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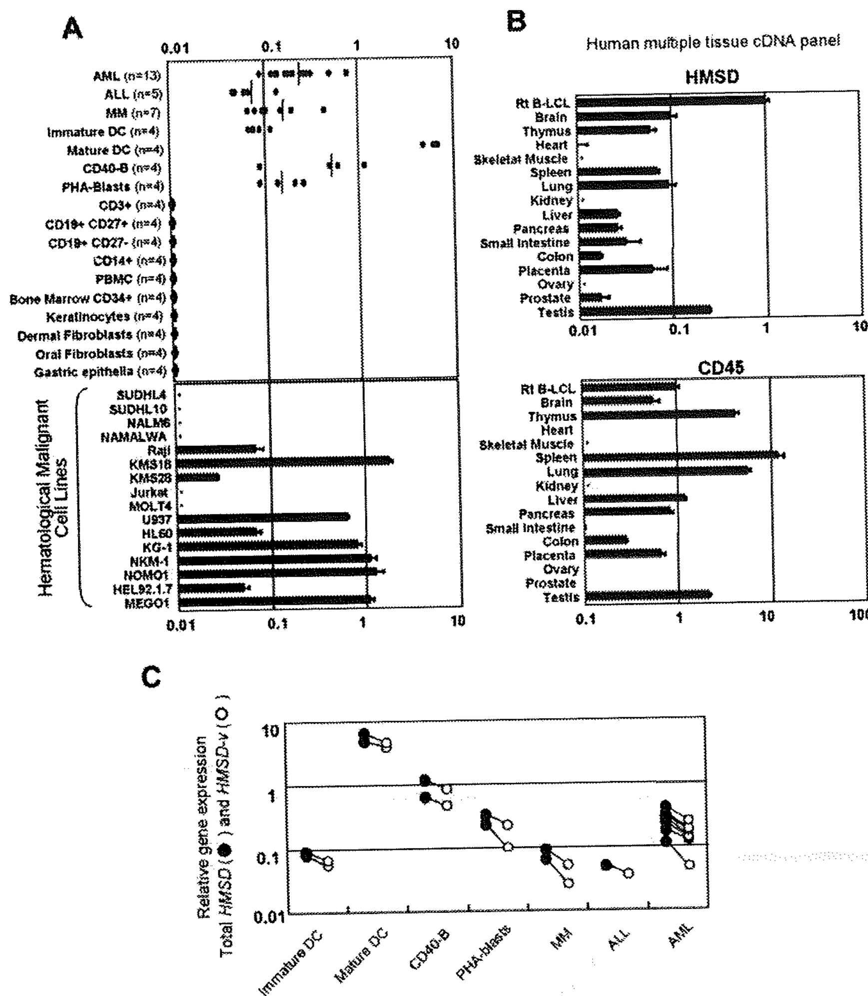

Figure 5. Selective mRNA expression of *HMSD* and *HMSD-v*. (A) Total *HMSD* expression was determined by real-time quantitative PCR in various normal tissues and malignant hematopoietic cell lines using a primer-probe set that detects the exon 3-4 boundary. Targeted mRNA expression in the recipient B-LCL is set as 1.0. In the top dotted plot graph, cDNAs prepared from CD34$^+$ subsets of primary leukemic cells and CD138$^+$ subsets of primary MM cells, freshly isolated hematopoietic cells, their subpopulations, immature and mature DCs, activated B and T cells, freshly isolated CD34$^+$ bone marrow cells, and primary cell cultures were similarly analyzed. Values in the parentheses indicate the number of the individuals tested. In the bottom and middle panels, cDNAs prepared from 16 hematologic malignant cell lines are shown. SUDHL4 and SUDHL10 are derived from B-cell non-Hodgkin lymphoma; NALM6 from acute B-lymphocyte leukemia; NAMALWA and Raji from Burkitt lymphoma; KMS18 and KMS28 from multiple myeloma (MM); Jurkat and MOLT4 from acute T-lymphocyte leukemia; U937 from histiocytic lymphoma; HL60, KG-1, NKM-1, NOMO1, and HEL92.1.7 from acute myeloid leukemia; and MEGO1 from chronic myeloid leukemia (blast crisis). (B) cDNAs of 15 normal tissue samples purchased from Clontech (MTC panels human I and II) were analyzed for total *HMSD* expression (top panel) and CD45 mRNA expression (bottom panel). Messenger RNA expression in the recipient B-LCL is set as 1.0. (C) *HMSD-v* expression levels (○) were compared with total *HMSD* expression levels (●) using a primer-probe set that detects the exon 1-3 boundary specific for *HMSD-v* mRNA. Among primary hematopoietic cells shown in the top of panel A, cells that were found to be heterozygous for ACC-6 allele were further selected and tested. Paired samples are linked.

CTL-2A12 (Figure 6A). The mRNA expression level of total *HMSD* in these AML cells was 47% (AML-1), 28% (AML-2), and 24% (AML-3) of that in the ACC-6-heterozygous recipient B-LCL, respectively.

Next, to determine whether the ACC-6 mHA recognized by CTL-2A12 is indeed expressed on LSCs and thus might have been involved in a GVL effect in AML patient UPN-027, we performed the LSC engraftment assay as previously reported[27] but substituted the significantly immunodeficient NOG mice because the absence of NK activity in NOG mice has been shown to facilitate the engraftment level of xenogenic human hematopoietic cells.[22] The CD34$^+$ fractions of primary AML cells that were lysed by CTL-2A12 (AML-2 in Figure 6A) were selected for this assay, since it was found to be negative for the HLA-B*4403-restricted mHA ACC-2^{D}[6] and not lysed by the ACC-2^{D}-specific clone CTL-3B5 (data not shown), which was used as an irrelevant control. These AML CD34$^+$ cells were incubated in vitro for 16 hours either alone or in the presence of CTL-2A12 or control CTL-3B5 at a T-cell/AML cell ratio of 5:1. Subsequently the mixtures were inoculated into NOG mice. After 5 weeks, flow cytometric analysis of BM and PBMCs was conducted to study the expression of human CD45, CD34, and CD8. Representative flow cytometric profiles are shown in Figure 6B. BM cells of control mice receiving AML-2 cells cultured in medium alone or with control CTL-3B5 before inoculation were found to contain 2.79% to 25.44% (mean, 20.29%) human CD45$^+$ CD34$^+$ cells, whereas PBMCs of the same 2 groups of mice contained 2.97% to 9.69% human cells. In contrast, human cells were not detectable in either BM or PBMCs of the mice inoculated with AML cells precultured with CTL-2A12. Percentage AML engraftment at 5 weeks after inoculation under these conditions is summarized in Figure 6C, indicating that CTL-2A12 eradicated AML stem cells with repopulating capacity ($P = .015$ for BM).

Follow-up of ACC-6–specific CTLs in peripheral blood from an AML patient (UPN-027)

To detect ACC-6–specific CTLs in peripheral blood from AML patient UPN-027 and from his donor, we performed real-time quantitative PCR (Figure 7A) using a set of primers and a fluorogenic probe specific for the unique CDR3 sequence of the CTL-2A12 TCR β chain at several time points. Although ACC-6-specific CTLs were not detected in blood samples from the donor and the patient before HCT, they became detectable in patient samples after HCT at frequencies of 0.11%, 0.23%, 0.83%, and 0.16% among CD3$^+$ cells at days 29, 91, 197, and 548, respectively (Figure 7B). During this period of time, there were no documented clinical manifestations of recurrent disease, and only grade 1 acute GVHD was noted.

Discussion

Antigenicity of the majority of previously identified human mHAs is generated by differences in amino-acid sequence between donor

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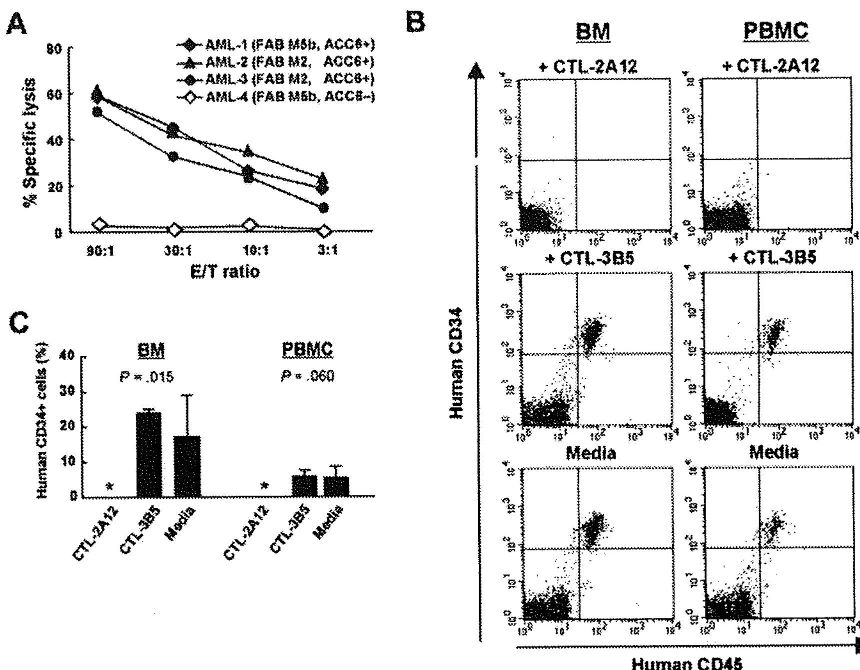

Figure 6. Inhibition of human AML stem cell engraftment in severely immunodeficient NOG mice by CTL-2A12. (A) Specific lysis by CTL-2A12 of primary leukemia cells. A standard 4-hour ^{51}Cr release assay was conducted at the indicated E/T ratios. The CD34$^+$ fraction of 3 primary AML cells positive for HLA-B*4403 and the ACC-6$^+$ allele by genotyping (AML-1, -2 and -3; the expression level of *HMSD* was 47%, 28%, and 24% of that in the recipient B-LCL, respectively) and 1 HLA-B*4403$^+$, ACC-6 allele-negative (AML-4) were tested. FAB denotes French-American-British classification. (B) Representative flow cytometric profiles of peripheral blood and BM cells from AML-inoculated NOG mice for the expression of human CD45 and CD34. Peripheral blood and BM cells were obtained 5 weeks after inoculation from mice receiving 7.0 × 10^6 AML-2 CD34$^+$ cells (negative for ACC-2^D mHA) that had been incubated with either CTL-2A12 (top), control CTL-3B5 (middle; HLA-B*4403-restricted, ACC-2^D mHA-specific CTL), or culture medium alone (bottom) at a T-cell/AML cell ratio of 5:1. (C) Summary of results from engraftment experiments. Mean (± SD) percentage of CD45 and CD34 double-positive cells of 3 mice in each group at 5 weeks after inoculation and the *P* values examined by 1-way ANOVA test are shown. Asterisk indicates that CD45 and CD34 double-positive cells were not detectable in NOG mice inoculated with AML-2 cells preincubated with CTL-2A12.

and recipient due to nonsynonymous SNPs. In this study, we identified a novel HLA-B44–restricted mHA epitope (ACC-6) encoded by an allelic splice variant of *HMSD* (*HMSD-v*) in which exclusion of exon 2 due to alternative splicing was completely controlled by an intronic SNP at IVS2+5. Indeed, by RT-PCR, the novel *HMSD-v* was not detected in cDNA samples from mHA$^-$ B-LCLs, whereas it was detectable in mHA$^+$ B-LCLs. An interesting question is why the splicing of exon 2 was completely controlled by the intronic SNP. In general, during intron splicing reactions, U1snRNA first binds the 5′ splice site of an intron, spliceosome assembly starts, lariat formation is made with several other factors, and thereafter the intron is spliced out (reviewed in Valadkhan[28]). Here U1snRNA is an important initiator of the cascade. It has been shown that aberrant splicing can result from mutations that either destroy or create splice-site consensus sequences at the 5′ splice site such that approximately half of the observed aberrant splicing is exon skipping while intron retention is rarely observed.[29] In this case, we speculate that the G-to-A substitution of the intronic SNP at nucleotide 5 in intron 2

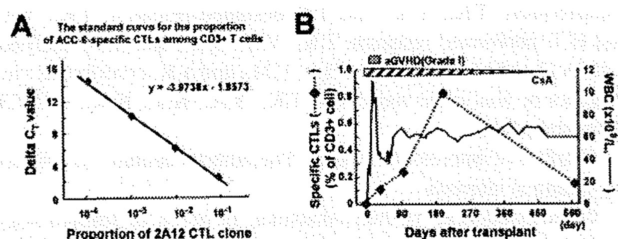

Figure 7. Detection of ACC-6–specific CTLs in peripheral blood from the AML patient (UPN-027) by real-time quantitative PCR using a set of primers and fluorogenic probe specific for the CTL-2A12 CDR3 sequence. (A) The standard curve for the proportion of ACC-6–specific CTL-2A12 serially diluted into CD3$^+$ cells from healthy donors using the comparative C_T (threshold cycle) method. The y-axis is delta C_T value. The x-axis is the log proportion of ACC-6–specific CTLs among CD3$^+$ T cells. (B) The frequency of T cells carrying the CDR3 sequence of CTL-2A12 over a period of 1.5 years after HCT. The percentages of such T cells among CD3$^+$ T cells (left y-axis) were estimated by using a standard curve in panel A and are indicated before HCT and after HCT at day 29, day 91, day 197, and day 548, respectively (diamonds with dotted line). Also noted are white blood cell (WBC) counts (right y-axis), acute GVHD (gray bar), and immunosuppressive therapy with cyclosporine A (CsA; hatched bar) during the same time period.

(IVS2+5G>A, 5′-GUACAU-3′), in addition to the presence of nonconsensus IVS2+4C (underlined), which is commonly observed in both mHA$^+$ and mHA$^-$ alleles and thus is likely to be permissive, completely disrupts the consensus alignment sequence critical for U1snRNA binding (5′-GUAAGU-3′) such that U1snRNA cannot stably bind the 5′ end of intron 2 in the precursor mRNA from the mHA$^+$ allele. A similar mutation (IVS3+5G>C, 5′-GUAACU-3′) and resultant exon 3 skipping was reported as a disease-causing mutation in the *NF1* gene.[30] Accordingly, intron 2 cannot be spliced out; a large lariat consisting of intron 1, exon 2, and intron 2 is formed; and then the large lariat is spliced out. In the latter case, 1 nucleotide (IVS1+4) does not match the U1snRNA sequence, but this mismatch is again likely to be permissive. Indeed, it has been shown that a mismatch at nucleotide 3, 4, or 6 of the 5′ splice site is not critical compared with others.[31,32] To our knowledge, this is the first demonstration of an mHA whose antigenicity is controlled by alternative splicing due to an intronic SNP, which may represent an important mechanism for the generation of mHAs.

The novel epitope was located on exon 3 and was transcribed from a reading frame different from the *HMSD* transcripts (Figure 4B). Although exon 3 is shared by *HMSD* and *HMSD-v*, it is speculated that polypeptide including the epitope was not being translated from *HMSD*, because donor B-LCL was not lysed by CTL-2A12. In general, ribosomes initiate translation from the first AUG start codon, but sometimes second or other AUG codons downstream can serve as start codons due to "leaky scanning."[33] However, it seems this is not the case for *HMSD* because the donor B-LCL homozygous for this allele was not lysed at all. This identification of an mHA unexpectedly generated from a previously unknown alternative transcript due to SNP has important implications for the identification of other new mHAs.

LSCs, which are present at very low frequencies, have a particularly strong capacity for proliferation, differentiation, and self-renewal[34] and likely play an important role in disease refractoriness or relapse after chemotherapy and transplantation. Thus, complete eradication of such stem cells is critical for cure in any treatment modalities. The LSC engraftment assay of AML cells in

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immunodeficient mice has been shown to be a powerful method for testing the effect of treatment, here mHA-specific CTLs, on LSCs. In addition, preliminary analysis has shown that CTL-2A12 lysed the CD34$^+$CD38$^-$ fraction of AML cells (Figure S1, available on the *Blood* website; see the Supplemental Figures link at the top of the online article), which is considered to contain leukemic stem-like cells.[35] These data clearly demonstrate that ACC-6 mHA is expressed on such stem cells and may serve as target for cognate CTL-2A12 in vivo.

We performed quantitative RT-PCR analyses for *HMSD* transcripts in various tissues with great interest because cytotoxicity assays suggested its limited expression in hematopoietic cells. Notably, *HMSD* showed selective expression in several hematopoietic primary tumor cells (especially those of myeloid lineage), mature DCs, and activated B and T cells. Since high expression was observed in mature DCs as in the case of *HMHA1* encoding HA-1 mHA,[36] immune responses to *HMSD*-derived mHAs may induce not only a GVL effect[37] against hematopoietic tumor cells but also GVHD,[38] since recipient DCs are responsible for initiating GVHD after HCT. Collectively, our data suggest that this novel mHA, ACC-6, might be a good target for immunotherapy inducing GVL if potential GVHD induction can be managed until recipient DCs have been eliminated early after HCT. Finally, relatively high expression of *HMSD* in the CD138$^+$ fraction of MM cells and their susceptibility to 2A12-CTL (Figure S2) suggest that ACC-6 may serve as a potential target for immunotherapy of multiple myeloma.

It is of interest to correlate clinical outcomes with ACC-6-specific T-cell kinetics after HCT using reagents such as tetramers. The preparation of HLA-B44 tetramer, however, is known to be very difficult,[39] so we used real-time quantitative RT-PCR using CTL-2A12 CDR3 sequence-specific primers/probe, because Yee et al[40] have previously shown strong concordance between semiquantitative RT-PCR analysis of a clone-specific CDR3 region and tetramer analysis used to monitor the fate of adoptively infused CTL clones for the treatment of melanoma. The highest frequency of 0.83% among CD3$^+$ cells was obtained at day 197 after HCT, concordant with the fact that CTL-2A12 was generated from the PBMCs collected at that time. This magnitude is somewhat lower than that observed in the case of LRH-1-specific T cells (1.6% of CD8$^+$ T cells) at the peak level after donor lymphocyte infusion (DLI)[16] but similar to that observed in the case of HA-1-specific T cells (1000 to 6000 tetramer-positive cells per mL blood, corresponding to 0.2% to 1.0% among CD3$^+$ cells).[41] The possibility that the ACC-6 mHA might preferentially induce GVL is supported by the fact that ACC-6–specific CTLs were detectable in the recipient's peripheral blood at a relatively high level after resolution of mild acute GVHD and that LSCs could be eradicated as shown in the NOG mice model. Whether or not ACC-6 mismatching in donor-recipient pairs may be associated with an increased risk of GVHD or morbidity would need to be studied using a large cohort of patients.

The therapeutic applicability of particular mHAs, calculated from the disparity rate and restricting HLA allele frequency, is an issue of interest.[42] The observed frequency of this ACC-6$^+$ phenotype was approximately 35% (n = 48/135) in healthy Japanese donors (data not shown) and HLA-B*4403 is present in around 20% of Japanese populations, so that ACC-6 incompatibility is expected to occur in approximately 4.6% of HCT recipient-donor pairs. Because CTL-2A12 lysed HLA-B*4402$^+$ B-LCLs possessing the ACC-6$^+$ phenotype derived from white individuals, this novel epitope peptide can also bind to HLA-B*4402, which is a relatively common allele (around 20%) in white populations. Actually, data from the HapMap Project[43] demonstrate that the genotype frequency of carrying at least one IVS2+5A (ACC-6$^+$) allele is 0.381 for individuals registered in the Centre d'Etude du Polymorphisme Humain (CEPH) cell bank,[44] thus this mHA should also be applicable to white patients. These results together suggest that *HMSD*-derived products could be attractive targets for immunotherapy and that given the possible role of intronic SNPs, a mechanism of alternative splicing should be also taken into consideration when searching for novel mHAs.

Acknowledgments

The authors thank Dr W. Ho for critically reading the manuscript; Dr T. Otsuki for myeloma cell lines; Dr Yoshihisa Morishita; Dr Tetsuya Tsukamoto for gastric mucosa and Dr Yoshitoyo Kagami for primary leukemia cells; Dr Eisei Kondo and Dr Hidemasa Miyauchi for helpful discussion; Dr Keitaro Matsuo, Dr Hiroo Saji, Dr Etsuko Maruya, Dr Kazuhiro Yoshikawa, Ms Hisano Wakasugi, Ms Yumi Nakao, Ms Keiko Nishida, Dr Ayako Demachi-Okamura, and Ms Hiromi Tamaki for their expert technical assistance.

This study was supported in part by Grants-in-Aid for Scientific Research (C; no.17591025) and Scientific Research on Priority Areas (B01; no.17016089) from the Ministry of Education, Culture, Science, Sports, and Technology, Japan; Research on Human Genome, Tissue Engineering Food Biotechnology and the Second and Third Team Comprehensive 10-year Strategy for Cancer Control (no. 30) from the Ministry of Health, Labor, and Welfare, Japan; a Grant-in-Aid from Core Research for Evolutional Science and Technology (CREST) of Japan; Science and Technology Corporation (JST); Daiko Foundation; and Nagono Foundation.

Authorship

Contribution: T.K., Y.A., and T.T. designed research; T.K., Y.A., and H.T. performed research; T.K., Y.A., S.O., and S.M. analyzed data; A.O., M.M., A.T., K.M., H.I., Y.M., and Y.K. contributed vital reagents or analytical tools; and T.K., Y.A., K.T., K.K., and T.T. wrote the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Possible Association between Obesity and Posttransplantation Complications Including Infectious Diseases and Acute Graft-versus-Host Disease

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Both obesity and malnutrition are considered risk factors for complications after bone marrow transplantation (BMT). To elucidate the impact of pretransplantation body mass index (BMI) on clinical outcome, we performed a retrospective cohort study with registration data from the Japan Marrow Donor Program (JMDP). Between January 1998 and December 2005, a total of 3935 patients received unrelated BMT through the JMDP; of these, 3827 patients for whom pretransplantation height and weight data were available were included in the study. Patients were stratified according to pretransplantation BMI values (low BMI: BMI < 18 kg/m^2, n $= 295$; normal BMI: $18 \leq$ BMI < 25 kg/m^2, n $= 2906$; overweight: $25 \leq$ BMI <30 kg/m^2, n $= 565$; obese: 30 kg/m$^2 \leq$ BMI, n $= 61$). In a univariate analysis, pretransplantation BMI was associated with a significantly greater risk of grade II-IV acute graft-versus-host disease (GVHD; $P = .03$). Multivariate analysis showed that pretransplantation BMI tended to be associated with an increased risk of grade II-IV acute GVHD ($P = .07$). Obesity was associated with an increased risk of infection compared with normal BMI (odds ratio = 1.9; 95% confidence interval = 1.1 to 3.2; $P = .02$). Our findings demonstrate a correlation between pretransplantation BMI and posttransplantation complications. Although BMI depends strongly on multiple factors, the effect of obesity on clinical outcome should be evaluated in a prospective study.

Biol Blood Marrow Transplant 15: 73-82 (2009) *© 2009 American Society for Blood and Marrow Transplantation*

KEY WORDS: Obesity, Allogeneic transplantation, Infection, Acute graft-versus-host disease

INTRODUCTION

Both obesity and malnutrition are considered risk factors for complications and increased relapse and nonrelapse mortality in hematopoietic stem cell transplantation (HSCT). An inferior outcome after allogeneic HSCT has been reported in obese adult patients in both allogeneic [1,2] and autologous HSCT [3-5]. Furthermore, our group recently reported that hyperglycemia during the neutropenic period is associated with an increased risk of acute graft-versus-host disease (GVHD) and subsequent nonrelapse mortality [6]. Obesity obviously is associated with an increased risk of hyperglycemia [7], which can lead to an inferior outcome after allogeneic HSCT. Recently, obesity was reported to be associated with low-grade systemic inflammation and was identified as a possible risk factor for autoimmune diseases [8-10]. Alternatively, malnutrition has been reported to be associated with an increased risk of early death after allogeneic HSCT [11,12]. Several reports have noted an association between malnutrition and a high incidence of infectious disease in conventional chemotherapy settings [13-15].

Although we can speculate that these infectious complications may be associated with nonrelapse mortality in HSCT, there is currently no agreement

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Financial disclosure: See Acknowledgments on page 81.

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Received September 12, 2008; accepted October 29, 2008
1083-8791/09/151-0001$36.00/0
doi:10.1016/j.bbmt.2008.10.029

regarding a suitable target range of pretransplantation body mass index (BMI) for clinical management. Previous studies have included various kinds of stem cell sources, and some have included HSCT with T cell depletion. The aim of the present study was to retrospectively evaluate the impact of pretransplantation BMI on the clinical outcome after unrelated bone marrow transplantation (BMT) for hematologic malignancies, using registration data from the Japan Marrow Donor Program (JMDP). The results should provide insight into how to better manage nutritional support for patients undergoing HSCT.

PATIENTS AND METHODS

A total of 3935 patients with various hematologic malignancies underwent BMT through the JMDP between January 1998 and December 2005. Data from 3827 of these patients for whom pretransplantation height and weight data were available were included in the present study. Patient characteristics are summarized in Table 1. The median patient age was 39 years (range, 18 to 72 years), and diagnoses included acute myeloid leukemia (AML; n = 1165), acute lymphoblastic leukemia (ALL; n = 755), myelodysplastic syndrome/myeloproliferative disease (MDS/MPD; n = 597), malignant lymphoma (ML; n = 500) and chronic ML (CML; n = 576), other leukemia (n = 69), multiple myeloma (n = 71), and other (n = 94). Standard risk included acute leukemia in first complete remission (CR1), CML in first chronic phase, MDS in refractory anemia, and lymphoma in CR1. The rest of the patients were categorized as a high-risk group. Bone marrow was the sole stem cell source for transplantation. Total body irradiation (TBI) was used in 2849 patients. GVHD prophylaxis included cyclosporine (CSP)-based (n = 1520) and tacrolimus (TAC)-based regimens (n = 2155), or other combinations (n = 152), with the addition of low-dose antithymocyte globulin (ATG) in 205 patients. Alleles at the HLA-A, -B, and -DRB1 loci were identified by high-resolution DNA typing. The median follow-up period was 565 days. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and the study design was approved by the JMDP's Institutional Review Board.

The study's primary endpoints were nonrelapse mortality at 100 days and 1 year, overall survival at 1 year, and progression-free survival at 1 year. For nonrelapse mortality, an event was death without disease progression after BMT. For overall survival, an event was death from any cause after BMT. For progression-free survival, an event was disease progression or death after BMT. Secondary endpoints were the incidence of infection (bacterial, viral, fungal, and others); incidence of lung organ toxicity including interstitial pneumonia, adult respiratory distress syndrome, bronchiolitis obliterans, pulmonary hemorrhage, and others, excluding pneumonia with obvious infectious diseases; and incidence of hepatic toxicity, including veno-occlusive disease and drug toxicity. Acute GVHD was classified as grade 0, I, II, III, or IV according to established criteria [16]. The probability of acute GVHD, nonrelapse mortality rate, overall survival, progression-free survival, and relapse rate were estimated using the Kaplan-Meier method. Death without acute GVHD was treated as censoring in the analysis of acute GVHD, and death without progression was treated as censoring in the analysis of relapse. Dichotomous variables between groups were compared using the χ^2 test, and survival times were compared using the log-rank test. An order-restricted version of the log-rank test (a log-rank trend test) was used to test ordered differences between the estimated survival curves. Multivariate analyses were performed using a logistic regression model or a Cox proportional hazards model, as appropriate. The following covariates were included in the univariate analysis: BMI (BMI < 18 kg/m^2, 18 ≤ BMI < 25 kg/m^2, 25 ≤ BMI < 30 kg/m^2, and 30 kg/m^2 ≤ BMI), sex (donor–recipient pairs), patient age (age < 30 years, 30 ≤ age < 50 years, age ≥ 50 years), donor age (age < 40 years, age ≥ 40 years), type of disease, risk of leukemia relapse (standard vs high), conditioning (TBI-based vs non–TBI-based), GVHD prophylaxis (CSP-based vs TAC-based), genotypic HLA match versus HLA mismatch, ABO match versus mismatch (major mismatch vs minor mismatch vs major/minor mismatch vs match), cell dose in the graft (dose < 3.0 × 10^8/kg, 3.0 ≤ dose < 5.0 × 10^8/kg, ≥ 5.0 × 10^8/kg), and use of ATG/antilymphocyte globulin (ALG) (ATG/ALG vs no ATG/ALG). All *P* values were 2-sided. A *P* value < .05 was considered statistically significant.

RESULTS

Patient Characteristics

Table 1 gives the BMI distribution of the study group. Patients were classified into 4 groups based on pretransplantation BMI values according to consensus weight designations from the World Health Organization [17] and the National Heart Lung and Blood Institute Expert Panel [18], as follows: low BMI (BMI < 18 kg/m^2; n = 295), normal BMI (18 ≤ BMI < 25 kg/m^2; n = 2906), overweight (25 ≤ BMI < 30 kg/m^2; n = 565), and obesity (30 kg/m^2 ≤ BMI; n = 61). The prevalence of obesity was quite low compared with that in previous reports from Western countries [1-4]. Significant differences in patient characteristics were observed with regard to age, sex disparity, total nucleated cells (TNCs) per body weight, and primary disease. The low-BMI group

Table 1. Patient Characteristics

	n (%)				
	BMI < 18 kg/m²	18 ≤ BMI <25 kg/m²	25 ≤ BMI < 30 kg/m²	30 kg/m² ≤ BMI	
	(n = 295)	(n = 2906)	(n = 565)	(n = 61)	*P* value
Recipient age, years					
< 30	116 (39)	734 (25)	90 (16)	14 (23)	< .0001
30 ≤ age < 50	121 (41)	1473 (51)	322 (57)	36 (59)	
> 50	58 (20)	699 (24)	153 (27)	11 (18)	
Donor age, years					
< 40	217 (74)	2099 (72)	385 (68)	38 (62)	.27
≥ 40	75 (25)	741 (25)	162 (29)	18 (30)	
Sex, donor/recipient					
Match	181 (61)	1833 (63)	374 (66)	39 (64)	< .0001
Male/female	70 (24)	519 (18)	87 (15)	9 (15)	
Female/male	43 (16)	495 (17)	87 (15)	9 (15)	
TNC (× 10^{-8}/kg)					
TNC < 3.0	12 (4)	323 (11)	160 (28)	31 (51)	< .0001
3.0 ≤ TNC< 5.0	60 (20)	1085 (37)	267 (47)	16 (26)	
5.0 ≤ TNC	187 (63)	1191 (41)	78 (14)	1 (2)	
Year of transplantation					
1998	12 (4)	83 (3)	11 (2)	0 (0)	.18
1999	21 (7)	248 (9)	39 (7)	0 (0)	
2000	26 (9)	363 (12)	81 (14)	7 (11)	
2001	43 (15)	398 (14)	74 (13)	7 (11)	
2002	47 (16)	409 (14)	72 (13)	14 (23)	
2003	50 (17)	404 (14)	87 (15)	10 (16)	
2004	40 (14)	463 (16)	88 (16)	12 (20)	
2005	56 (19)	538 (19)	113 (20)	11 (18)	
Diagnosis					
Acute leukemia	186 (63)	1469 (51)	304 (54)	29 (48)	.02
CR1/CR2/>CR2	81/33/65	594/301/541	113/79/107	7/4/18	
Chronic leukemia	30 (10)	449 (15)	84 (15)	13 (21)	
CP1/CP2/AP/BC	16/5/5/3	251/66/65/53	53/8/12/11	5/3/3/2	
MDS/MPD	37 (13)	462 (16)	87 (15)	11 (18)	
RA/RAEB/others	7/12/10	99/155/166	25/33/20	7/3/1	
ML	35 (12)	400 (14)	62 (11)	4 (7)	
CR/>CR	10/19	138/230	24/33	1/3	
MM	5 (2)	56 (2)	10 (2)	0 (0)	
CR/>CR	1/1	10/33	1/7	0/0	
Disease stage*					
Standard	110 (37)	1034 (36)	202 (36)	19 (31)	.67
High	158 (54)	1686 (58)	324 (57)	38 (62)	
Blood type disparity					
Match	146 (49)	1477 (51)	276 (49)	28 (46)	.98
IA	8 (3)	103 (4)	18 (3)	2 (3)	
MA	71 (24)	650 (22)	127 (22)	13 (21)	
MI	65 (22)	586 (20)	121 (21)	14 (23)	
HLA disparity					
HLA allele match	185 (63)	1660 (57)	342 (61)	36 (59)	.01
HLA allele mismatch	70 (24)	857 (29)	149 (26)	18 (30)	
1 allele mismatch	59 (20)	728 (25)	118 (21)	11 (18)	
2 allele mismatch	10 (3)	116 (4)	31 (5)	6 (10)	
3 allele mismatch	1 (0)	13 (0)	0 (0)	1 (2)	
Conditioning regimen					
Conventional	235 (80)	2308 (79)	443 (78)	52 (85)	.25
Reduced-intensity	59 (20)	539 (19)	105 (19)	5 (8)	
TBI for conditioning					
No	80 (27)	654 (23)	146 (26)	12 (20)	.14
Yes	214 (73)	2188 (75)	402 (71)	45 (74)	
ATG for conditioning					
No	268 (91)	2670 (92)	517 (92)	55 (90)	.21
Yes	23 (8)	155 (5)	25 (4)	2 (3)	
GVHD prophylaxis					
CSP-based	137 (46)	1141 (39)	226 (40)	16 (26)	.19
TAC-based	153 (52)	1651 (57)	312 (55)	39 (64)	
Others	3 (1)	48 (2)	7 (1)	2 (3)	
Comorbidity					
Liver dysfunction					
No	239 (81)	2436 (84)	481 (85)	49 (80)	.78
Yes	41 (14)	360 (12)	66 (12)	7 (11)	

(Continued)

Table 1. (*Continued*)

	n (%)				
	BMI < 18 kg/m^2	18 ≤ BMI <25 kg/m^2	25 ≤ BMI < 30 kg/m^2	30 kg/m^2 ≤ BMI	
	(n = 295)	(n = 2906)	(n = 565)	(n = 61)	*P* value
Renal dysfunction					
No	273 (93)	2706 (93)	528 (93)	54 (89)	.90
Yes	7 (2)	90 (3)	19 (3)	2 (3)	
Heart dysfunction					
No	260 (88)	2601 (90)	519 (92)	54 (89)	.32
Yes	20 (7)	195 (7)	28 (5)	2 (3)	
Pulmonary dysfunction					
No	267 (91)	2709 (93)	528 (93)	56 (92)	.27
Yes	13 (4)	87 (3)	19 (3)	0 (0)	

*Disease stage: Standard risk stage included CR1 in acute leukemia, first chronic phase in CML, and CR1 in lymphoma. Others were classified as high-risk stage.

included more young patients, patients receiving high TNCs per body weight, patients with acute leukemia, and male patients with a female donor.

Clinical Outcomes

The incidence of grade II-IV acute GVHD was 42% in the low-BMI group, 45% in the normal-BMI group, 48% in the overweight group, and 58% in the obesity group (Figure 1A). Thus, increased BMI was significantly associated with a higher incidence of grade II-IV acute GVHD (*P* = .03 by the log-rank trend test). Other factors associated with a higher incidence of grade II-IV acute GVHD were HLA allele disparity, GVHD prophylaxis with CSP (vs with TAC), and donor age ≥ 40 years. Multivariate analysis showed that pretransplantation BMI tended to be associated with an increased risk of grade II-IV acute GVHD (*P* = .07, log-rank trend test) (Table 2). The incidence of grade III-IV acute GVHD was 17% in the low-BMI group, 17% in the normal-BMI group, 19% in the overweight group, and 25% in the obesity group (Figure 1B). An increase in BMI tended to be associated with a higher incidence of grade III-IV acute GVHD, but this trend was not significant (*P* = .087, log-rank trend test). Multivariate analysis showed no association between pretransplantation BMI and the incidence of grade III-IV acute GVHD (*P* = .19, log-rank trend test) (Table 3).

Nonrelapse mortality was 29% in the low-BMI group, 31% in the normal-BMI group, 32% in the overweight group, and 40% in the obesity group at 1 year after BMT (*P* = .19, log-rank trend test) (Figure 2A). Overall survival was 61% in the low-BMI group, 58% in the normal-BMI group, 59% in the overweight group, and 53% in the obesity group at 1 year after BMT (*P* = .98, log-rank trend test) (Figure 2B). Progression-free survival was 54%, 52%, 56%, and 47% (*P* = .72, log-rank trend test), and the relapse rate was 24%, 24%, 18%, 21%, respectively, in the 4 groups at 1 year after BMT (*P* = .04 by log-rank trend test) (Figure 2C and D). The incidence of systemic infectious diseases, including bacterial, fungal, and viral infections, was 39%, 43%, 46%, and 59%, respectively, in the 4 groups (Figure 3). Obesity was significantly associated with increased incidence of infectious disease compared with normal

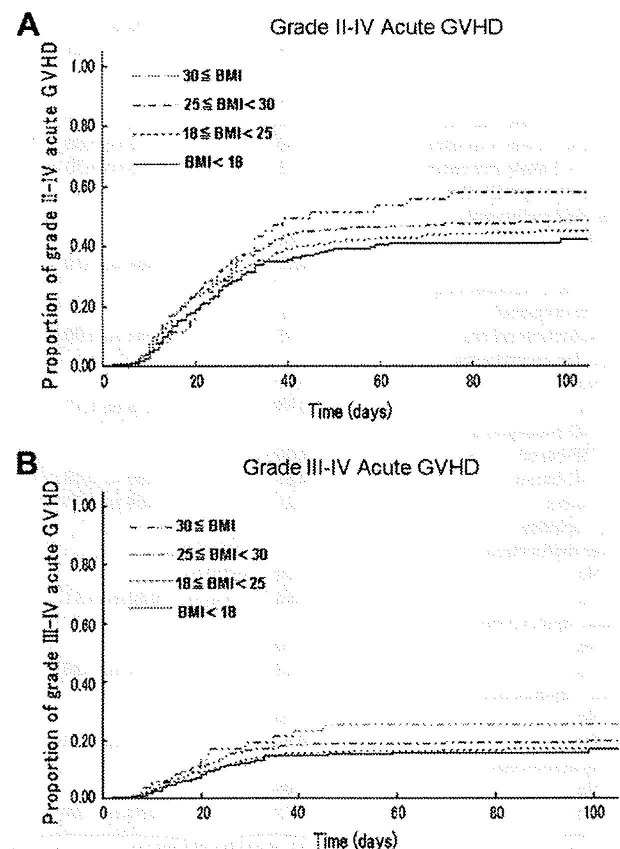

Figure 1. Probability of grade II-IV acute GVHD (A) and grade III-IV acute GVHD (B).

Table 2. Univariate and Multivariate Analyses of Risk Factors for Grade II-IV Acute GVHD

	Univariate Analysis			Multivariate Analysis		
Covariates	HR	95% CI	*P* value	HR	95% CI	*P* value
Recipient BMI						
18 ≤ BMI < 25 kg/m^2	1.00		.026*	1.00		.066*
BMI < 18 kg/m^2	0.91	0.75 to 1.10		1.06	0.85 to 1.31	
25 ≤ BMI < 30 kg/m^2	1.11	0.97 to 1.27		1.19	0.93 to 1.52	
30 ≤ BMI kg/m^2	1.28	0.89 to 1.85		1.29	0.82 to 2.03	
Recipient age, years						
<30	1.00		.85*			
30 ≤ age < 50	1.00	0.88 to 1.13				
≥ 50	0.99	0.86 to 1.14				
Donor age, years						
< 40	1.00		< .0001	1.00		< .0001
≥ 40	1.30	1.17 to 1.45		1.28	1.13 to 1.44	
Sex, donor/recipient						
Match	1.00		.053	1.00		.20
Male/female	1.12	0.98 to 1.27		1.09	0.95 to 1.26	
Female/male	1.15	1.01 to 1.32		1.12	0.97 to 1.30	
TNC (× 10^{-8}/kg)						
TNC <3.0	1.00		.76*			
3.0 ≤ TNC < 5.0	1.03	0.88 to 1.20				
5.0 ≤ TNC	0.99	0.85 to 1.16				
Diagnosis						
Acute	1.00		.28			
Chronic	1.08	0.93 to 1.24				
MDS/MPD	1.01	0.87 to 1.16				
ML	1.17	1.01 to 1.35				
MM	0.92	0.62 to 1.36				
Blood type disparity						
M	1.00		.15	1.00		.49
IA	1.19	0.92 to 1.55		1.14	0.85 to 1.52	
MA	1.03	0.90 to 1.16		1.02	0.89 to 1.17	
MI	1.14	1.00 to 1.29		1.11	0.96 to 1.27	
HLA disparity						
HLA allele match	1.00		< .0001	1.00		< .0001
HLA 1 allele mismatch	1.30	1.16 to 1.47		1.36	1.21 to 1.54	
HLA 2 allele mismatch	1.49	1.18 to 1.88		1.51	1.19 to 1.93	
HLA 3 allele mismatch	2.23	1.16 to 4.30		2.23	1.15 to 4.31	
Conditioning regimen:						
TBI for conditioning						
No	1.00		.67			
Yes	0.98	0.87 to 1.10				
Intensity of conditioning:						
Conventional	1.00		.42			
Reduced-intensity	0.95	0.84 to 1.08				
ATG for conditioning						
No	1.00		.58			
Yes	0.94	0.75 to 1.17				
GVHD prophylaxis						
CSP-based	1.00		.025	1.00		.0003
TAC-based	0.89	0.80 to 0.98		0.80	0.71 to 0.89	
Others	1.21	0.84 to 1.75		0.99	0.66 to 1.49	
Comorbidity						
Liver dysfunction						
No	1.00		.52			
Yes	0.95	0.82 to 1.11				
Renal dysfunction						
No	1.00		.84			
Yes	1.03	0.77 to 1.38				
Heart dysfunction						
No	1.00		.58			
Yes	0.94	0.77 to 1.16				
Lung dysfunction						
No	1.00		.15	1.00		.09
Yes	1.22	0.93 to 1.60		1.29	0.96 to 1.74	

*The log-rank trend test was used for calculating *P* values.

Table 3. Univariate and Multivariate Analyses of Risk Factors for Grade III-IV Acute GVHD

	Univariate Analysis			Multivariate Analysis		
Covariate	HR	95% CI	P value	HR	95% CI	P value
Recipient BMI						
$18 \leq$ BMI < 25 kg/m^2	1.00		.086*	1.00		.19*
BMI < 18 kg/m^2	0.99	0.73 to 1.35		1.01	0.72 to 1.43	
$25 \leq$ BMI < 30 kg/m^2	1.16	0.94 to 1.45		1.16	0.79 to 1.72	
$30 \leq$ BMI kg/m^2	1.56	0.90 to 2.71		1.42	0.70 to 2.87	
Recipient age, years						
< 30	1.00		.97*			
$30 \leq$ age <50	0.98	0.81 to 1.19				
≥ 50	1.00	0.79 to 1.25				
Donor age, years						
< 40	1.00		< .0001	1.00		< .0001
≥ 40	1.52	1.28 to 1.79		1.53	1.27 to 1.84	
Sex, donor/recipient						
Match	1.00		.057	1.00		.15
Male/female	1.21	0.99 to 1.49		1.13	0.90 to 1.42	
Female/male	1.23	1.00 to 1.51		1.24	0.99 to 1.56	
TNC ($\times 10^{-8}$/kg)						
TNC < 3.0	1.00		.56*			
3.0 < TNC < 5.0	0.90	0.71 to 1.15				
5.0 < TNC	0.91	0.72 to 1.16				
Diagnosis						
Acute leukemia	1.00		.66			
Chronic leukemia	1.13	0.90 to 1.41				
MDS/MPD	0.99	0.78 to 1.24				
ML	0.98	0.77 to 1.26				
MM	0.71	0.35 to 1.43				
Blood type disparity						
M	1.00		.55			
IA	1.04	0.67 to 1.61				
MA	1.11	0.91 to 1.13				
MI	1.15	0.94 to1.40				
HLA disparity						
HLA allele match	1.00		.0002	1.00		< .0001
HLA 1 allele mismatch	1.36	1.13 to 1.64		1.43	1.18 to 1.74	
HLA 2 allele mismatch	1.57	1.10 to 2.24		1.57	1.07 to 2.30	
HLA 3 allele mismatch	1.49	0.48 to 4.66		1.47	0.47 to 4.60	
Conditioning regimen						
TBI for conditioning						
No	1.00		.49			
Yes	0.94	0.78 to 1.13				
Intensity of conditioning regimen						
Conventional	1.00		.29			
Reduced-intensity	1.11	0.91 to 1.36				
ATG for conditioning						
No	1.00		.26			
Yes	0.80	0.55 to 1.18				
GVHD prophylaxis						
CSP-based	1.00		.029	1.00		.02
TAC-based	0.93	0.79 to 1.10		0.81	0.67 to 0.97	
Others	1.78	1.09 to 2.91		1.27	0.71 to 2.28	
Comorbidity						
Liver dysfunction						
No	1.00		.95			
Yes	0.99	0.78 to 1.27				
Renal dysfunction						
No	1.00		.20			
Yes	1.32	0.87 to 1.99				
Heart dysfunction						
No	1.00		.26			
Yes	0.82	0.57 to 1.16				
Lung dysfunction						
No	1.00		.11	1.00		.20
Yes	1.39	0.93 to 2.07		1.36	0.86 to 2.16	

*The log-rank trend test was used for calculating P values.

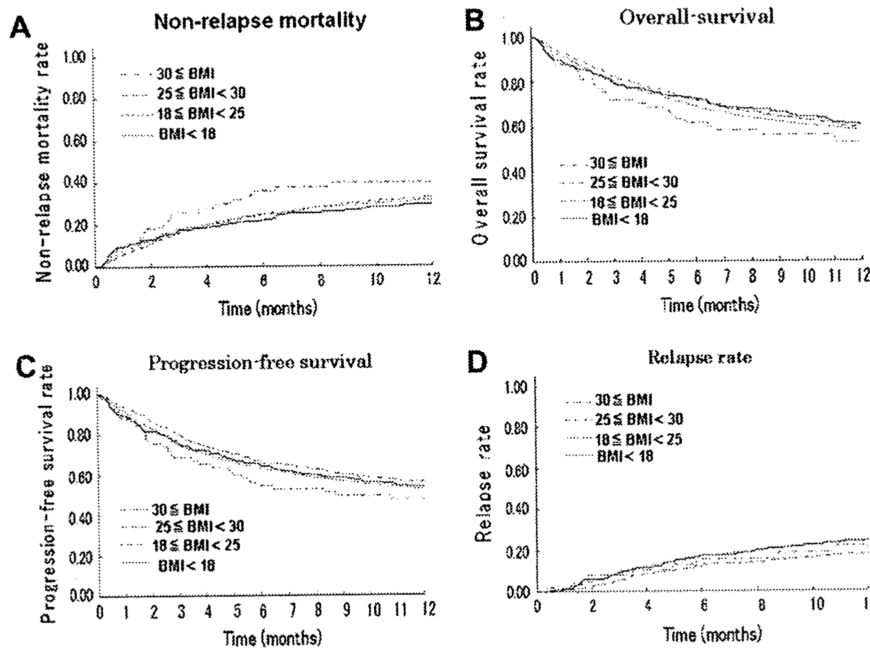

Figure 2. Probability of nonrelapse mortality (A), progression-free survival (B), overall survival (C), and relapse/progression (D).

weight (odds ratio = 1.9; 95% confidence interval [CI] = 1.1 to 3.2; P = .02). The incidence of liver dysfunction, including sinusoidal occlusive syndrome, was 19% in the low-BMI group, 20% in the normal-BMI group, 21% in the overweight group, and 25% in the obesity group; the differences were not statistically significant. The incidence of interstitial pneumonia, excluding obvious infectious diseases such as cytomegalovirus or *Pneumocystis jirovecii* pneumonia, was 13% in the low-BMI group, 13% in the normal-BMI group, 12% in the overweight group, and 15% in the obesity group; again, the differences are not statistically significant. The causes of death are given in Table 4. More infections and GVHD-related deaths were seen in the obesity group. If only early mortality is considered, then the nonrelapse mortality within 100 days was 17% in the low-BMI group, 18% in the normal-BMI group, 17% in the overweight group, and 25% in the obesity group. Obesity tended to be associated with greater early nonrelapse mortality, but this difference was not statistically significant (P = .83). The incidence of infection-related mortality within 100 days was 5%, 5%, 4% and 8%, respectively, in the 4 groups. Bacterial infection was the main cause of infection-related mortality, with 6 cases (40%) in the low-BMI group, 91 cases (67%) in the normal-BMI group, 17 cases (74%) in the overweight group, and 3 cases (60%) in the obesity group.

To investigate whether pretransplantation BMI had an additional impact on outcome in the patients who developed acute GVHD, we stratified the patients according to the grade of acute GVHD and analyzed the association between pretransplantation BMI and early nonrelapse mortality. We found that pretransplantation BMI had no additional impact on early nonrelapse mortality.

DISCUSSION

Both obesity and malnutrition are considered risk factors for complications, especially infectious diseases. To elucidate the impact of pretransplantation BMI on the clinical outcome, in this study we retrospectively reviewed the data of patients who underwent unrelated BMT, stratified according to recipient BMI, and found results similar to those reported previously [1,2]. The present study has an obvious limitation,

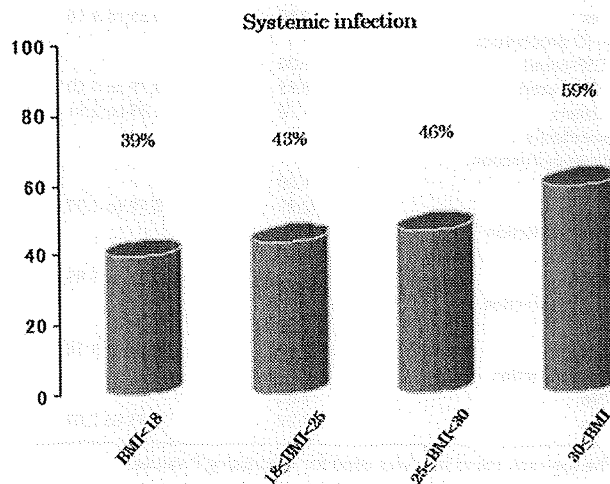

Figure 3. Incidence of systemic infections.

Table 4. Causes of Death

	BMI < 18 kg/m²	18 ≤ BMI < 25 kg/m²	25 ≤ BMI < 30 kg/m²	30 kg/m² ≤ BMI
	(n = 295)	(n = 2906)	(n = 565)	(n = 61)
Relapse, n (%)	66 (22%)	609 (21%)	95 (17%)	8 (13%)
Nonrelapse mortality, (%)	97 (33%)	988 (34%)	206 (36%)	24 (39%)
Infection, n (%)	25 (8%)	276 (9%)	56 (10%)	8 (13%)
Bacterial, n	13	151	34	4
Fungal, n	3	37	7	1
Viral, n	4	39	5	1
Mixed, n	2	16	3	1
Others, n	3	33	6	1
Acute GVHD, n (%)	9 (3%)	78 (3%)	26 (5%)	4 (7%)
Chronic GVHD, n (%)	4 (1%)	45 (2%)	13 (2%)	2 (3%)
Graft failure, n (%)	2 (1%)	31 (1%)	6 (1%)	1 (2%)
Organ dysfunction, n (%)	47 (16%)	395 (14%)	83 (15%)	7 (11%)
Others, n (%)	10 (3%)	163 (6%)	22 (4%)	2 (3%)

lacking concise data regarding weight-based dose adjustment of chemotherapy, which is critical for analyzing the incidence of organ dysfunction. Dosing schemes for preparative chemotherapy regimens vary widely among transplantation centers. In addition, centers differ in their use of ideal body weight, actual body weight, and compensatory calculations that yield doses between the actual and ideal weights [19,20]. Another limitation of this study is that low prevalence of obesity in Japan makes the study's statistical power less reliable. For example, patients with morbid obesity (BMI > 35 kg/m²), considered a significant comorbidity in a hematopoietic cell transplantation-specific comorbidity index, are quite rare in Japan [21]. Similar analyses need to be performed in Western countries to clarify the impact of obesity, especially morbid obesity, after allogeneic HSCT.

Our findings demonstrate that obesity is associated with an increased risk of infectious disease compared with normal weight. Hyperglycemia, caused primarily by insulin resistance in obesity, can lead to increased incidence of infectious disease. As reported by Sheean et al. [22], hyperglycemia after HSCT may be a risk factor for infectious disease. Recently, Derr et al. [23] reported an association between hyperglycemia before a neutropenic period and increased risk of infectious diseases during a neutropenic period after HSCT. In our study, an increased incidence of acute GVHD was associated with an increased risk of infectious disease. On the other hand, low BMI, which suggests the presence of malnutrition, was not associated with an increased risk of infectious diseases or transplantation-related mortality, inconsistent with previous reports [10,11]. This could be because the incidence of acute GVHD was lower and the dose of TNC per body weight was higher in the low-BMI group. Even if we further divide the BMI < 18 kg/m² group into 3 subgroups (BMI < 16 kg/m², 16 ≤ BMI < 17 kg/m², and 17 ≤ BMI < 18 kg/m²), we find no differences in the incidence of acute GVHD or infectious disease, or in clinical outcomes (data not shown). It is possible that in the Japanese population, BMI < 18 kg/m² may not directly reflect a malnutritional status.

Importantly, our findings also suggest an association between increased BMI and a significantly increased incidence of acute GVHD grade II-IV. This observation is based on multiple factors, and no single clear scientific explanation for it exists, but several mechanisms can be hypothesized. First, the dose of the conditioning regimen and GVHD prophylaxis could be improperly adjusted in obese patients, possibly leading to increased tissue damage or poorer GVHD prophylaxis and, ultimately, a higher incidence of acute GVHD. With regard to the conditioning regimen, the relapse rate was lower in the overweight and obese patients compared with the low-BMI and normal-BMI patients, but the incidence of regimen-related toxicity (ie, liver dysfunction and interstitial pneumonitis) did not differ significantly among these groups. With regard to GVHD prophylaxis, there might not have been any significant difference in drug exposure, because dose adjustment of the calcineurin inhibitor usually is done through serial monitoring of drug concentration. Second, the stem cell dose could influence the incidence of acute GVHD. But in this study, the stem cell dose was analyzed independently, and no association was found between stem cell dose and the incidence of acute GVHD. Third, there was an obvious selection bias in each group. For example, it is possible that obese patients may be less likely to find an unrelated donor with an adequate dose of cells for transplantation. While the donor search continued, the number of chemotherapy courses could increase, and the patient's general condition (including disease status and organ function) could become worse. Finally, even though there were no direct data regarding glucose levels in this study, obesity is likely associated with hyperglycemia [7-9], possibly resulting in elevated levels of several cytokines [24-27], inducing a vicious cycle [28-30]. Our group previously reported an association

between hyperglycemia during neutropenia and the development of acute GVHD [6], possibly due to the augmented production of cytokines stimulated by the conditioning regimen. Furthermore, recently it has become clear that adipocytokines, which are secreted mainly from adipocytes, play important roles in the control of immunity [31-33]. In particular, the level of leptin has been found to be proportional to body fat weight and to affect T regulatory cell (Treg) proliferation and function [34,35]. Thus, it could be hypothesized that in obese patients, a higher leptin level suppresses Treg activity, increasing the risk of acute GVHD. These mechanisms are based on the results of animal models, however, and await confirmation in human studies.

The clinical significance of our findings merits careful consideration, because pretransplantation BMI is one of the few factors that can be properly managed and corrected during the unstable, fast-moving pretransplantation period. On the other hand, malnutrition can be corrected by appropriate nutritional support, and obesity can be controlled through an appropriate diet and exercise program during chemotherapy. This study suggests that such a pretransplantation nutritional support program can improve clinical outcomes after allogeneic BMT.

In conclusion, this retrospective analysis of registration data found an association between pretransplantation obesity and increased risk of infectious disease, possibly leading to increased risk of mortality. Although body weight is affected by multiple clinical factors, the effect of obesity on clinical outcome, as suggested here, needs to be confirmed by a prospective study to identify better patient management approaches.

ACKNOWLEDGMENTS

Financial disclosure: This study was supported by Health and Labor Science Research Grant from Ministry of Health, Labor and Welfare of Japan. We thank the staff members of the transplantation center, donor centers, and JMDP office for their generous cooperation, and thank Drs Kawase and Morishima for providing data. Author contributions: S.F., S.K., K.Y., S.M. participated in the conception of this study; H.A., S.O., H.S., J.T., N.K., S.M. performed the execution for transplantation; K.Y. performed statistical data analysis; S.F., S.K., K.Y., S.M. wrote the paper; and all authors checked the final version of the paper.

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Bone Marrow Transplantation (2009) 44, 105–111
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www.nature.com/bmt

ORIGINAL ARTICLE

Intensive glucose control after allogeneic hematopoietic stem cell transplantation: a retrospective matched-cohort study

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Some studies have shown that intensive glucose control (IGC) improves outcome in the intensive care unit setting. However, it is the benefit of IGC in hematopoietic SCT (HSCT) that is not well defined. Between June 2006 and May 2007, IGC was maintained prospectively after allogeneic HSCT and clinical outcomes were compared with a cohort matched for conditioning regimen, source of stem cells, age and relation to donor. A stratified Cox regression model was used. There were no significant differences in baseline clinical characteristics. The median age was 43.5 years in both groups. The primary diagnosis was a hematologic malignancy. Patients in the IGC group had a lower glucose level (least-square mean, 116.4 vs 146.8 mg per 100 ml, $P<0.001$) compared to the standard glucose control group. The incidences of documented infections and bacteremia were significantly lower in the IGC group (14 vs 46%, $P=0.004$, 9 vs 39%, $P=0.002$, respectively). IGC tended to reduce the incidence of renal dysfunction (19 vs 37%, $P=0.36$) and the elevation of C-reactive protein (18 vs 38%, $P=0.13$). This study suggests that IGC has may have a beneficial effect after HSCT. IGC should be evaluated further in a large prospective, randomized study.
Bone Marrow Transplantation (2009) **44,** 105–111; doi:10.1038/bmt.2008.431; published online 19 January 2009
Keywords: intensive glucose control; allogeneic transplantation; hyperglycemia; C-reactive protein

Introduction

Previous studies showed that intensive glucose control (IGC), in which the target blood glucose level was set within 80–110 mg per 100 ml, reduced infections, dysfunction of organs including the liver and kidney and mortality compared to patients who received standard glucose control.[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 IGC rather than to any pharmacological activity of administered insulin *per se*.[3,4]

Recipients of allogeneic hematopoietic SCT (HSCT), which is the most drastic therapeutic modality in patients with hematological malignancies, often suffer from serious complications including infectious diseases, GVHD and multiple organ failure. They are also at higher risk of hyperglycemia because of the use of steroids for the treatment of GVHD, the use of total parenteral nutrition (TPN), immunosuppressive drugs and infectious complications,[10,11] which makes them further susceptible to numerous serious complications including infectious diseases and multiple organ failure.[12–14] Our group previously reported that hyperglycemia during neutropenia was associated with an increased risk of acute GVHD and nonrelapse mortality (NRM) after myeloablative allogeneic HSCT,[15] and that hyperglycemia during neutropenia was associated with a higher incidence of subsequent acute GVHD. It is well known that an increase in the levels of circulating cytokines may aggravate hyperglycemia, and hyperglycemia itself could increase the levels of cytokines. This vicious cycle could lead to elevated cytokine levels, which could lead to subsequent acute GVHD. With this background, it can be hypothesized that IGC would reduce the incidence of infectious diseases, acute GVHD and organ dysfunctions after allogeneic HSCT. Therefore, we prospectively investigated the effect of IGC after allogeneic HSCT, and compared the clinical outcomes to those in a matched cohort to address whether IGC following allogeneic HSCT could improve the clinical course of patients, that is, reduction of infectious diseases and organ dysfunction, as has been shown in the intensive care unit (ICU) setting.

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Received 22 May 2008; revised 28 October 2008; accepted 21 November 2008; published online 19 January 2009

Patients and methods

Patients
From June 2006 to May 2007, a total of 73 patients received allogeneic HSCT at the National Cancer Center Hospital (Tokyo, Japan); 60 patients were eligible for participation in this trial. Finally, 22 patients (36.7%) were enrolled in this IGC study to keep the blood glucose level at 80–110 mg per 100 ml, as shown in Figure 1.

Study center and organization
The National Cancer Center Hospital in Tokyo holds 600 beds. The transplant team consists of 4 full-time physicians and 26 nursing staff who oversee 26 beds in the HSCT, and the entire ward is covered by high-efficiency particulate air-filters. We regularly perform 90–120 transplants per year: 80% allogeneic and 20% autologous.

Study design
This was a case–control study to investigate the clinical benefits of comprehensive nutritional support including IGC and parenteral nutrition (PN) management, which was approved by the Institutional Review Board. A matching control group was selected among patients who received HSCT from January 2002 to March 2007 (ratio of 1:2 compared to the study group) according to the following criteria: (1) conditioning regimen (conventional myeloablative or reduced intensity), (2) source of stem cells (BM, peripheral blood or cord blood), (3) age and (4) source of donor (related or unrelated). Criteria (1–4) were essential for inclusion. As a result, 42 matched controls were selected, and a total of 64 patients were subjected to further analysis (Table 1).

Exclusion criteria
Exclusion criteria were as follows: (1) patients who received a reduced-intensity conditioning regimen for an HLA-matched related donor, as we applied GVHD prophylaxis without short-term MTX in this setting, and they had much less need for TPN and less need for intense glucose control,[16] (2) those with a poor performance status (Eastern Cooperative Oncology Group) ⩾2, (3) those with uncontrolled infectious diseases at the beginning of the conditioning regimen and (4) those with preexisting neutropenia. We previously reported that the incidence of severe stomatitis (Common Terminology Criteria for Adverse Events (CTCAE) grade (3) was 0% after reduced-intensity SCT (RIST) from a related HLA-matched donor.[16] In this situation, the need for TPN and the incidence of hyperglycemia were quite low, compared to RIST from an unrelated donor, which included additional low-dose TBI or antithymocyte globulin (ATG) and short-term MTX or conventional SCT with a myeloablative regimen. Hence, we only included patients who received a RIST regimen from an unrelated donor, who had a higher probability of glucose-control intervention, to evaluate the beneficial effects of IGC.

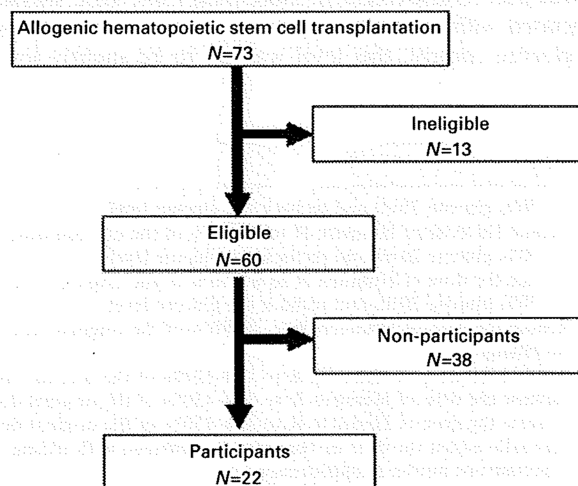

Figure 1 Trial profile.

Table 1 Patients' characteristics

Variable	N (%)/median (range)		P-*value*
	Intensive glucose control (n = 22)	*Standard glucose control* (n = 42)	
Age (years)	43.5 (17–64)	43.5 (20–66)	
<40	8 (36)	18 (43)	0.62
⩾40	14 (64)	24 (57)	
Sex			
Male	9 (41)	22 (52)	0.38
Female	13 (59)	20 (48)	
Disease risk[a]			
Standard	6 (27)	16 (38)	0.39
High	16 (73)	26 (62)	
Conditioning			
CST	14 (64)	27 (64)	
BU/CY	9 (40)	18 (43)	
CY/TBI (12 Gy)	4 (18)	6 (14)	
Other	1 (5)	3 (7)	
RIST	8 (36)	15 (36)	0.96
2CdA/BU	1 (5)	1 (2)	
Flu/BU	7 (32)	14(33)	
Low-dose TBI (2–4 Gy)	3 (14)	7 (17)	
Low-dose ATG	5 (23)	10 (24)	0.92
GVHD prophylaxis			
Cyclosporin-based	7 (32)	27 (64)	
Tacrolimus-based	15 (68)	15 (36)	0.01
Short-term MTX (+)	22 (100)	40 (95)	0.30
Relation to donor			
Related	6 (27)	12 (29)	
Unrelated	16 (73)	30 (71)	0.91
Stem cell source			
Bone marrow	15 (68)	30 (71)	
PBSC	5 (23)	10 (24)	
Cord blood	2 (9)	2 (5)	0.19
HLA match			
Match	11 (50)	28 (67)	
Mismatch	11 (50)	14 (33)	0.19

Abbreviations: ATG = antithymocyte globulin; 2CdA = cladribine; CST = conventional stem cell transplantation; Flu = fludarabine; RIST = reduced-intensity stem cell transplantation.
[a]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.

Transplantation procedures
Forty-one patients received a myeloablative conditioning regimen that included BU (orally 4 mg/kg per day × 4 days or i.v. 3.2 mg/kg per day × 4 days) plus CY (60 mg/kg per day × 2 days, $n = 27$), CY plus 12 Gy TBI ($n = 10$) or other ($n = 4$). Twenty-three patients received a reduced-intensity conditioning regimen that included fludarabine (30 mg/m^2 per day × 6 days) or cladribine (0.11 mg/kg per day × 6 days) plus BU (oral 4 mg/kg per day × 2 days or i.v. 3.2 mg/kg per day × 2 days). Low-dose TBI (2 or 4 Gy, $n = 10$) and/or low-dose ATG (total dose 5–10 mg/kg ATG-F or 5 mg/kg thymoglobulin, $n = 15$) were added. GVHD prophylaxis included CYA- ($n = 13$) and tacrolimus-based regimens ($n = 51$), with an additional short course of MTX. 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 after the beginning of the conditioning regimen until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis after the beginning of the conditioning regimen. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and VZV after the beginning of the conditioning regimen until immunosuppressive agents were discontinued. 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 or other cephalosporin, 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 ANC exceeded 0.5×10^9 per l.

Glucose management protocol
In the IGC group, the blood glucose level was routinely tested every morning to adjust the dose of insulin so as to keep the level within the range of 80–110 mg per 100 ml. Owing to the presence of fewer nursing staff in the HSCT unit than in the ICU, we replaced the continuous infusion of insulin with the addition of Humulin R to the bottle of PN to control the glucose level within the target range. In TPN, we universally added at least 1 unit of Humulin R per 10 g glucose. In patients who had an elevated blood glucose level, we also added Humulin R to the bottle of PN. We monitored the glucose level at least once a day in the morning as long as the level remained within the target range of 80–110 mg per 100 ml. When the glucose level became elevated, we increased the frequency of monitoring up to 2–4 times daily. In most patients, we adjusted the dose of insulin added to the bottle of PN as described in Table 2. When the blood glucose level was >180 mg per 100 ml or the dose of insulin was high, we manually adjusted the dose of Humulin R and administered insulin subcutaneously according to the attending physician's discretion. S.c. insulin administration usually consisted of 3–5 units at the beginning, and, if this was insufficient, the dose was manually adjusted by 2–4 units. When the patients received high-dose systemic steroid such as methylprednisolone 1–2 mg/kg per day for GVHD, we used the preprandial s.c. injection of insulin Aspart (NovoRapid) three times daily to avoid postprandial hyperglycemia and adjusted the dose according to the amount of food intake and the postprandial glucose level. When patients exhibited nausea, anorexia or vomiting, the amount of food intake became unstable. In such situations, insulin Aspart was injected immediately after the meal. When food intake was <50%, the dose was reduced or discontinued. Routine glucose monitoring was continued until PN was stopped, whereas the blood glucose level was maintained within the target range. Daily caloric intake was calculated by the dietitians. We tried to maintain oral intake as much as possible by using a suitable diet in jelly or liquid form. A dietitian adjusted the dose of supplemental PN to maintain the total caloric intake over 1.0 × basal energy expenditure (BEE), and if the glucose level was stable, the nutritional intake could be increased up to 1.5 × BEE. The glucose concentration in PN was usually started at 7.5% glucose as supplemental PN. The concentration was gradually increased to 12%, and, if necessary, this was further increased up to 18% to meet the target caloric intake. A lipid emulsion was also used to supply 10–30% of total caloric intake. The minimal total nutritional intake was set at 1.0 × BEE because a retrospective analysis at our institute showed that caloric intake of more than 1.0 × BEE was not associated with clinically significant wt loss.[17] To improve the glucose control, this level was set to be slightly lower

Table 2 Protocol for adjustment of Humulin R

Glucose level (mg per 100 ml)	*Adjustment of Humulin R*
BS≤40	i.v. 50% glucose 20 ml and recheck the glucose level Reduce the dose of Humulin R to 40–60% of the original dose
40≤BS<60	i.v. 50% glucose 20 ml and recheck the glucose level Reduce the dose of Humulin R to 60–80% of the original dose
60≤BS<80	i.v. 50% glucose 20 ml and recheck the glucose level Reduce the dose of Humulin R to 70–90% of the original dose
80≤BS≤110	No change
110<BS<130	Increase the dose of Humulin R to 110–120% of the original dose
130≤BS<150	Increase the dose of Humulin R to 120–130% of the original dose
150≤BS<180	Increase the dose of Humulin R to 130–150% of the original dose
BS≥180	Manually adjust the dose of Humulin R combined with sliding subcutaneous insulin administration

Abbreviation: BS = blood sugars.

than the recommendation in the HSCT setting (1.3–1.5 × BEE[18]). There are two beneficial aspects of this protocol: we could maintain the minimal caloric intake with supplemental PN and we could immediately start insulin as required after the introduction of PN. The SGC group was managed without a specific protocol for nutrition practice and glucose control, although we routinely monitored blood glucose at least three times weekly to avoid severe hyperglycemia (blood glucose >200 mg per 100 ml).

Outcome measures
Serially monitored glucose values were compared between the IGC group and the SGC group. We also analyzed the association between the mean glucose level during monitoring and the infection rate in both the SGC group and IGC group. Mean glucose levels were estimated for each patient and were categorized as follows: 80–110, 111–140, 141–179 and >180. Glycemic variability, defined as the s.d. of the mean glucose value, was also analyzed. The outcome measures were time to the occurrence of documented infectious complications within 100 days after HSCT, time to each organ dysfunction defined as described below, time to grades II–IV and grades III–IV acute GVHD and time to NRM. These were calculated from the date of the start of the conditioning regimen. Organ dysfunction was defined with reference to van den Berghe[5–7] as follows: (1) hypercreatininemia; serum creatinine level ⩾2.0 mg per 100 ml or more than twice the baseline, (2) hyperbilirubinemia; serum total bilirubin level ⩾2.0 mg per 100 ml and (3) increased inflammatory markers; serum C-reactive protein (CRP) level ⩾15 mg per 100 ml. In our institute, the CRP level was routinely monitored at least three times a week, as we previously reported that the preengraftment CRP level may predict a subsequent occurrence of acute GVHD and NRM after allogeneic HSCT.[19] These results suggested that CRP might be useful not only as a marker of infectious diseases but also as a surrogate marker for produced cytokines. Therefore, the serial changes of CRP level were compared between the two groups. Acute GVHD was graded by the consensus criteria.[20]

Statistical analyses
Baseline characteristics were summarized using descriptive statistics. The Student's t, χ^2 and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. The probability of documented infectious complications and organ dysfunction were calculated using Kaplan–Meier estimates. A stratified Cox regression model, which accounts for the matched-cohort design, was used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs). On the basis of 64 patients, the study has an approximately 80% power to detect a HR of 0.5 for documented infections. The glucose values, measured repeatedly, were compared between groups using a repeated-measure analysis with a linear mixed-effect model. A level of $P<0.05$ was defined as statistically significant. All P-values are two-sided. All analyses were performed using SAS version 9.1.3 (Cary, NC, USA).

Results

Patient characteristics
Table 1 lists the patients' clinical and transplantation characteristics. Patients and transplantation characteristics were well balanced with the application of matching criteria. Nevertheless, in the IGC group, more patients received tacrolimus for GVHD prophylaxis (68 vs 36%, $P=0.01$) and more had a previous transplantation (32 vs 7%, $P=0.01$). The median duration of follow-up in surviving patients was 299 days (range, 78–607 days) in the IGC group and 1146 days (range, 329–1774 days) in the SGC group.

Glycemic control
Duration of monitoring and number of tests. The median duration of glucose monitoring and intervention in the IGC group was 38 days (range, 24–70 days) after the start of the conditioning regimen. The total number of glycemic monitorings was 867 and 1094 in the SGC group and IGC group, respectively.

Mean values and distribution of values. Patients in the IGC group had a lower glucose level (least-square mean, 116.4 vs 146.8 mg per 100 ml, $P<0.001$) than the SGC group. The trend of the glucose value is shown in Figure 2a. All glycemic results for the SGC and IGC groups were stratified into six levels: <40, 40–79, 80–110, 111–140, 141–179 and ⩾180, as shown in Figure 2b.

Hypoglycemia
In the IGC group, the incidence of mild hypoglycemia (CTCAE grades 1–2, glucose level 40–69 mg per 100 ml) was significantly higher than that in the SGC group (11 vs 3 patients, $P<0.001$). Although one patient (4.5%) in the IGC group who was diagnosed as type 2 diabetes mellitus developed severe hypoglycemia (CTCAE grade 3, glucose level 30–39 mg per 100 ml) with faintness, no patient developed seizure or loss of consciousness.

Glycemic variability
The mean glycemic variability in the SGC group and IGC group was 37.2 mg per 100 ml (range, 10.1–121.7 mg per 100 ml) and 27.5 mg per 100 ml (range, 11.3–46.6 mg per 100 ml), respectively, and glycemic variability in the IGC group tended to be lower than that in the SGC group ($P=0.07$).

TPN and insulin dosing
The percentage of patients who received TPN was 60% (25 patients) and 77% (17 patients) in the SGC group and the IGC group, respectively. The mean duration of TPN was 9 days (range, 0–35) and 13 days (range, 0–38) in the SGC group and IGC group, respectively. There was a tendency for more patients in the IGC group to receive TPN compared to the SGC group, but this difference was not statistically significant. The mean maximal dose of insulin (median (range), 51 (0–100) vs 2 (0–110) IU, $P<0.001$) and the mean maximal dose of insulin per 1 g parenteral glucose

were significantly higher in the IGC group (median (range), 0.22 (0–0.71) vs 0.003 (0–0.4) IU/g glucose, $P<0.001$).

Infections

Table 3 summarizes the results. In the IGC group, dramatically fewer patients developed documented infections within 100 days compared to the SGC group, as shown in Figure 3.

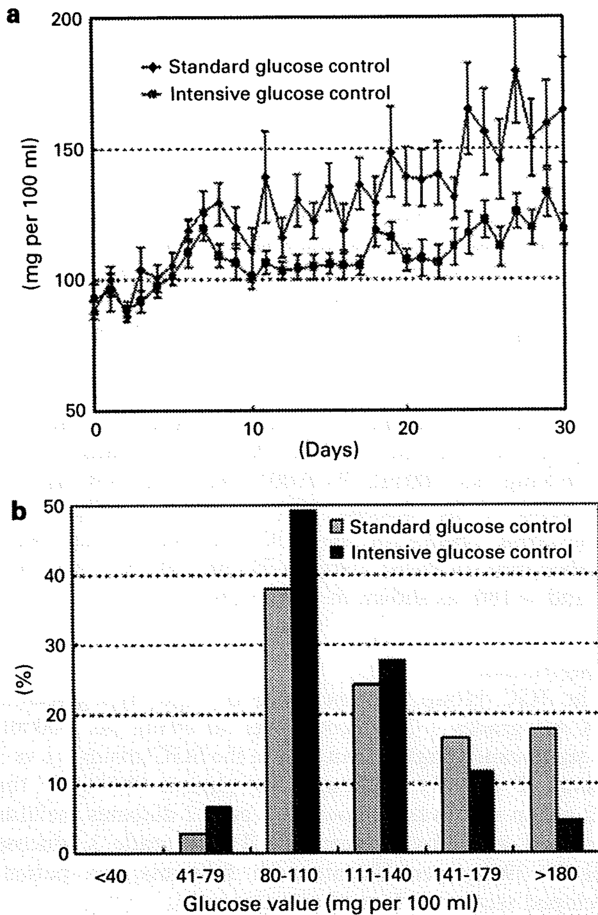

Figure 2 Serial changes in the mean glucose level in the intensive glucose control (IGC) and standard glucose control (SGC) groups. Values are mean + s.e. (**a**). The distribution of the glucose values in IGC and SGC is shown as a histogram (**b**).

Relation to mean glucose level

We also analyzed the association between the mean glucose level during monitoring and the infection rate in both the SGC and IGC groups. The incidence of infection was 34, 17, 67 and 40%, respectively, with mean glucose levels of 80–110, 111–140, 141–179 and ⩾180. When we compared a lower glucose-level group (mean glucose level of 80–140) with a higher glucose-level group (mean glucose level of >140), the incidence of infection was significantly higher in the latter group (28 vs 57%, $P=0.042$). When we assessed only patients with a lower glucose level, the IGC group tended to show a lower incidence of infectious diseases than the SGC group (14 vs 41%, $P=0.061$).

Relation to glycemic variability

We also analyzed the association between glycemic variability and the infection rate. The mean glycemic variability in patients with and without infection was 34.6 mg per 100 ml (range, 10.5–121.7 mg per 100 ml) and 33.3 mg per 100 ml (range, 10.1–110.6 mg per 100 ml), respectively, with no significant difference. As the importance of glycemic variability could vary among patients

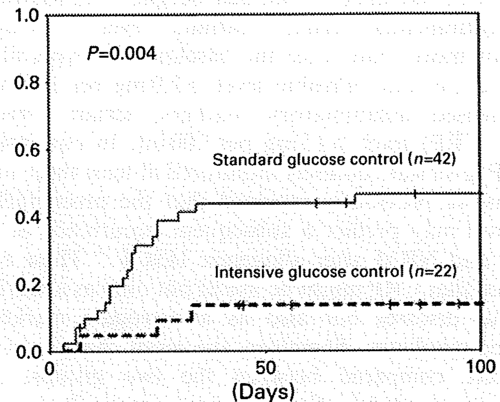

Figure 3 Probability of documented infections in the IGC and SGC groups.

Table 3 Incidence of infectious diseases and organ dysfunction

Variable	*N (%)/median (range)*			
	Intensive glucose control n = *22 (%)*	*Standard glucose control* n = *42 (%)*	*HR (95% CI)*	P-*value*
Documented infection	13	46	0.17 (0.04–0.75)	0.004
Bacteremia	9	39	0.10 (0.01–0.74)	0.002
Organ dysfunction				
Hypercreatininemia[a]	19	37	0.60 (0.19–1.88)	0.36
Hyperbilirubinemia[b]	28	31	1.05 (0.38–2.91)	0.93
Increased inflammatory markers[c]	18	38	0.45 (0.15–1.37)	0.13

Abbreviations: CI = confidence interval.
[a]Serum creatinine level ⩾2.0 mg per 100 ml or more than twice of baseline.
[b]Serum bilirubin level ⩾2.0 mg per 100 ml.
[c]Serum C-reactive protein level ⩾15 mg per 100 ml.

with different mean glucose levels,[21] we divided the patients into two groups based on mean glucose level 80–140 or 140+ and then determined whether glycemic variability was associated with an increased incidence of infections. However, there was no significant association between glycemic variability and the incidence of infections in both groups.

CRP levels

Figure 4 shows serial changes in the CRP level. Even though there was no difference in the CRP level between the two groups at the beginning of the conditioning regimen, the CRP level was significantly elevated in the SGC group compared to that in the IGC group 15 days after the beginning of the conditioning regimen, and this trend continued up to 40 days ($P<0.05$). The maximal CRP level during the neutropenic period in the IGC group was significantly lower than that in the SGC group (median (range), 6.9 (0.9–16.3) vs 11.5 (1.6–37.3), $P=0.007$).

Other clinical outcomes

The probability of grades II–IV acute GVHD within 100 days was 28 and 37% in the IGC and SGC groups (HR 1.05, 95% CI 0.38–2.91, $P=0.93$). The incidences of grades III–IV acute GVHD and NRM within 100 days were low in both groups (one and two patients, and one and one patient, in the IGC and SGC groups, respectively).

Discussion

This is the first study to evaluate the outcomes in allogeneic HSCT patients who were treated with a glucose management protocol. A salient finding of this study is that the incidence of documented infections, especially the incidence of bacteremia, was significantly lower in the IGC group than in the SGC group, as in a previous report in the ICU setting.[1] Moreover, there tended to be fewer organ dysfunctions in the IGC group, albeit this difference was not statistically significant. Furthermore, the CRP level,

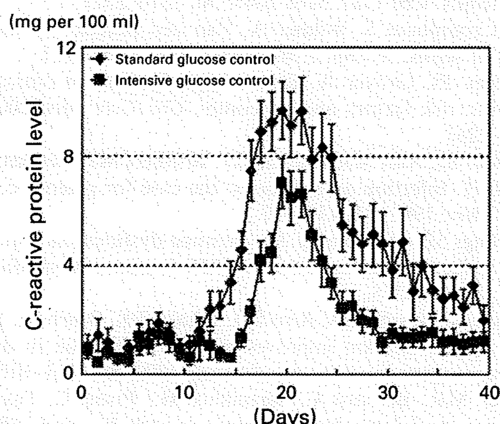

Figure 4 Serial change in the CRP level in the IGC and SGC groups. Values are mean + s.e.

which might be a surrogate marker for produced cytokines,[19] was significantly lower in the IGC group than in the SGC group, as shown in Figure 4. Even though this study did not have enough power to detect a decrease in acute GVHD and NRM, it could be anticipated that IGC could reduce the CRP level, which would lead to a reduced incidence of acute GVHD and NRM.

This study has several limitations. One limitation is that only 64 patients were analyzed with no sufficient power to demonstrate any statistically significant changes in the incidences of organ dysfunctions, which was similar to the result in a previous report in the ICU.[1,2] An additional limitation was that the control of the glucose level could be suboptimal. This could be because of the glucose control protocol, which included monitoring of glucose level and the administration of insulin. With regard to the administration of insulin, we replaced the continuous infusion of insulin with the addition of Humulin R to the bottle of PN to control the glucose level within the target range because of the presence of fewer nursing staff in the HSCT unit than in the ICU. This could delay the normalization of hyperglycemia. Even though severe hyperglycemia (>180 mg per 100 ml) was reduced, a glucose value within the normal range (80–110 mg per 100 ml) could be achieved in only 49% of the IGC group as shown in Figure 1b. From a methodological point of view, it might be inappropriate to simply count the number of glucose value measurements, as patients with hyperglycemia were monitored more frequently, as defined in this protocol. Furthermore, as the mode of glucose monitoring was quite different between the IGC group and the SGC group, it could be inappropriate to compare the glucose values. A future protocol should include a more appropriate monitoring of glucose level and administration of insulin system that assures the fine tuning of glucose levels within the target range. Finally, there was a possible selection bias that may have affected the results, as this study was not a randomized-control study and there were many nonparticipants. However, the incidence of documented infections in nonparticipants within 100 days after allogeneic HSCT was 42%. Therefore, the reduction in the incidence of documented infections in the IGC group could not simply be explained by other causes such as the selection of antibiotics or catheter management.

With these limitations in mind, we took several steps to improve the quality of the study. First, we carefully matched patients and transplantation characteristics. Second, the IGC strategy was applied prospectively. Third, the low rate of patients who developed clinically significant hypoglycemia should be emphasized. As previously reported, the IGC procedure becomes very difficult in the medical ICU, especially in patients who have sepsis, a high APACHE score or mechanical ventilation.[1,2,22,23] The low rate of hypoglycemia could be because the medical acuity of our patients were relatively mild compared to those of patients in the medical ICU. Moreover, patients undergoing HSCT are younger and might have better β-cell function. The low rate of hypoglycemia could be important for maximizing the benefit of IGC because severe hypoglycemia could be associated with an increased risk of mortality.[23]