, Artz A, Smith S, et al. Fludarabine, melphalan, and alemtuzumab conditioning in adults with standard-risk advanced acute myeloid leukemia and myelodysplastic syndrome. J Clin Oncol. 2005;23:5728–38. doi:10.1200/JCO.2005.15.602.
27. Lim ZY, Ho AY, Ingram W, et al. Outcomes of alemtuzumab-based reduced intensity conditioning stem cell transplantation using unrelated donors for myelodysplastic syndromes. Br J Haematol. 2006;135:201–9. doi:10.1111/j.1365-2141.2006.06272.x.
28. Maruyama D, Fukuda T, Kato R, et al. Comparable anti-leukemia/lymphoma effects in non-remission patients undergoing allogeneic hematopoietic cell transplantation with a conventional cytoreductive or reduced-intensity regimen. Biol Blood Marrow Transplant. 2007;13:932–41. doi:10.1016/j.bbmt.2007.04.004.

Biology of Blood and Marrow Transplantation 14:510-517 (2008)
© 2008 American Society for Blood and Marrow Transplantation
1083-8791/08/1405-0001$32.00/0
doi:10.1016/j.bbmt.2008.02.008

Preengraftment Serum C-Reactive Protein (CRP) Value May Predict Acute Graft-versus-Host Disease and Nonrelapse Mortality after Allogeneic Hematopoietic Stem Cell Transplantation

Shigeo Fuji, Sung-Won Kim, Takahiro Fukuda, Shin-ichiro Mori, Satoshi Yamasaki, Yuriko Morita-Hoshi, Fusako Ohara-Waki, Yuji Heike, Kensei Tobinai, Ryuji Tanosaki, Yoichi Takaue

Department of Hematology and Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

Correspondence and reprint requests: Yoichi Takaue, MD, Department of Medical Oncology, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan (e-mail: ytakaue@ncc.go.jp).

Received September 29, 2007; accepted February 10, 2008

ABSTRACT

In a mouse model, inflammatory cytokines play a primary role in the development of acute graft-versus-host disease (aGVHD). Here, we retrospectively evaluated whether the preengraftment C-reactive protein (CRP) value, which is used as a surrogate marker of inflammation, could predict posttransplant complications including GVHD. Two hundred twenty-four adult patients (median age, 47 years; range: 18-68 years) underwent conventional stem cell transplantation (CST, n = 105) or reduced-intensity stem cell transplantation (RIST, n = 119). Patients were categorized according to the maximum CRP value during neutropenia: the "low-CRP" group (CRP < 15 mg/dL, n = 157) and the "high-CRP" group (CRP ≥ 15 mg/dL, n = 67). The incidence of documented infections during neutropenia was higher in the high-CRP group (34% versus 17%, $P = .004$). When patients with proven infections were excluded, the CRP value was significantly lower after RIST than after CST ($P = .017$) or after related than after unrelated transplantation ($P < .001$). A multivariate analysis showed that male sex, unrelated donor, and HLA-mismatched donor were associated with high CRP values. The high-CRP group developed significantly more grade II-IV aGVHD ($P = .01$) and nonrelapse mortality (NRM) ($P < .001$), but less relapse ($P = .02$). The present findings suggest that the CRP value may reflect the net degree of tissue damage because of the conditioning regimen, infection, and allogeneic immune reactions, all of which lead to subsequent aGVHD and NRM.
© 2008 American Society for Blood and Marrow Transplantation

KEY WORDS

C-reactive protein • Allogeneic transplantation • Acute graft-versus-host disease • Nonrelapse mortality

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is associated with high treatment-related mortality (TRM) because of acute graft-versus-host disease (aGVHD) and infections [1,2]. Inflammatory cytokines, for example, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and IL-6 [3-11], are produced following conditioning and play a primary role in activating T cells, leading to GVHD and resultant target tissue destruction [12,13]. An acute-phase protein, C-reactive protein (CRP), is produced by hepatocytes downstream of IL-6 [14] and is widely used as a reliable surrogate marker of infectious diseases [15-19]. This process is further stimulated by other cytokines including TNF-α [12,13]. After allogeneic HSCT, the elevation of CRP was observed with infectious complications, but not in uncomplicated aGVHD [8,20]. On the other hand, elevation of CRP has been shown to be associated with TRM [21-24]. Nevertheless, these previous studies adopted the sporadic measurement of CRP and mostly focused on patients undergoing conventional HSCT (CST) with a myeloablative regimen. It has been hypothesized that recently developed reduced-intensity HSCT (RIST) decreases regimen-related toxicities and, hence, may reduce inflammation

that augments the subsequent allogeneic immune reaction to induce GVHD and nonrelapse mortality (NRM).

In this study, the correlation between the preengraftment CRP value and subsequent clinical events was analyzed to test whether high CRP reflected the degree of tissue damage because of the conditioning regimen, infections, and allogeneic immune reactions and/or inflammation, all of which could contribute to subsequent aGVHD and NRM.

MATERIALS AND METHODS

Patient Characteristics

The data from a cohort of 224 consecutive adult patients with hematologic malignancies, who were treated between January 2002 and July 2006 at the National Cancer Center Hospital (NCCH, Tokyo, Japan), were reviewed retrospectively. Patients who developed graft failure or who had previous allogeneic transplantation were excluded. Their characteristics are listed in Table 1. The median age of the patients was 47 years (range: 18-68 years), and their diagnosis included acute myeloid leukemia (AML, n = 94), acute lymphoblastic leukemia (ALL, n = 23), non-Hodgkin lymphoma (NHL, n = 62), myelodysplastic syndrome (MDS, n = 27) and chronic myeloid leukemia (CML, n = 12). Standard risk included acute leukemia in first complete remission, chronic leukemia in the first chronic phase, MDS in refractory anemia, and NHL in complete remission, with the rest of the patients categorized as a high-risk group. Stem cell sources used for transplantation included bone marrow (BM, n = 108), peripheral blood stem cells (PBSC, n = 98) and cord blood cells (CB, n = 18). One-hundred five patients received a CST regimen including total-body irridiation (TBI)-based (n = 50) and non-TBI-based busulfan-containing regimens (n = 55), whereas 119 patients received a RIST regimen including fludarabine or cladribine plus busulfan or melphalan (Table 1). CMV serostatus was positive in 157 patients and negative in 67 patients. The median age of the patients was 49 years in the high-CRP group (range: 19-67) and 47 years in the low-CRP group (range: 18- 68). Written informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

GVHD prophylaxis included cyclosporine- (n = 174) and tacrolimus-based regimens (n = 50), with an additional short course of methotrexate (MTX) in 165 patients. Granulocyte colony-stimulating factor (G-CSF) was administered in all patients from day +6 of transplantation until engraftment was confirmed. Most patients received ciprofloxacin (200 mg orally 3 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 was provided with 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 discontinuation of immunosuppressive agents. Patients with fever during the neutropenic period were treated with cefepime, and additional agents including vancomycin and 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. In our institute, the CRP level was serially measured as part of our routine checkup at least 3 times a week. Hence, all serially admitted patients were subjected to this analysis. Every patient had started CRP measurement

Table 1. *Patients' Characteristics*

	N (%)/ Median		
Variable	Low CRP Group CRP < 15 mg/dL n = 157	High CRP Group CRP ≥ 15 mg/dL n = 67	*P* Value
Age (year)	47 (18-68)	49 (19-67)	.85
<40	53 (34)	26 (39)	
≥40	104 (66)	41 (61)	.47
Patient sex			
Male	84 (54)	48 (72)	
Female	73 (46)	19 (28)	.01
Donor sex			
Male	81 (52)	30 (45)	
Female	76 (48)	37 (55)	.35
CMV serostatus			
Positive	140 (89)	64 (96)	
Negative	17 (11)	3 (4)	.20
Disease risk			
Standard	35 (22)	17 (25)	
High	122 (78)	50 (75)	.62
Conditioning			
CST	72 (47)	33 (50)	
RIST	85 (53)	34 (50)	.64
GVHD prophylaxis			
Cyclosporin-based	122 (78)	52 (78)	
Tacrolimus-based	35 (22)	15 (22)	.99
Short term MTX (+)	107 (68)	58 (87)	.004
Relation to donor			
Related	94 (60)	13 (19)	
Unrelated	63 (40)	54 (81)	<.001
Stem cell source			
Bone marrow	63 (40)	45 (67)	
PBSC	87 (55)	11 (16)	
Cord blood	7 (5)	11 (16)	<.001

CRP indicates C-reactive protein; CMV, cytomegalovirus; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; MTX, methotrexate; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen.

Table 2. *Comparison of Preengraftment CRP Value Stratified According to the Conditioning Regimen (CST versus RIST) and the Relation to Donor (Related versus Unrelated)*

Patients' Characteristics	CRP Value Median (Range)
All patients	8.9 (0.1-42.7)
CST	10.5 (0.3-31.3)*
Related	9.4 (0.6-30.0)†
Unrelated	10.6 (0.3-31.3)†
RIST	6.2 (0.1-42.7)*
Related	1.6 (0.1-9.7)‡
Unrelated	16.2 (0.5-42.7)‡

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation.
*P = .017.
†P = .33.
‡P < .001.

before the initiation of the conditioning regimen, and the median pretransplant CRP level was 0.3 mg/dL (range: 0.0-20.5 mg/dL). The median maximum CRP value during neutropenia was 8.9 mg/dL (0.1-42.7, Table 2).

The "maximum CRP level" was determined by measuring both the CRP level and the neutrophil count, as shown in the example in Figure 1A. The average number of levels assessed for each patient was 8 (range: 1-30). The median day of the maximum CRP level was day 10 of HSCT (range: 0-25), with 79% of patients developing this in later days (≥8 days). The patients were categorized according to the maximum CRP level after the threshold CRP level was determined following a preliminary analysis of the maximum CRP level after CST using an ROC curve analysis (data not shown). The "low-CRP" group (CRP <15 mg/dL) included 157 patients and the "high-CRP" group (CRP ≥15 mg/dL) included 67 patients.

Statistical Analyses

The primary endpoint of this study was the occurrence of grade II-IV and grade III-IV aGVHD, according to the Consensus Criteria [25]. The secondary endpoints were overall survival (OS) and nonrelapse mortality (NRM). Standard descriptive

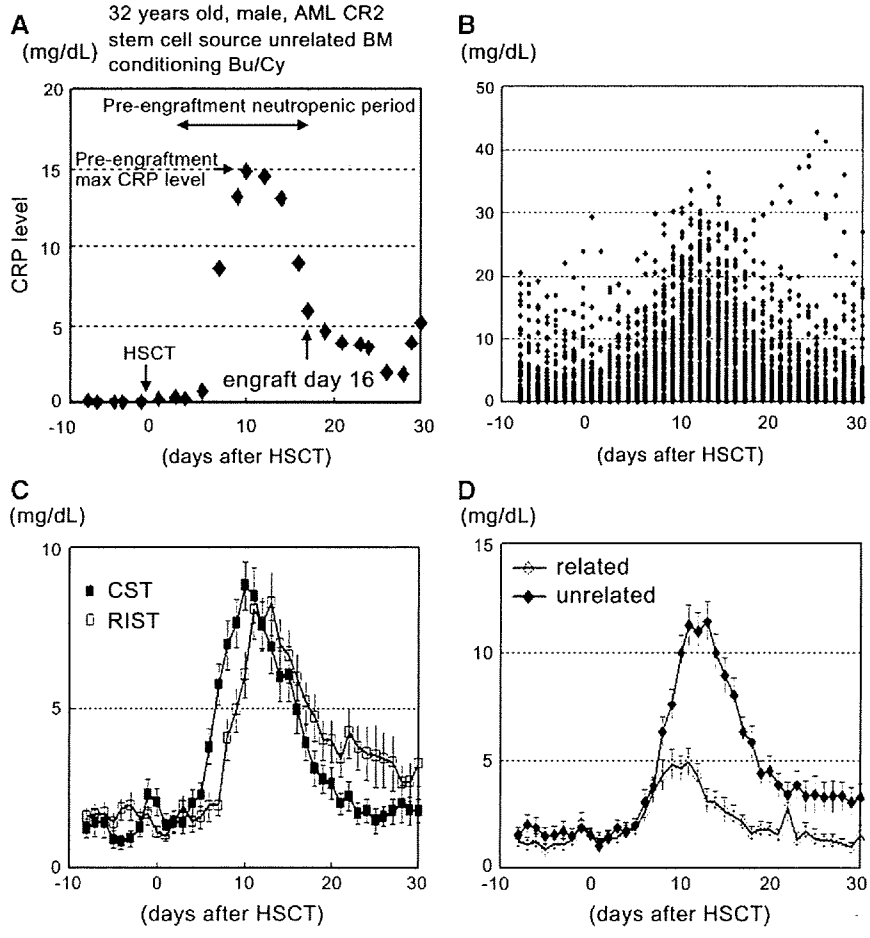

Figure 1. An example of how we measured CRP in a representative patient (A). Dot plot of the CRP level. All patients (B), CST versus RIST (C) and related versus unrelated (D).

statistics were used. Student *t*, chi-square, Fisher's exact test, and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. To analyze the pretransplant risk factors for a high CRP level, logistic analysis was used. OS was estimated using Kaplan-Meier curves. The cumulative incidence of aGVHD and NRM was estimated based on a Cox regression model for cause-specific hazards by treating progressive disease or relapse as a competing event. Cox proportional hazard models were used for the multivariate analysis of variables in aGVHD, NRM, and OS after HSCT. Clinical factors that were assessed for their association with aGVHD included patient age, patient sex, donor sex, CMV serostatus, conditioning regimen (CST versus RIST), donor (human leukocyte antigen [HLA]-matched versus HLA-mismatched, related versus unrelated), GVHD prophylaxis (cyclosporine-based versus tacrolimus-based, short-term MTX versus no MTX) and disease risk (standard versus high risk). NRM and OS were also assessed for their association with these factors. Factors with $P < .10$ in the univariate analyses were subjected to a multivariate analysis using a multiple logistic analysis and Cox proportional hazard modeling. In Japan, only BM and CB are allowed for unrelated transplantation, and most transplantations with a related donor use PBSC as a stem cell source. Therefore, the stem cell source was not included as a factor in the multivariate analysis. A level of $P < .05$ was defined as statistically significant. All P values are 2-sided. All analyses were made with SPSS ver 10.0 statistical software (Chicago, IL). This analysis was approved by the institutional review board.

RESULTS

Infections

The median duration of follow-up in surviving patients was 965 days (61 to 1432 days) in the high-CRP group and 915 days (76 to 1803 days) in the low-CRP group, and the incidence of total documented infections during neutropenia was, respectively, 23 cases in the high-CRP group (34%) and 27 cases in the low-CRP group (17%, $P = .004$). The incidence of bacteremia was, respectively, 20 cases (30%) and 20 cases (13%, $P = .002$), and the incidence of pneumonia was 7 cases (10%) and 4 cases (3%, $P = .01$). The incidence of central venous catheter infection was, respectively, 4 cases (6%) and 7 cases (4%, $P = .63$).

Serial changes in the CRP level are shown in Figure 1B; in most cases, the CRP level was elevated within 2 weeks of HSCT. Stratified data according to conditioning regimen (CST versus RIST) or relation to donor (related versus unrelated) are shown in Figure 1C and D, respectively.

To clarify the pretransplant risk factors for high CRP values during neutropenia, we performed a logistic regression analysis, which showed that male, unrelated donor, stem cell source with BM or CB transplantation (versus PBSCT), HLA-mismatched donor, and immunosuppression with MTX were associated with high CRP values during neutropenia (Table 1). Factors that showed significant associations ($P < .1$) were subjected to a multiple logistic regression analysis, and the results showed that unrelated donor, HLA mismatch and male sex were associated with high CRP ($P < .001$, $P = .005$, $P = .028$, respectively), as shown in Table 3. The median CRP levels after CST and RIST were 10.5 (0.3-31.3) and 6.2 (0.1-42.7), respectively, with a significant difference ($P = .017$) (Table 2). Notably, within the RIST group, the median CRP level was significantly lower in related than in unrelated transplantation (1.6 mg/dL [0.1-9.7] veruss 16.2 mg/dL [0.5-42.7]: $P < .001$). However, the logistic analysis failed to disclose any overall significant difference between CST and RIST.

Primary Outcomes

The cumulative incidences of aGVHD grade II-IV and grade III-IV are shown, respectively, in Figure 2A and B. Grade II-IV and grade III-IV aGVHD were both more frequent in the high-CRP group than in the low-CRP group ($P = .001$ and $P = .04$, respectively). A Cox proportional hazard model showed that a high CRP level and CMV serostatus were associated with an increased risk of grade II-IV aGVHD (Table 4). Similar results were obtained when we included only the patients who received a myeloablative conditioning regimen (grade II-IV aGVHD 25% in the low-CRP group and 58% in the high-CRP group, $P < .001$, grade III-IV aGVHD 7% in the low-CRP group and 21% in the high-CRP group, $P = .047$).

Secondary Outcomes

OS and NRM are shown, respectively, in Figure 3A and B. OS was significantly worse in the

Table 3. *Multiple Logistic Regression Analysis of Risk Factors for High CRP during Neutropenia*
*Factors with P < .10 in a Multivariate Analysis Was Shown**

	Multiple Logistic Regression Analysis		
Outcomes and Variables	Odds	95% CI	P Value
Unrelated donor	4.6	2.2-9.6	<.001
HLA mismatch	2.6	1.3-5.0	.005
Patient sex (male)	2.1	1.1-4.2	.0028

CRP indicates C-reactive protein; CI, confidence interval; HLA, human leukocyte antigen; CMV, cytomegalovirus.
*Factors included in univariate analysis: patient sex, donor sex, CMV serostatus, use of short-term MTX, relation to donor, HLA mismatch, conditioning, GVHD prophylaxis, stem cell source.

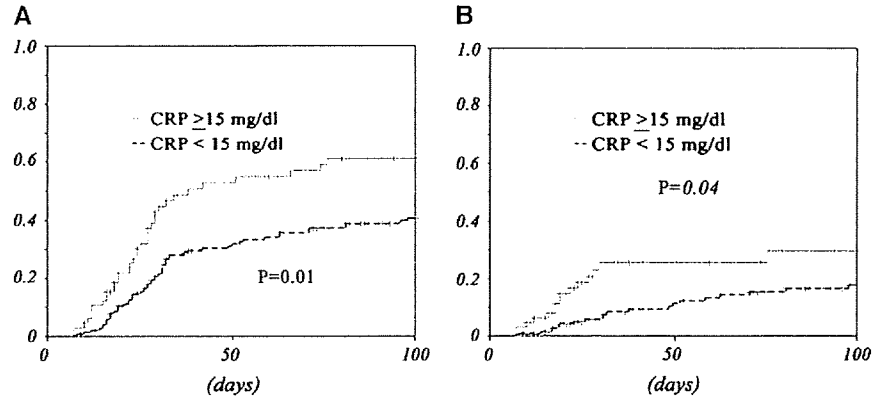

Figure 2. Cumulative incidence of grade II-IV aGVHD (A) and grade III-IV aGVHD (B) stratified according to the maximal CRP level during neutropenia.

high-CRP group than in the low-CRP group (1-year OS 47% versus 75%, $P = .001$). NRM was significantly higher in the high-CRP group than in the low-CRP group (1-year NRM 47% versus 13%, $P < .001$). Similar results were obtained when we included only patients who received a myeloablative conditioning regimen (1-year NRM 8% in the low-CRP group and 38% in the high-CRP group, $P = .007$). A Cox proportional hazard model showed that the risk factors for poor OS were high CRP ($P = .002$, hazard ratio [HR] 2.0, 95% confidence interval [CI] 1.3-3.1) and high-risk disease ($P = .015$, HR 2.2, 95% CI 1.2-4.0), whereas those for high NRM were high CRP ($P < .001$, HR 4.0, 95% CI 2.0-8.0) and high-risk disease ($P = .029$, HR 2.6, 95% CI 1.1-6.2), as shown in Table 4. When the threshold was set at 15 mg/dL, the sensitivity and specificity of the CRP level for prediction of grade II-IV aGVHD, NRM, or OS were 37% and 75%, 59% and 79%, and 40% and 78%, respectively. The relapse rate was significantly lower in the high-CRP group than in the low-CRP group (1-year relapse 21% versus 33%, $P = .02$).

Causes of death are summarized in Table 5. A total of 57 patients (36%) in the low-CRP group and 39 patients (58%) in the high-CRP group died ($P = .002$, OR 2.4 [1.4-4.4]). Six patients (4%) in the low- and 5 (7%) in the high-CRP group died because of aGVHD, for example, death because of infectious diseases associated with aGVHD and its treatment. Seven patients (4%) in the low- and 11 (16%) in the high-CRP group ($P = .003$, OR 4.2 [1.6-11.4]) died because of chronic GVHD (cGVHD), including death because of infectious diseases associated with cGVHD and its treatment. No patient (0%) in the low- and 5 (7%) in the high-CRP group ($P = .002$) died because of infectious diseases excluding infectious disease concomitant with GVHD. No patient in the low-CRP group and 4 (6%) in the high-CRP group ($P = .008$) died because of multiple-organ failure (MOF) excluding MOF because of GVHD and infectious disease.

DISCUSSION

The results of this retrospective study suggested that higher CRP values during the neutropenic period may reflect net inflammation secondary to tissue damage because of the conditioning regimen, infection, and subsequent allogeneic immune reactions, all of which lead to aGVHD/cGVHD and ultimate NRM. In a mouse model, the concept that the production of inflammatory cytokines plays an important role in the development of aGVHD, by affecting the afferent and effector phase [12,13], has been accepted. Cooke et al. [26] showed that LPS antagonism reduced aGVHD in a mouse model, as indicated by Ferrara et al. [4]. However, in human studies, the value of determining individual levels of cytokines to monitor aGVHD has not been fully explored, because this approach is very costly and requires sophisticated techniques, which impedes its universal applicability. On the other hand, CRP is already being widely used

Table 4. *Multiple Variate Analysis for aGVHD, NRM, and OS**

Outcomes and Variables	Hazard Ratio	95% CI	P value
Grade II-IV aGVHD			
High CRP	1.7	1.1-2.6	.02
CMV positivity	3.1	1.0-9.8	.5
Disease risk (high)	1.6	0.9-2.7	.10
NRM			
High CRP	4.0	2.0-8.0	<.001
Age (≥40 years old)	1.9	0.9-3.9	.07
Disease risk (high)	2.6	1.1-6.2	.03
OS			
High CRP	2.0	1.3-3.1	.002
Disease risk (high)	2.2	1.2-4.0	.02

CRP indicates C-reactive protein; CI, confidence interval; CMV, cytomegalovirus; GVHD, graft-versus -host disease; TBI, total body irradiation; NRM, nonrelapse mortality; OS, overall

*Factors included in univariate analysis: patient sex, donor sex, CMV serostatus, use of short-term MTX, relation to donor, HLA mismatch, conditioning, GVHD prophylaxis, stem cell source

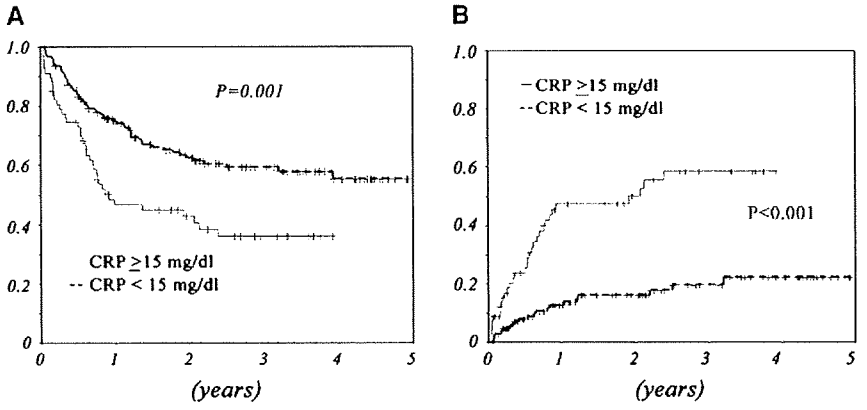

Figure 3. OS stratified according to the maximal CRP level during neutropenia (A). Cumulative incidence of TRM stratified according to the maximal CRP level during neutropenia (B).

worldwide, especially in Japan, to distinguish bacterial infections from other causes of fever [15-19]. Based on this practice, we reviewed the value of the CRP level after HSCT, and our data suggest that it might be useful to monitor the CRP value as a net surrogate marker for produced cytokines, and for predicting the subsequent development of aGVHD and NRM.

Our patients had various interacting backgrounds, and it is still difficult to predict whether a patient with a high CRP level is destined to suffer from GVHD or major infectious complications. Infectious diseases were previously reported to be a primary cause of elevated CRP [8,20], which might, in turn, affect the severity of aGVHD. In this study, we made every effort, including intense culture studies, to exclude infection as a primary cause of increased CRP, and showed that there were significantly more documented infections in the high-CRP group than in the low-CRP group. Current practice for the prevention of infection mostly focuses on the effective control of Gram-negative bacteria, considering the potent immediate pathologic effect of the organisms. However, if the hypothesis that decreasing the net production of cytokines is important for the prevention of subsequent GVHD is correct, more effort should be paid to broadly cover other types of organisms or even clinically less significant infection, that is, stomatitis, at least during the early period of neutropenia, particularly in patients carrying risk factors for high CRP, which included unrelated donor, HLA mismatch, BM, and CB transplantation in this study. The addition of other markers, such as procalcitonin, may be useful for identifying the risk of major infectious complications [24].

Table 5. *Causes of Death Stratified According to CRP Value during Neutropenia*

Causes of death	Low CRP Group CRP < 15 mg/dL n = 157	High CRP Group CRP ≥ 15 mg/dL n = 67	*P* Value
Total	57 (36%)	39 (58%)	.002
Relapse/progressive disease	34 (22%)	8 (12%)	.09
acute GVHD (total)	6 (4%)	5 (7%)	.25
acute GVHD	5 (3%)	3 (5%)	.63
acute GVHD + infection	1 (1%)	2 (3%)	.16
chronic GVHD (total)	7 (4%)	11 (16%)	.003
chronic GVHD	3 (2%)	7 (10%)	.005
chronic GVHD + infection	4 (3%)	4 (6%)	.21
Infection*	0 (0%)	5 (7%)	.002
MOF†	0 (0%)	4 (6%)	.008
Respiratory failure‡	3 (2%)	4 (6%)	.11
Others	Stroke 2	VOD 1	
	VOD 2	Myocardial infarction 1	
	Secondary cancer 1		
	Unknown 2		

CRP indicates C-reactive protein; GVHD, graft-versus-host disease; TBI, total-body irradiation; MOF, multiple organ failure; VOD, veno-occlusive disease.

*Excluding infection during GVHD or GVHD treatment.

†Excluding MOF due to GVHD, infection.

‡Excluding respiratory failure because of GVHD, infection, and MOF.

Tissue damage caused by the conditioning regimen, complicated infections, and allogeneic immune reactions are the primary factors that are associated with the initial elevation of CRP early in the course of allogeneic HSCT. Consequently, it can be speculated that a reduced-intensity conditioning regimen results in decreased cytokine release and a resultant lower CRP value, which may lead to less chance of developing GVHD. Although the RIST regimens we used were relatively dose-intense, in this retrospective review we still found that CRP levels tended to be decreased after RIST compared to conventional myeloablative transplantation, particularly in a related compared to an unrelated transplantation setting. Because augmentation of allogeneic immune and inflammation reactions may induce a higher CRP value, we speculate that the benefit of RIST is diminished when a strong allogeneic reaction is induced, as in cases of unrelated transplantation.

To further evaluate the relationship between a higher CRP value during neutropenia and common risk factors associated with transplantation, we performed a multivariate analysis and showed that unrelated donor, HLA mismatch, and male sex were associated with higher CRP values. Additionally, from the finding in the multivariate analysis that unrelated donor and HLA mismatch were independently associated with high CRP, we surmised that the degree of genetic disparity might be associated with higher CRP during neutropenia. Based on a consideration of these findings together, we think that a higher CRP value may reflect the degree of tissue damage because of the transplant regimen and the subsequent magnitude of allogeneic immune reactions. Nevertheless, our analysis was hampered, because in Japan only BM and CB are allowed for unrelated transplantations, and most transplantations with a related donor use PBSC as a stem cell source. In these settings, a theoretically longer neutropenic period after unrelated BM or CB transplantation might be associated with a higher risk of infection, which could lead to higher CRP, as shown in this study.

In this study, the primary causes of death in the low-CRP group were mainly relapse and progression, whereas in the high-CRP group this was NRM. Notably, the observation that the relapse rate was higher in the low-CRP group than in the high-CRP group, as previously suggested by Min et al. [23], may further support our hypothesis that serum CRP values represent overall inflammation and cytokine production, which paves the way to GVHD and related graft-versus-leukemia (GVL) effects. A possible reason for this finding is that a low CRP level resulted in a lower incidence of GVHD and a resultant decrease in the GVL effect, or the high-CRP group developed earlier and more-frequent death from NRM compared to the low-CRP group, which left fewer patients for evaluation of the later occurrence of relapse.

In conclusion, our results suggest that the CRP value in the neutropenic period before engraftment in patients undergoing allogeneic HSCT may be a net surrogate marker of early inflammation that leads to the development of aGVHD/cGVHD and subsequent NRM, as has been proposed in mouse models. The intensity of the conditioning regimen, infectious diseases, and degree of allogeneic immune response attributed to HLA compatibility and the stem cell source may be the major factors that predict higher CRP values. Based on the results of this retrospective study, future clinical studies to evaluate the feasibility of earlier intervention and adjustment of the procedure for preventing GVHD and NRM based on monitoring of the early CRP value are warranted.

ACKNOWLEDGMENTS

This work was presented in part as a poster presentation at the annual Meeting of EBMT, Lyon, March 2007. This study was supported in part by grants from the Ministry of Health, Labor and Welfare, and Advanced Clinical Research Organization, Japan. There is no potential conflict of interest to declare.

REFERENCES

1. Wojnar J, Giebel S, Krawczyk-Kulis M, et al. Acute graft-versus-host disease. The incidence and risk factors. *Ann Transplant.* 2006;11:16-23.
2. Weisdorf D, Hakke R, Blazar B, et al. Risk factors for acute graft-versus-host disease in histocompatible donor bone marrow transplantation. *Transplantation.* 1991;51:1197-1203.
3. Krenger W, Hill GR, Ferrara JL. Cytokine cascades in acute graft-versus-host disease. *Transplantation.* 1997;64:553-558.
4. Ferrara JL. The cytokine modulation of acute graft-versus-host disease. *Bone Marrow Transplant.* 1998;21(Suppl 3):S13-S15.
5. Cooke KR, Olkiewicz K, Erickson N, Ferrara JL. The role of endotoxin and the innate immune response in the pathophysiology of acute graft versus host disease. *J Endotoxin Res.* 2002;8: 441-448.
6. Toren A, Novick D, Or R, Ackerstein A, Slavin S, Nagler A. Soluble interleukin-6 receptors in hematology patients undergoing bone marrow transplantation. *Transplantation.* 1996;62:138-142.
7. Liem LM, van Houwelingen HC, Goulmy E. Serum cytokine levels after HLA-identical bone marrow transplantation. *Transplantation.* 1998;66:863-871.
8. Schwaighofer H, Herold M, Schwarz T, et al. Serum levels of interleukin 6, interleukin 8, and C-reactive protein after human allogeneic bone marrow transplantation. *Transplantation.* 1994; 58:430-436.
9. Chasty RC, Lamb WR, Gallati H, Roberts TE, Brenchley PE, Yin JA. Serum cytokine levels in patients undergoing bone marrow transplantation. *Bone Marrow Transplant.* 1993;12:331-336.
10. Lange A, Karabon L, Klimczak A, et al. Serum interferon-gamma and C-reactive protein levels as predictors of acute graft-vs-host disease in allogeneic hematopoietic precursor cell (marrow or peripheral blood progenitor cells) recipients. *Transplant Proc.* 1996;28:3522-3525.
11. Symington FW, Symington BE, Liu PY, Viguet H, Santhanam U, Sehgal PB. The relationship of serum IL-6 levels

to acute graft-versus-host disease and hepatorenal disease after human bone marrow transplantation. *Transplantation*. 1992;54: 457-462.

12. Fowler DH, Foley J, Whit-Shan Hou J, et al. Clinical "cytokine storm" as revealed by monocyte intracellular flow cytometry: correlation of tumor necrosis factor alpha with severe gut graft-versus-host disease. *Clin Gastroenterol Hepatol*. 2004;2: 237-245.
13. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. *Blood*. 1992;80:2964-2968.
14. Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. *Biochem J*. 1990;265:621-636.
15. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med*. 1999;340:448-454.
16. Santolaya ME, Cofre J, Beresi V. C-reactive protein: a valuable aid for the management of febrile children with cancer and neutropenia. *Clin Infect Dis*. 1994;18:589-595.
17. Manian FA. A prospective study of daily measurement of C-reactive protein in serum of adults with neutropenia. *Clin Infect Dis*. 1995;21:114-121.
18. Persson L, Engervall P, Magnuson A, et al. Use of inflammatory markers for early detection of bacteraemia in patients with febrile neutropenia. *Scand J Infect Dis*. 2004;36:365-371.
19. von Lilienfeld-Toal M, Dietrich MP, Glasmacher A, et al. Markers of bacteremia in febrile neutropenic patients with hematological malignancies: procalcitonin and IL-6 are more reliable than C-reactive protein. *Eur J Clin Microbiol Infect Dis*. 2004; 23:539-544.
20. Rintala E, Remes K, Salmi TT, Koskinen P, Nikoskelainen J. The effects of pretransplant conditioning, graft-versus-host disease and sepsis on the CRP levels in bone marrow transplantation. *Infection*. 1997;25:335-338.
21. Schots R, Kaufman L, Van Riet I, et al. Monitoring of C-reactive protein after allogeneic bone marrow transplantation identifies patients at risk of severe transplant-related complications and mortality. *Bone Marrow Transplant*. 1998;22:79-85.
22. Schots R, Van Riet I, Ben Othman T, et al. An early increase in serum levels of C-reactive protein is an independent risk factor for the occurrence of major complications and 100-day transplant-related mortality after allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2002;30:441-446.
23. Min CK, Kim SY, Eom KS, et al. Patterns of C-reactive protein release following allogeneic stem cell transplantation are correlated with leukemic relapse. *Bone Marrow Transplant*. 2006;37: 493-498.
24. Pihusch M, Pihusch R, Fraunberger P, et al. Evaluation of C-reactive protein, interleukin-6, and procalcitonin levels in allogeneic hematopoietic stem cell recipients. *Eur J Haematol*. 2006; 76:93-101.
25. Przepiorka D, Weisdorf D, Martin P, et al. 1994 consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1995;15:825-828.
26. Cooke KR, Gerbitz A, Crawford JM, et al. LPS antagonism reduces graft-versus-host disease and preserves graft-versus-leukemia activity after experimental bone marrow transplantation. *J Clin Invest*. 2001;107:1581-1589.

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

Shigeo Fuji,[1] Sung-Won Kim,[1] Shin-ichiro Mori,[1] Takahiro Fukuda,[1] Shigemi Kamiya,[2] Satoshi Yamasaki,[1] Yuriko Morita-Hoshi,[1] Fusako Ohara-Waki,[1] Osamu Honda,[3] Setsuko Kuwahara,[2] Ryuji Tanosaki,[1] Yuji Heike,[1] Kensei Tobinai,[1] and Yoichi Takaue[1,4]

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.

(*Transplantation* 2007;84: 814–820)

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-

Supported in part by grants from the Ministry of Health, Labor and Welfare, Japan.

This paper was presented in part as a poster presentation at the tandem Meeting of ASBMT and IBMTR, Keystone, Colorado, February 2007.

[1] Department of Hematology and Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan.

[2] Division of Nutritional Management, National Cancer Center Hospital, Tokyo, Japan.

[3] Tokyo Anesthesiology Group, Tokyo, Japan.

[4] Address correspondence to: Yoichi Takaue, M.D., Department of Hematology, National Cancer Center Hospital., 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan.

E-mail: ytakaue@ncc.go.jp

Received 12 June 2007. Revision requested 10 July 2007.

Accepted 18 July 2007.

Copyright © 2007 by Lippincott Williams & Wilkins

ISSN 0041-1337/07/8407-814

DOI: 10.1097/01.tp.0000282790.66889.a5

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

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.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

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.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

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

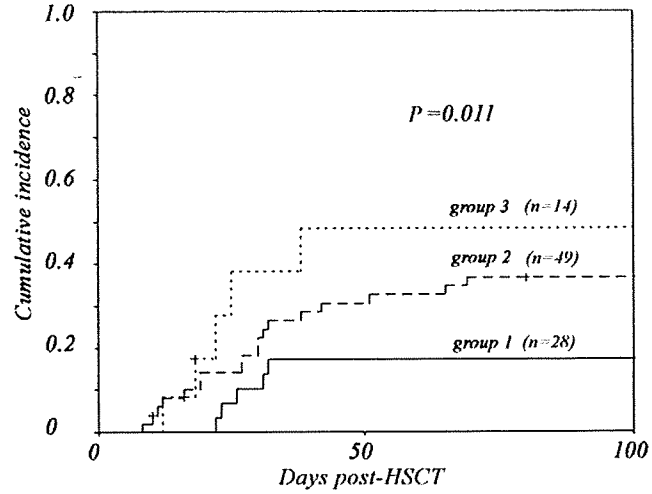

FIGURE 1. Cumulative incidence of acute GVHD grade II–IV stratified according to the mean glucose level during neutropenia. Group 1 included patients with normoglycemia, group 2 included patients with mild hyperglycemia, and group 3 included patients with moderate and severe hyperglycemia.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

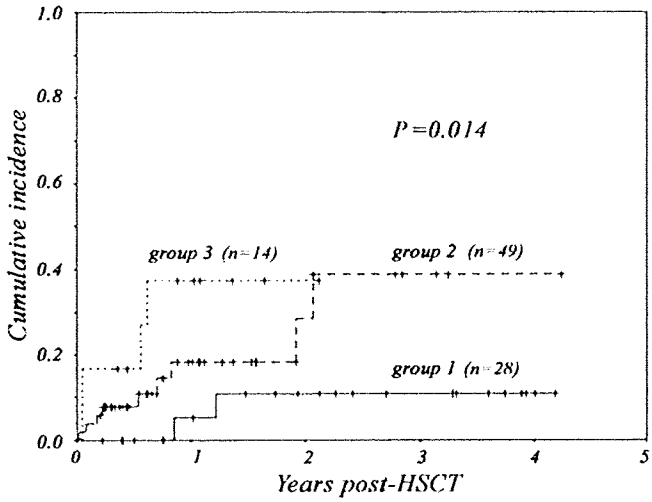

FIGURE 2. Cumulative incidence of treatment-related mortality stratified according to the mean glucose level during neutropenia.

(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.

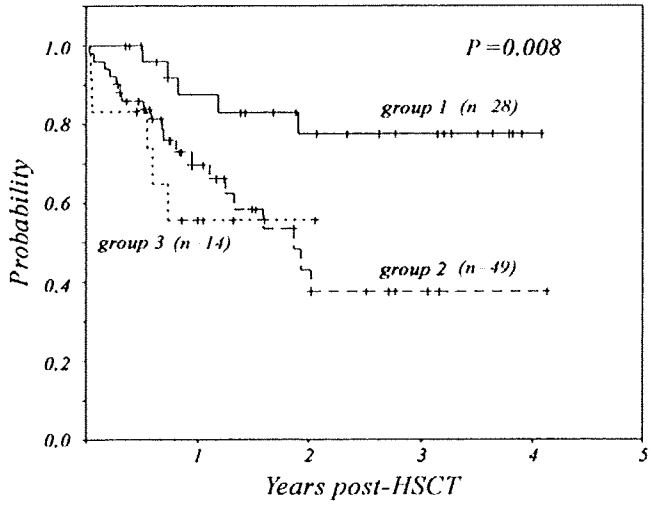

FIGURE 3. Overall survival stratified according to the mean glucose level during neutropenia.

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

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

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 posttransplant 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.

ACKNOWLEDGMENTS

We thank the medical, nursing, data processing, laboratory, and clinical staffs at the National Cancer Center Hospital for their important contributions to this study through dedicated care of the patients. The authors are indebted to Y. Iisaka and M. Kurita for their assistance with data collection. We also thank S. Saito for helping to prepare the manuscript.

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

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

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.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

Biology of Blood and Marrow Transplantation 13:932-941 (2007)
© 2007 American Society for Blood and Marrow Transplantation
1083-8791/07/1308-0001$32.00/0
doi:10.1016/j.bbmt.2007.04.004

Comparable Antileukemia/Lymphoma Effects in Nonremission Patients Undergoing Allogeneic Hematopoietic Cell Transplantation with a Conventional Cytoreductive or Reduced-Intensity Regimen

Dai Maruyama, Takahiro Fukuda, Ruri Kato, Satoshi Yamasaki, Eiji Usui, Yuriko Morita-Hoshi, Sung-Won Kim, Shin-ichiro Mori, Yuji Heike, Atsushi Makimoto, Kinuko Tajima, Ryuji Tanosaki, Kensei Tobinai, Yoichi Takaue

Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan

Correspondence and reprint requests: Takahiro Fukuda, MD, PhD, Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo, 104-0045, Japan (e-mail: tafukuda@ncc.go.jp).

Received February 1, 2007; accepted April 12, 2007

ABSTRACT

To evaluate the potential of allogeneic hematopoietic cell transplantation (HCT) with a reduced-intensity conditioning regimen (RIST) for the treatment of patients with hematologic malignancies not in remission, we retrospectively reviewed the medical records of 132 patients (89 leukemia or myelodysplastic syndrome, 40 malignant lymphoma, and 3 others) who received conventional myeloablative HCT (CST, n = 52) or RIST (n = 80). The median age of the RIST group was significantly higher than that of the CST group (53 years versus 40 years, $P < .01$). The RIST group also included a higher proportion of patients with an HCT-specific comorbidity index (HCT-CI) of 1 or more than the CST group (65% versus 37%, $P = .03$). The probabilities of achieving complete remission and the incidences of grades II-IV and III-IV acute graft-versus-host disease (aGVHD) in the CST and RIST groups were, respectively, 77% and 64%, 50% and 50%, and 23% and 28%, with no significant differences. Similarly, there was no difference in the 2-year probabilities of nonrelapse mortality (NRM, 36% and 38%), progressive disease or relapse (PD 51% and 49%), overall survival (OS, 31% and 38%), and progression-free survival (PFS, 28% and 29%). Multivariate analyses revealed that a higher HCT-CI score and transplant from donors other than HLA-matched relatives were associated with increased risks of NRM and poor OS, and patients who received chemotherapy within 2 months before HCT were associated with increased risks of PD, poor OS, and PFS after transplantation. After adjusting for these variables, the risks of NRM, PD, OS, and PFS in the RIST group were not significantly different from those in the CST group. In conclusion, these results suggest that the antileukemia/lymphoma effect associated with RIST is comparable to that associated with CST. RIST appears to be feasible for the treatment of hematologic malignancies not in remission.
© *2007 American Society for Blood and Marrow Transplantation*

KEY WORDS

Transplantation • Leukemia • Lymphoma

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) has the potential to achieve long-term cure of hematologic malignancies by pretransplant conditioning and a graft-versus-leukemia/lymphoma (GVL) effect. It has been well established that the disease status at the time of transplantation is the most important prognostic factor, and the rates of relapse and nonrelapse mortality (NRM) significantly increase in patients with hematologic malignancies who were not in remission. Therefore, conventional stem cell transplantation (CST) using a myeloablative conditioning

regimen has been universally used in the hope of maximally reducing the tumor burden before HCT in patients not in remission. However, CST may not be an option for many patients because of their older age or associated comorbidities. Alternatively, over the past few years, nonmyeloablative and reduced-intensity conditioning stem cell transplantation (RIST) have been offered to these patients undergoing HCT, on the assumption that RIST would be better tolerated [1-4].

There have been several reports that the outcome of older patients who underwent RIST while in remission was comparable to that of patients who received CST [5-9], which suggests that the GVL effect associated with RIST might be adequate for controlling chemosensitive or slowly progressing disease. On the other hand, it still remains controversial whether RIST is feasible for patients not in remission, although small pilot studies have shown that RIST was unsuccessful for advanced hematologic malignancies [3,10-14]. To address this issue, we retrospectively analyzed 132 patients who were not in remission at the time of CST or RIST.

PATIENTS AND METHODS

Study Patients

We retrospectively reviewed the medical records of 132 patients with various hematologic malignancies who underwent allogeneic HCT (CST, n = 52; RIST, n = 80) while not in remission at our institution from January 2000 to December 2004. Patients with chronic myelogenous leukemia (CML) in the chronic phase, myelodysplastic syndrome (MDS)-refractory anemia, and those with lymphoma in partial remission (PR) were not included because the response to treatment and the outcome of these patients is generally considered to be similar to those in patients who are in complete remission (CR). Bone marrow or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) were harvested from donors according to protocols approved by the guidelines of the Japan Marrow Donor Program, the Japanese Society for Hematopoietic Cell Transplantation, and the Japanese Society of Blood Transfusion. Informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

The conditioning regimens used in CST included the combination of cyclophosphamide (CY; 60 mg/kg i.v. daily for 2 days) and fractionated total body irradiation (TBI; 12 Gy in 6 fractions over 3 days) in 34 patients, CY and oral busulfan (BU; 16 mg/kg divided over 4 days) in 13 patients, and other combinations in 5 patients (Table 1). Targeted dose adjustment of BU was not performed. Patients who underwent RIST were older than 50 years of age or those who had comorbidities or prior transplantation. The conditioning regimens for RIST consisted of fludarabine (30 mg/m^2 i.v. daily for 6 days) or cladribine (0.11 mg/kg i.v. daily for 6 days) plus 8 mg/kg of oral BU [15] with (n = 27) or without (n = 53) 4 Gy TBI. In Japan, only bone marrow is permitted as a stem cell source in transplantation from an unrelated healthy volunteer donor. In the setting of nonmyeloablative stem cell transplantation from an unrelated donor, the sustained engraftment rate has been reported to be lower for recipients of bone marrow than for those given PBSC [13]. Therefore, low-dose TBI was also added to the conditioning regimen for RIST from an unrelated donor to facilitate engraftment.

Day 0 was defined as the day of stem cell infusion. G-CSF was administered after transplantation in all patients until neutrophil engraftment. Most patients who underwent CST were given cyclosporine (CSP) with methotrexate (MTX) [16], and all patients who underwent RIST were given CSP with or without MTX for graft-versus-host disease (GVHD) prophylaxis (Table 1). GVHD was treated with 1 to 2 mg/kg/day prednisolone equivalents, resumption of full-dose CSP administration if applicable, or both. Initial doses of corticosteroids and tapering schedules of immunosuppressive medications were modified at the discretion of the attending physicians according to the presence or absence of malignant cells and the severity of GVHD. Treatment for relapse after transplantation was left to the discretion of the attending physicians.

All patients received ciprofloxacin (200 mg orally 3 times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Patients who had positive serologic test results for herpes simplex virus or varicella zoster virus received prophylactic low-dose acyclovir until the cessation of immunosuppressive agents [17]. Prophylaxis against *Pneumocystis jiroveci* infection was provided with trimethoprim-sulfamethoxazole 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 were monitored with weekly cytomegalovirus (CMV) pp65 antigenemia testing, and positive antigenemia was treated with ganciclovir as described previously [18,19].

Definitions

Chemotherapy within 2 months before HCT was defined as chemotherapy to control the disease except for rituximab alone for lymphoma and imatinib mesylate alone for CML. Pretransplantation comorbidities were determined by the HCT-specific comorbidity index (HCT-CI) [20] with a minor modification [21]. Neutrophil engraftment was defined as the first

Table 1. *Patient Characteristics*

	CST	RIST	P-Value
No. of patients	52	80	
Sex, male/female	25/27	50/30	
Median age, years (range)	40 (3-55)	53 (20-68)	<.01
Disease status at conditioning, N (%)			
AML	20 (38)	15 (19)	
Relapse 1	12	6	
Relapse ≥2	5	5	
Primary refractory	3	4	
MDS (including overt AML)	15 (29)	24 (30)	
Relapse 1	1	2	
Untreated	6	11	
Primary refractory	8	11	
ALL	5 (10)	2 (3)	
Relapse 1	4	1	
Relapse 2	1	1	
CML	5 (10)	3 (4)	
Accelerated phase	3	0	
Blastic crisis	2	3	
NHL	7 (13)	33 (40)	
Relapse 1	2	6	
Relapse ≥2	2	16	
Primary refractory	3	11	
Others*	0	3 (4)	
Chemotherapy within 2 months before HCT, N (%)	33 (63)	52 (65)	
Leukemia/MDS	30	23	
Lymphoma	3	26	
Others*	0	3	
HCT-CI score, N (%)			.03
0	33 (63)	28 (35)	
1-2	11 (21)	31 (39)	
≥3	8 (16)	21 (26)	
Conditioning regimen, N (%)			
TBI/CY	34 (65)	0	
BU/CY	13 (25)	0	
Fludarabine-based (± TBI)†	0	68 (85)	
Cladribine-based (±TBI)‡	0	12 (15)	
Others	5 (10)	0	
Donor type, N (%)			.1
HLA-matched related donor	17 (33)	41 (51)	
HLA-mismatched related donor	5 (9)	7 (9)	
Unrelated donor	30 (58)	32 (40)	
Stem cell source, N (%)			<.01
G-CSF mobilized PBSC	21 (40)	49 (61)	
BM	27 (52)	20 (25)	
CB	4 (8)	11 (14)	
GVHD prophylaxis, N (%)			<.01
Cyclosporine§	1 (2)	52 (65)	
Cyclosporine/MTX¶	49 (94)	28 (35)	
Tacrolimus	1 (2)	0	
Tacrolimus/MTX	1 (2)	0	
Prior HCT, N (%)	4 (8)	8 (10)	.65

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; TBI, total-body irradiation; CY, cyclophosphamide; BU, busulfan; HLA, human leukocyte antigen; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral blood stem cell; BM, bone marrow; CB, cord blood; GVHD, graft-versus-host disease; MTX, methoterexate.

*Others included 1 chronic lymphocytic leukemia and 2 multiple myeloma patients.

†Twenty-three patients received 4 Gy TBI.

‡Four patients received 4 Gy TBI.

§Including 7 with antithymocyte globulin.

¶Including 10 with antithymocyte globulin.