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「適応拡大に向けた臍帯血移植の先進化による成績向上と普及に関する研究」

移植成績の解析と臨床試験の支援・遂行に関する研究

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研究要旨

競合イベントに対する回帰モデルとしてよく用いられている Subdistribution hazard Model (Fine and Gray, 1999) と競合イベントの累積発症率関数の擬似観察値に対する回帰モデル (Klein and Andersen, 2005) との比較検討を行った。シミュレーションや、実際の臍帯血移植データを用いて試行的に解析を試み実データへの適用可能性について検討した結果、両者に大きな違いはみられなかった。

A. 研究目的

移植データの統計解析においてはいわゆる競合イベントの問題が存在する。競合イベントに対する回帰モデルとしてよく用いられているのが Fine and Gray (1999) が提案した Subdistribution hazard model であるが、一方で Klein and Andersen (2005) が競合イベントの累積発症率関数の擬似観察値に対する回帰モデル (Regression model of competing risks data based on pseudo-values of the cumulative incidence function) を提案しており有用な方法の一つと考えられている。本研究では Klein ら (2008) が SAS マクロを提供していることから、それらを参考に実際の臍帯血移植データを用いて、試行的に解析を試み実データへの適用可能性について検討した。

B. 研究方法

Klein ら (2008) をもとに、SAS マクロを作成、実データに適用する。Fine and Gray (1999) の方法などとの比較も試みる。ソフトウェアのデータへの適用可能性について検討する。

C. 研究結果

SAS マクロを作成し、実データに適用した。いくつかのシミュレーションの結果も含めて、Cumulative incidence function の推定などについては、Fine and Gray (1999) の方法などの方法と結果に大きな違いはみられなかった。

D. 考察

Klein and Andersen (2005) の方法は Cumulative incidence function を直接モデル化する方法であり、モデルの自由度が大きい。パラメータ推定は一般化推定方程式 (GEE) を用いることで問題なく行うことが可能であり、また、GEE についてはたくさんの先行研究が存在することからその性能について十分明らかであるという利点がある。他の方法との比較検討結果につい

ては、Haller ら (2012) の結論と相違なかったが、今後も追加のシミュレーションや他の臍帯血データを追加するなどして、競合イベントを考慮している他の統計モデルとの比較検討を続ける予定である。

E. 結論

競合イベントの累積発症率関数の擬似観察値に対する回帰モデルの解析プログラムを作成し、実際の臍帯血移植データに適用した。

F. 研究発表

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G. 知的財産権の出願・登録状況

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Ⅶ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Ⅷ. 研究成果の刊行物・別刷

Biol Blood Marrow Transplant 19 (2013) 486–491

Prospective Multicenter Study of Single-Unit Cord Blood Transplantation with Myeloablative Conditioning for Adult Patients with High-Risk Hematologic Malignancies

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A B S T R A C T

Although the use of cord blood transplantation (CBT) is increasing, the optimal methods for conditioning and graft-versus-host disease (GVHD) prophylaxis remain to be established. Among previous reports, the Institute of Medical Science, University of Tokyo (IMSUT) has reported remarkably favorable results of CBT for hematologic malignancies as a single-institute experience. The aim of the present multicenter prospective study was to assess the safety and efficacy of CBT performed precisely according to IMSUT transplantation procedures. Thirty-three adult patients with hematologic malignancies, such as acute leukemia, chronic myelogenous leukemia, or myelodysplastic syndrome, either lacking an HLA-identical sibling/HLA-matched unrelated donor or requiring urgent transplantation were enrolled. Conditioning consisted of total body irradiation (12 Gy), cytarabine, and cyclophosphamide. Cyclosporine A and methotrexate were used for GVHD prophylaxis. Diagnoses were acute leukemia in 26 patients, chronic myelogenous leukemia in 4, and myelodysplastic syndrome in 3; 12 patients were in first complete remission, and the others were in advanced stages at the time of CBT. Thirty-one patients achieved engraftment, and the cumulative incidence of grade II-IV acute GVHD was 45% (95% confidence interval, 28%-62%). With a median follow-up of 46.2 months in 16 surviving patients, the 1-year cumulative incidence of nonrelapse mortality was 15% (95% confidence interval, 5%-30%). Causes of nonrelapse mortality were infection (n = 4) and graft failure (n = 1). The overall and disease-free survival rates were 51% (95% CI, 34%-68%) and 42% (95% CI, 26%-59%), respectively. These results suggest that the IMSUT CBT procedures can safely provide a high disease-free survival rate in patients with high-risk hematologic malignancies.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the most promising curative treatment for hematologic malignancies. Several hematopoietic stem cell sources are now available, and the use of cord blood transplantation (CBT) has been increasing dramatically [1]. However, the outcomes of CBT are not necessarily satisfactory, because of the high nonrelapse mortality (NRM). Conditioning and prophylaxis against graft-versus-host disease (GVHD) used in CBT have varied significantly among previous studies, and the optimal approaches remain to be established [2-6]. Among those studies, the outcomes of CBT for hematologic diseases at the Institute of Medical Science, University of Tokyo (IMSUT) were notably favorable and in fact were superior to the outcomes of allogeneic bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT) from related and unrelated donors at the same institution [7,8].

The IMSUT transplantation procedures involve a myeloablative conditioning regimen using total body irradiation (TBI), cyclophosphamide (CY), and high-dose cytarabine. Cytarabine is combined with granulocyte colony-stimulating factor (G-CSF) for myeloid malignancies. In addition, cyclosporine A is given over 10 hours with short-term methotrexate (MTX) for GVHD prophylaxis. To date, however, no study has systematically assessed whether IMSUT's favorable results would be reproduced if CBT for hematologic malignancy were performed precisely according to IMSUT's transplantation procedures. Accordingly, we designed and performed a multi-institutional study to evaluate the safety

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and efficacy of single-unit CBT for hematologic malignancies by strictly following these procedures.

PATIENTS AND METHODS

This study is a multi-institutional prospective study of the Kanto Study Group for Cell Therapy. The protocol was approved by the Institutional Review Boards of the 9 participating institutions and registered at http://clinicaltrials.gov (NCT00270881). No patients were enrolled from IMSUT. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Patient Eligibility and Cord Blood Unit Selection

Eligibility criteria for this study included (1) age 20-55 years; (2) diagnosis of acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), or myelodysplastic syndrome (MDS) suitable for allogeneic HSCT; (3) lack of an available 5/6 or fully HLA-matched related donor; (4) either lack of an available fully 6/6 HLA-matched unrelated donor or the need for immediate HSCT based on the features of the disease as judged by the treating physician; (5) availability of a 6/6, 5/6, or 4/6 serologically HLA-matched cord blood unit with a minimum of 2×10^7 total nucleated cells per kilogram of recipient body weight before cryopreservation in the Japan Cord Blood Bank Network; (6) Eastern Cooperative Oncology Group performance status of 0 or 1; (7) adequate function of main organs, including the liver, kidneys, lungs, and heart; and (8) lack of anti-HLA (class I and/or II) antibody. Patients with a previous history of HSCT, active infection, or active central nervous system disease or psychiatric disorders were excluded. This protocol was only for patients receiving single-unit CBT. HLA disparity was determined based on the antigen level of HLA-A, -B, and -DR loci specified by low- or high-resolution techniques.

Conditioning Regimen

All patients received the same myeloablative conditioning as described previously [7-9]. TBI 12 Gy was delivered in 4 or 6 fractions for 2 or 3 days (days -8, -7, and -6 or days -7 and -6). After completion of TBI, cytarabine at a dose of 2 or 3 g/m^2 was administered i.v. over 2 hours every 12 hours for 2 consecutive days (days -5 and -4). All patients received steroid eye drops for prophylaxis against keratoconjunctivitis due to cytarabine. For myeloid malignancies (AML, MDS, and CML), recombinant human granulocyte colony-stimulating factor (G-CSF; lenograstim) was given by continuous infusion at a daily dose of 5 μg/kg, starting 12 hours before the first dose of cytarabine and continuing until the last dose of cytarabine. Then CY 60 mg/kg was administered i.v. over 2 hours for 2 consecutive days (days -3 and -2). Cytarabine could be omitted in exceptional cases based on factors in the patient's background, such as a history of allergic reaction. No patient received antithymocyte globulin as part of the conditioning regimen.

Infusion of Cord Blood, GVHD Prophylaxis, and Supportive Care

Two days after the completion of CY administration (day 0), patients received CBT. The cord blood graft was thawed and immediately infused without washing. GVHD prophylaxis was provided with short-term methotrexate (MTX; 15 mg/m^2 on day 1, and 10 mg/m^2 on days 3 and 6) and cyclosporine A (CsA). Leucovorin was given i.v. to ameliorate its toxicity. CsA was given i.v. over 10 hours starting on day -1. The CsA dose was adjusted at the discretion of the physician only if the trough level of CsA was <100 ng/mL or adverse events associated with CsA developed.

Each patient was isolated in a laminar air-flow or high-efficiency particulate air-filtered room. The administration of lenograstim at a dose of 5 μg/kg was started 1 day after CBT and continued until neutrophil recovery was achieved. Prophylactic fluoroquinolone and fluconazole (200 mg/day) were given orally, starting 14 days before transplantation. For *Pneumocystis* pneumonia prophylaxis, cotrimoxazole was given for 14 consecutive days before transplantation and recommenced on a schedule of 2-3 days per week after sustained hematopoietic recovery was confirmed. Oral acyclovir at a dose of 1000 mg/day was given from day -7 to day 35. Cytomegalovirus (CMV) reactivation was routinely monitored by CMV antigenemia assay or PCR soon after neutrophil recovery, which triggered preemptive therapy with ganciclovir. Intravenous immunoglobulin was given in patients with a serum immunoglobulin G level <500 mg/dL.

Assessment of Chimerism, Engraftment, and GVHD

The chimerism study was performed on whole bone marrow cells at 1 month, 2 months, and 3 months after CBT. Analyses were performed by fluorescein in situ hybridization for X and Y chromosomes or by microsatellite PCR as appropriate. The day of myeloid engraftment was defined as the first day of 3 consecutive days when the absolute neutrophil count exceeded 0.5 × 10^9/L. The day of platelet engraftment was defined as the day when the absolute platelet count exceeded 20 × 10^9/L without platelet transfusion. Primary graft failure was defined as lack of myeloid engraftment until day 42; secondary graft failure, as a persistent loss of myeloid engraftment after having achieved engraftment. Both acute and chronic GVHD were diagnosed and graded based on published criteria [10,11].

Table 1
Patient and Transplant Characteristics (n = 33)

Characteristic	Value
Age, years, median (range)	37 (21-54)
Sex, males/females, n	21/12
Body weight, kg, median (range)	55.0 (39.1-97.0)
Diagnosis, n	
AML	20
ALL	6
CML	4
MDS	3
Disease status, n	
CR1	12
CR2, chronic phase 2	5
Not in CR, blast crisis	16
Conditioning, n	
TBI + cytarabine + G-CSF + CY	27
TBI + cytarabine + CY	5
TBI + CY	1
Cord blood units	
HLA disparity, n	
4-antigen match	29
5-antigen match	3
6-antigen match	1
Nucleated cells per kg body weight, median (range)	2.66 (2.00-4.58)
GVHD prophylaxis with CsA + short-term MTX, n	33

Statistical Analysis

The primary endpoint of this study was 1-year NRM, and secondary endpoints were engraftment, acute and chronic GVHD, infectious complications, day +100 NRM, relapse rate, disease-free survival (DFS), and overall survival (OS). Survival rates were calculated by the Kaplan-Meier method. Probability of acute GVHD, disease relapse, and NRM were estimated on the basis of cumulative incidence curves to accommodate the following competing events: death without GVHD and second transplantation for graft failure for acute GVHD, death for relapse, and relapse for NRM [12]. Comparisons were made using the log-rank test or Gray test as appropriate. Multivariate analyses were performed using the Cox proportional hazards model or the Fine and Gray proportional-hazards model as appropriate. $P < .05$ was considered to indicate statistical significance in all analyses.

RESULTS

Patient Characteristics

Thirty-three patients were enrolled and underwent CBT. Patient and transplant characteristics are summarized in Table 1. At the time of CBT, 12 patients with AML/ALL were in the first complete remission (CR1) and were defined as standard-risk patients. The remaining 19 patients had AML/ALL in CR2 or CML in chronic phase 2 (n = 3) or AML/ALL not in remission, MDS with an excess of blasts, or CML in blastic crisis (n = 16), and were defined as high-risk patients. Three patients in CR1 had Philadelphia chromosome–positive ALL. All but 1 patient received TBI, cytarabine, and CY with or without G-CSF as conditioning.

Engraftment and Chimerism

Myeloid engraftment was obtained at a median of 26 days (range, 18-60 days) in 31 patients. Platelet engraftment was obtained at a median of 44 days (range, 25-140 days) in 26 patients. Two patients experienced primary graft failure, caused by graft rejection in 1 patient and early disease progression in 1 patient. One case of secondary graft failure occurred after hemophagocytic syndrome. In the 27 patients who underwent chimerism analysis, full donor chimerism

Table 2
Infectious Complications

Infection	Number
Bacterial (n = 14)	
Bacteremia	10
Enteritis	1
Meningitis	1
Pneumonia	1
Cholecystitis	1
Fungal (n = 5)	
Candidemia	1
Invasive aspergillosis	3
Pneumocystis pneumonia	1
Viral (n = 28)	
CMV infection	23
CMV disease	1
HHV-6 central nervous system disorder	1
Parainfluenza virus pneumonia	1
Encephalitis*	2

HHV-6 indicates human herpesvirus 6.
* Causative virus was not identified.

was obtained at 1 month posttransplantation in 24 patients, at 2 months in 2 patients, and at 3 months in 1 patient.

Acute and Chronic GVHD

Acute GVHD developed in 21 of 31 evaluable patients with myeloid engraftment (grade I in 6 patients, grade II in 10, grade III in 3, and grade IV in 2). The cumulative incidence of grade II-IV acute GVHD up to day +100 post-transplantation was 46% (95% CI, 27.8%-61.5%). Among the 10 patients with grade II acute GVHD, 6 did not require systemic glucocorticoid in addition to CSA for the treatment of acute GVHD. Among the 27 patients who survived more than 100 days after transplantation, 6 patients developed chronic GVHD (2 with extensive type and 4 with limited type).

Infectious Complications

All but 1 patient experienced at least 1 episode of infectious complications after CBT (Table 2). The most common infective pathogen was viruses, including CMV infection, followed by bacteria and fungus. Four cases of infectious complications were fatal (2 with bacteremia and 2 with encephalitis). All of these patients had grade II-IV acute GVHD (1 with grade II, 1 with grade III, and 2 with grade IV) and were receiving systemic glucocorticoid therapy when infectious complications developed.

NRM, Relapse, and Survival

At a median follow-up of 46.2 months (range, 31.0-65.8 months), 16 patients were alive. Causes of death in the other 17 patients included relapse and complications associated with treatment of relapse after transplantation (12 patients), infectious complications (4 patients), and graft failure (1 patient). The cumulative incidence of NRM was 9% (95% CI, 2%-22%) at 100 days post-HSCT and 15% (95% CI, 5%-30%) at 1 year post-HSCT (Figure 1A). The cumulative incidence of NRM at 3 years post-HSCT did not differ significantly between standard-risk and high-risk patients (25% versus 10%; $P = .254$). In 14 patients, disease relapse or progression occurred at a median of 9 months (range, 0.9-23.0 months) post-HSCT. The 3-year relapse rate was 42% (95% CI, 25%-59%) (Figure 1B). OS was 51% (95% CI, 34%-68%), and DFS was 42% (95% CI, 26%-59%) (Figure 1C). The 16 patients who were alive without disease remained in good condition, with an Eastern Cooperative Oncology Group performance status

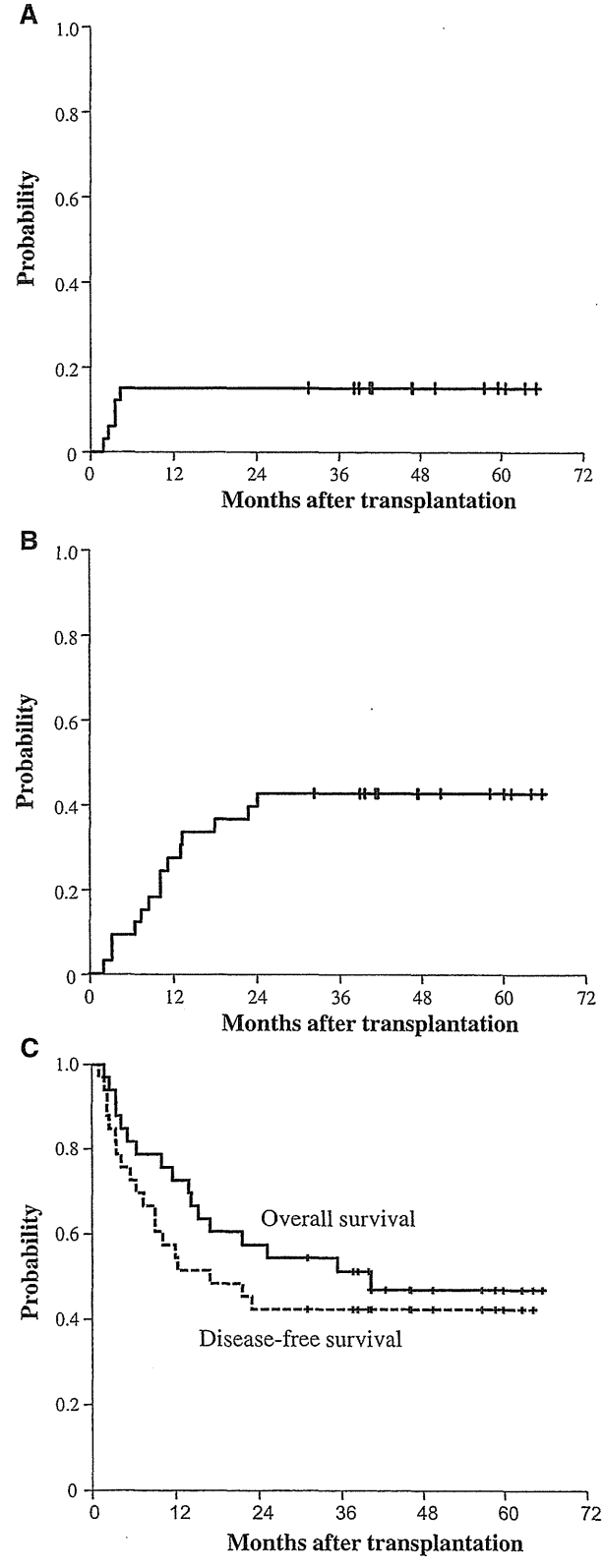

Figure 1. Cumulative incidences of NRM (A) and relapse rate (B), and Kaplan-Meier estimates of OS and DFS (C). "+" indicates a censored patient.

of 0 (n = 13) or 1 (n = 3). Two of the 3 patients who experienced primary or secondary graft failure and 4 of 14 patients who experienced disease relapse or progression subsequently underwent a second allogeneic HSCT. At the

time of this analysis, 2 patients were still alive without disease at 23 months and 62 months after the second HSCT.

As the possible factors affecting the rates of disease relapse, NRM, OS, and DFS, the following variables were analyzed: patient age (<40 years versus ≥40 years) and sex, risk categories based on disease status at transplantation (standard risk versus high risk), HLA disparity (4/6 versus 5/6 and full match), nucleated cell dose of cord blood graft ($<2.60 \times 10^7$/kg versus $\geq 2.60 \times 10^7$/kg), and development of acute GVHD (none or grade I versus grade II-IV). The development of grade II-IV acute GVHD had a negative impact on NRM (1-year NRM, 27% [95% CI, 8%-51%] versus 0%; $P < .05$); however, none of other variables, including patient age, had a significant impact on NRM, disease relapse, OS, and DFS.

DISCUSSION

The results of this prospective multicenter study demonstrate that single-unit CBT following myeloablative conditioning can provide favorable survival with low NRM in adult patients with high-risk hematologic malignancies. Our study is unique in that all patients received uniform conditioning, GVHD prophylaxis, and other supportive care. All of the CBT procedures applied in this study were identical to those reported by the IMSUT. In the IMSUT reports, the outcome of CBT was superior to that of allogeneic BMT or PBSCT from related and unrelated donors, which provided a notably favorable outcome compared with other studies [7,8]. Given the nature of the retrospective single-center study, we deemed it necessary to confirm the reproducibility of the results, and consequently planned and conducted a prospective multicenter study to evaluate the safety and efficacy of the IMSUT transplantation procedures. Of note, our study enrolled patients with high-risk hematologic malignancies regardless of disease status at transplantation, and indeed half of the patients enrolled were not in remission at the time of transplantation. Despite our use of such a high-risk cohort, the primary endpoint of 1-year NRM was 15%, which was comparable with the 9% reported by IMSUT and lower than those of other studies (30%~60%) [4-6,13-15]. The primary cause of NRM in the present study was infectious complications occurring after engraftment. All of those infectious complications occurred in patients who developed grade II-IV acute GVHD, and the sole factor significantly associated with the incidence of NRM was grade II-IV acute GVHD. Thus, to further reduce NRM, the optimal management of infectious complications should be explored, particularly in patients developing acute GVHD after CBT.

Disease relapse greatly interferes with the success of allogeneic HSCT, especially in patients with chemorefractory hematologic malignancies, and the intensity of conditioning plays a crucial role. Although TBI plus CY (TBI-CY) remains the most common myeloablative conditioning regimen for allogeneic HSCT, further intensification of conditioning by administering additional antileukemic agents has been attempted in an effort to reduce disease relapse. Although this further intensification can lead to more effective disease control, the benefit is generally offset by the higher rates of NRM, and thus the effect of this approach on survival remains controversial [16]. Cytarabine has been extensively investigated as an additional agent in this setting, but this drug is also associated with significant toxicity [17-22]. However, in a recent study we found favorable outcomes with low NRM with the use of TBI-CY plus cytarabine as conditioning in patients with ALL [9]. Based on these findings and the fact that the IMSUT protocol uses mainly TBI-CY plus cytarabine as conditioning for CBT [7,8], we followed the same regimen. In addition, we combined cytarabine with G-CSF infusion in patients with myeloid malignancies, based on the hypothesis that G-CSF increases the susceptibility of myeloid leukemic cells to cytarabine, thereby contributing to decreased relapse rate [23-30]. In the setting of allogeneic BMT and PBSCT, favorable outcomes of the conditioning consisting of TBI and G-CSF with cytarabine in patients with AML and advanced MDS have been reported [31-34]. In the present study, despite the high-risk features of the disease, approximately half of the patients achieved long-term DFS with this unique intensified myeloablative conditioning because of the low NRM and disease relapse, as expected, which seemed more favorable than the results of previous studies [5,14]. These results suggest that the myeloablative conditioning regimen used in the present study is safe and highly effective in eradicating leukemic cells even in patients with high-risk leukemia. However, the outcomes of our study patients seem inferior to those reported by IMSUT in terms of disease relapse and survival. The major difference is in the incidence of disease relapse, which was higher in the present study (40% versus 16%~17%) and consequently had an effect on DFS (42% versus 70%~74%). The most plausible explanation for this difference is the differing study designs (prospective multicenter versus retrospective single-center). Another possible explanation may be related to demographic differences in the patient cohorts. The standard variables, such as age and disease status at transplantation, seemed similar in the 2 studies. However, BMT from unrelated donors takes priority over CBT in clinical practice in all of the institutions participating in the present study. Thus, it is possible that high-risk patients, particularly those with diseases in CR, might be selectively enrolled into the present study based on the inclusion criteria defining the requirement of immediate HSCT based on disease features at the discretion of each treating physician. Disease status at transplantation cannot precisely reflect the risk of such features of the disease and could have significantly affected the outcome.

Along with conditioning, GVHD prophylaxis plays an important role in the outcomes of allogeneic HSCT. However, a standard GVHD prophylaxis regimen for CBT has yet to be established, and GVHD prophylaxis varies widely among previous studies, variously consisting of CsA or tacrolimus alone or in combination with MTX, glucocorticoids, mycofenolate mofetil (MMF), and/or antithymocyte globulin [2-6,13-15,35]. To avoid the toxicity and negative effects of MTX on hematopoietic reconstitution, several studies have applied non–MTX-containing GVHD prophylaxis. In contrast, CsA in combination with short-term MTX, which remains a common GVHD prophylaxis regimen in allogeneic HSCTs other than CBT, was used in the present study precisely according to the IMSUT protocol. Leucovorin was routinely given to ameliorate the toxicity of MTX. In addition, CsA was given over 10 hours at a dose of 3 mg/kg/day, with the dose adjusted only when the CsA trough level was <100 ng/mL or adverse events associated with CsA developed. With this relatively unique regimen, neutrophil engraftment was obtained at a median of 26 days after CBT, which is comparable with those in previous studies. The incidence of grade II-IV acute GVHD of 48% is identical to that reported by IMSUT (44%~52%) [7,8]. Of note, however, acute GVHD was manageable without systemic glucocorticoid administration in approximately 60% of patients with grade II acute GVHD, suggesting that the incidence of clinically

significant acute GVHD requiring systemic glucocorticoid therapy was actually lower with this prophylactic regimen. In addition, the development of acute GVHD requiring systemic glucocorticoid therapy was associated with NRM due to fatal infectious complications. Therefore, further investigations should focus on the more effective GVHD prophylaxis, although the balance between the immunosuppressive effect and graft-versus-tumor effect is also important. In this setting, less-toxic GVHD prophylaxis using MMF instead of MTX could be a good option, because it has a potential to allow faster engraftment and could decrease the risk of infectious complications.

Secondary graft failure due to graft rejection was observed in 1 patient, who was rescued by the second CBT and alive at 26 months after the second CBT. Other complications interfering with survival and quality of life, such as severe chronic GVHD and secondary malignancies, were seen in no patients during the follow-up period. All surviving patients were in good clinical condition without disease recurrence. These results demonstrate the long-term safety and tolerability of these CBT procedures despite the highly intense conditioning.

Two major limitations of the present study are the small number of patients evaluated and the heterogeneous disease background in these patients, including the types of disease, high-risk features, and disease status at transplantation. However, the study's primary endpoint was to evaluate 1-year NRM with respect to safety of the transplantation procedures. Thus, we believe that safety of the procedures can be confirmed even with our relatively small, heterogeneous cohort.

We conclude that the transplantation methods used in the present study, including intensified myeloablative conditioning, CsA with MTX as GVHD prophylaxis, and other supportive care measures, can provide low NRM and high survival rates in patients undergoing single-unit CBT for hematologic malignancies and could possibly become a standard treatment. However, because of the limited number of patients and high incidence of life-threatening infectious complications associated with GVHD, a larger-scale study with some modifications to GVHD prophylaxis is needed to establish the optimal CBT techniques.

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Acute kidney injury after myeloablative cord blood transplantation in adults: the efficacy of strict monitoring of vancomycin serum trough concentrations

H. Mae, J. Ooi, S. Takahashi, S. Kato, T. Kawakita, Y. Ebihara, K. Tsuji, F. Nagamura, H. Echizen, A. Tojo. Acute kidney injury after myeloablative cord blood transplantation in adults: the efficacy of strict monitoring of vancomycin serum trough concentrations. Transpl Infect Dis 2013: **15**: 181–186. All rights reserved

Abstract: *Background.* Acute kidney injury (AKI) is a common medical complication after myeloablative allogeneic stem cell transplantation (SCT). We have previously performed a retrospective analysis of AKI after cord blood transplantation (CBT) in adults, and found that the maximum of vancomycin (VCM) trough levels were significantly higher in patients with AKI. Following these results, we have monitored VCM serum trough concentrations more strictly, to not exceed 10.0 mg/L, since 2008.
Methods. In this report, we performed an analysis of AKI in a new group of 38 adult patients with hematological malignancies treated with unrelated CBT after myeloablative conditioning between January 2008 and July 2011.
Results. Cumulative incidence of AKI at day 100 after CBT was 34% (95% confidence interval 19–50). The median of the maximum value of VCM trough was 8.8 (4.5–12.2) mg/L. In multivariate analysis, no factor was associated with the incidence of AKI. No transplant-related mortality was observed. The probability of disease-free survival at 2 years was 83%.
Conclusion. These findings suggest that strict monitoring of VCM serum trough concentrations has a beneficial effect on outcomes of CBT.

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Key words: vancomycin; myeloablative conditioning; cord blood transplantation; acute kidney injury

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Acute kidney injury (AKI) is a common medical complication early after myeloablative allogeneic stem cell transplantation (SCT). The incidence of AKI, defined as a 2-fold rise in serum creatinine (sCr) concentration from baseline, has been reported ranging from 36% to 72% in SCT in a myeloablative setting (1–7), and about 20% required hemodialysis. We have previously reported a retrospective analysis of AKI in a group of 54 adult patients with hematological malignancies who received unrelated cord blood transplantation (CBT) after myeloablative conditioning between 2004 and 2007 (8). A statistically significant decrement of renal function from baseline was observed between days 11 and 20. Among the 54 patients, AKI occurred in 27.8% and was associated with a high mortality rate. Although no difference was seen in maximum cyclosporine (CYA) trough levels, the maximum vancomycin (VCM) trough levels were significantly higher in patients with AKI (8). Following these results, we have monitored VCM serum trough concentrations more strictly. In this report, we performed an analysis of AKI in a new group of 38 adult patients with hematological malignancies treated with unrelated CBT after myeloablative conditioning between January 2008 and

July 2011. The main purpose of this retrospective single-center study was to confirm the efficacy of strict monitoring of VCM serum trough concentrations, as well as to identify factors related to the incidence of AKI.

Patients and methods

Patients

This was a retrospective single-center analysis. Between January 2008 and July 2011, 39 consecutive adult patients with hematological malignancies were treated with unrelated CBT at The Institute of Medical Science, University of Tokyo. We excluded 1 patient who experienced primary engraftment failure. A total of 38 patients were analyzed. Patients qualified as standard risk if they were in first or second complete remission, had chronic-phase chronic myelogenous leukemia or refractory anemia of myelodysplastic syndrome, or had no high-risk cytogenetics. Patients in third complete remission, in relapse, or in refractory disease, with chronic myelogenous leukemia beyond chronic phase, or with high-risk cytogenetics were classified as high risk. Analyses of data were performed in December 2011. Written informed consent for treatment was obtained from all patients.

Conditioning

All patients received 4 fractionated 12 Gy total body irradiation on days −8 and −7, in addition to cytosine arabinoside (Ara-C) and cyclophosphamide. Ara-C was administered intravenously (IV) over 2 h at a dose of 3 g/m^2 every 12 h on day −5 and −4 (total dose 12 g/m^2). In patients with myeloid malignancies, recombinant human granulocyte colony-stimulating factor (G-CSF) was combined with Ara-C. G-CSF was administered by continuous infusion at a dose of 5 μg/kg/day. Infusion of G-CSF was started 12 h before the first dose of Ara-C and stopped at the completion of the last dose. Cyclophosphamide was administered IV over 2 h at a dose of 60 mg/kg once daily on days −3 and −2 (total dose 120 mg/kg). Two days after the completion of conditioning, patients received a CBT.

Graft-versus-host disease (GVHD) prophylaxis

All patients received standard CYA and methotrexate as GVHD prophylaxis. CYA was given IV every day starting on day −1 at a dose of 3 mg/kg/day. Methotrexate (15 mg/m^2 IV) was given on day 1, and 10 mg/m^2 on day 3 and 6. Once oral intake could be tolerated, patients were administered oral CYA at a dose of 1:2, in 2 divided doses per day, based on the last intravenous dose. CYA was reduced when sCr levels rose above 1.5 times baseline, or other serious agent-associated toxicities occurred. Physicians could freely modify the CYA dose for patients experiencing severe acute GVHD (aGVHD) or risk of disease relapse. Corticosteroid-based treatment was considered when grade II or higher severe aGVHD occurred (0.5–2 mg/kg).

Supportive care

All patients received G-CSF by intravenous infusion starting on day 1 until durable granulocyte recovery was achieved. The supportive care regimen, including prophylaxis for infection was the same as previously reported (8, 9).

Monitoring

All patients were monitored retrospectively 10 days before, and after the first 100 days, of CBT. Daily laboratory data collecting and the detecting method of VCM and CYA trough concentration were the same as previously reported (8). Therapeutic drug monitoring for VCM by assessing serum trough concentration was done twice in weekly, and modified to not exceed 10.0 mg/L.

End-points and definitions

AKI was defined as 2-fold rise in sCr concentration on daily laboratory results from the baseline (the average of days −10 to 0). Myeloid engraftment was defined as the first of 3 consecutive days, during which the absolute neutrophil count was at least 0.5 × 10^9/L. Platelet recovery time was achieved on the first of 3 days when the platelet count was higher than 50 × 10^9/L without transfusion support. The aGVHD was graded according to previously published criteria (10). Transplant-related mortality was defined as death from any cause except relapse. Relapse was defined by morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary sites. Disease-free survival was defined as the time from CBT to relapse, death, or the last observation.

Statistical analysis

Continuous variables are expressed as median and their range. For dichotomous variables, the frequencies of positive occurrence are given along with their corresponding percentages. Continuous variables were divided into high or low with their median values, and a single VCM trough concentration of 10.0 mg/L was defined as a threshold level for analysis. Cumulative incidence of AKI was estimated with competing risk setting, of which death and relapse were defined as competing risk events. Variables considered in univariate analysis were body weight, age, recipient gender, recipient cytomegalovirus serology, disease status at transplant (standard or high risk), total nucleated cell dose, CD34+ cell dose, baseline sCr levels, VCM use, VCM trough levels, CYA trough levels, foscarnet use, aminoglycosides use, days of neutrophil engraftment, aGVHD grade 3–4, and positive blood culture result. Variables with a *P*-value <0.1 for cumulative incidence of AKI were tested in multivariate analysis using Cox proportional hazards models, and *P*-values <0.05 were considered to be statistically significant. The probability of disease-free survival was estimated from the time of CBT according to the Kaplan–Meier method. Endpoints were calculated at the last contact, the date of the last follow-up being December 1, 2011. Statistical software R, version 2.12.2, was used for analysis.

Results

Characteristics of patients and cord blood units

The characteristics of 38 patients and cord blood units are shown in Table 1. Among the patients, the median age was 41.5 years (range, 18–52 years), the median weight was 59.5 kg (range, 39–76 kg), the median number of cryopreserved nucleated cells was 2.8×10^7/kg (range, 1.7–5.7 $\times 10^7$/kg), and the median number of cryopreserved CD34+ cells was 0.9×10^5/kg (range, 0.4–2.6 $\times 10^5$/kg). All patients received a single and human leukocyte antigen-mismatched cord blood unit.

Time courses of changing renal function

No patient had confirmed renal dysfunction before transplantation. The changes of renal function as variations (%) of sCr from baseline levels observed on days 11–20 were greatest and significant (+15.8%,

Characteristics and clinical course

Characteristics	
Patients, *n*	38
Male/Female, *n*	25/13
Median age, years (range)	41.5 (18–52)
Median weight, kg (range)	59.5 (39–76)
Median number of cryopreserved nucleated cells, $\times 10^7$/kg (range)	2.8 (1.7–5.7)
Median number of cryopreserved CD34+ cells, $\times 10^5$/kg (range)	0.9 (0.4–2.6)
Recipient CMV status, Positive/Negative, *n*	32/6
Diagnosis	
AML, *n*	12
MDS-related secondary AML, *n*	6
RAEB, *n*	3
RA, *n*	2
CML, *n*	3
ALL, *n*	11
NHL, *n*	1
Disease status at transplant	
Standard risk, *n*	10
High risk, *n*	28
Conditioning regimen	
TBI + Ara-C/G-CSF + CY, *n*	26
TBI + Ara-C + CY, *n*	12
GVHD prophylaxis	
CYA + MTX, *n*	38
Baseline sCr, mg/dL (range)	0.62 (0.33–0.87)
Neutrophil >0.5 $\times 10^9$/L, days (range)	21 (17–30)
Patients with positive blood culture, *n* (%)	6 (16)
Patients taking aminoglycosides, *n* (%)	32 (84)
Patients taking foscarnet, *n* (%)	10 (26)
Patients taking liposomal amphotericin, *n* (%)	16 (42)
Maximum CYA trough value, μg/L (range)	258.5 (40–453)
Patients taking VCM, *n* (%)	32 (84)
Duration of VCM therapy, days (range)	54 (6–100)
Maximum VCM trough value, mg/L (range)	8.8 (5.2–12.2)
Patients with maximum VCM trough value, >10.0 mg/L, *n* (%)	9 (24)
Patient requiring hemodialysis, *n* (%)	0 (0)

CMV, cytomegalovirus; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess blasts; RA, refractory anemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, recombinant human granulocyte colony-stimulating factor; CY, cyclophosphamide; GVHD, graft-versus-host disease; CYA, cyclosporine; MTX, methotrexate; sCr, serum creatinine; VCM, vancomycin.

Table 1

0.57 ± 0.18 mg/dL to 0.71 ± 0.24 mg/dL, $P < 0.001$). No obvious recovery occurred of declined renal function, which remained until day 100.

Incidence and risk factors of AKI

Cumulative incidence of AKI at day 100 after CBT was 34% (95% CI 19–50) (Fig. 1). The median of the maximum value of VCM trough was 8.8 (4.5–12.2) mg/L. In univariate analysis, baseline sCr levels and foscarnet use were associated with the incidence of AKI (Table 2). In multivariate analysis, no factor was associated with the incidence of AKI (Table 2).

Transplant outcomes

All patients had myeloid reconstitution, and the median time to >0.5 × 10^9/L absolute neutrophil count was 21 days (range, 17–30 days). A self-sustained platelet count >50 × 10^9/L was achieved in 37 patients at a median time of 45.5 days (range, 34–127 days). In 37 of 38 evaluable patients, aGVHD occurred. The grading of aGVHD was grade I in 7 patients, grade II in 25, grade III in 4, and grade IV in 1. No one experienced hepatic veno-occlusive disease. Six of 38 patients (16%) had positive blood culture; however, no one had confirmed hypotension, indicated with decrease in systolic blood pressure >10 mmHg to <90 mmHg. Of 6 patients with positive blood cultures, 4 patients were not administered VCM. The total number of positive blood cultures was 13 of 998 specimens. Ten of 13 bacterial pathogens from blood cultures were gram-positive cocci (Table 3). Vancomycin-resistant *Enterococci* were detected in 1 patient from blood culture, however, this had been continuously detected from stool specimens since admission. No patients required hemodialysis. Among the 38 patients, no patient died of transplant-related causes (transplant-related mortality 0%). Six patients relapsed. Of these 6 patients, 5 patients died of relapse. A total of 32 of 38 patients are alive and free of disease at between 139 and 1400 days (median: 634 days) after CBT. The probability of disease-free survival at 2 years was 83% and 77% at 3 years (Fig. 2).

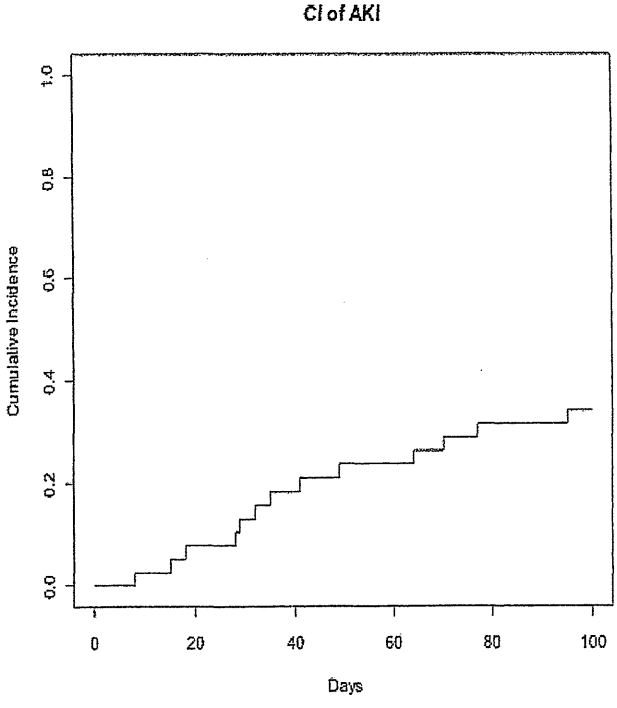

Fig. 1. Cumulative incidence (CI) of acute kidney injury (AKI).

Discussion

In this study, similar trends were observed in the time course of renal function changes as previously reported (8). However, the elevation in sCr was lower in this study, especially in days 11–20 (from 35.0% [8] to 15.8% in this study). Cumulative incidence of AKI was 34%; however, this was not assessed in our previous study (8). When we assessed the incidence of AKI with an identical definition to the previous

Univariate and multivariate analysis of factors associated with acute kidney injury

	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Baseline sCr, mg/dL				
>0.62	0.27 (0.08–0.98)	0.047	0.33 (0.08–1.32)	0.12
<0.62	1		1	
Foscarnet				
(+)	3.11 (1.07–9.05)	0.037	2.45 (0.71–8.42)	0.15
(−)	1		1	
VCM trough, >10.0 mg/L				
(+)	2.68 (0.89–8.09)	0.081	2.64 (0.76–9.19)	0.13
(−)	1		1	

CI, confidence interval; sCr, serum creatinine; VCM, vancomycin.

Table 2

Isolated bacterial pathogens from blood cultures

Pathogens	*n*
Enterococcus faecalis	3
Vancomycin-resistant *Enterococcus faecium*	3
Methicillin-resistant *Staphylococcus* species	1
Methicillin-resistant *Staphylococcus epidermidis*	3
Stenotrophomonas maltophilia	1
Bacillus species	1
Bacillus cereus	1

Table 3

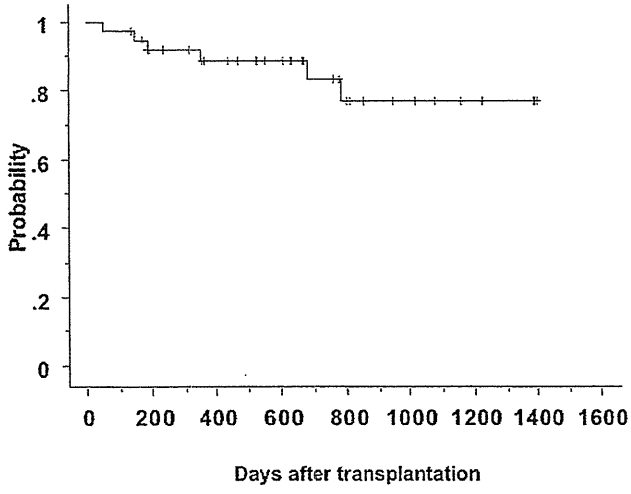

Fig. 2. Probability of disease-free survival after cord blood transplantation.

study, defined as just a 2-fold rise in sCr of 10 days average before and after transplantation, the incidence of AKI decreased to 11% in this study. In our previous study, the maximum VCM trough levels were significantly higher in patients with AKI (8); therefore, we have monitored VCM serum trough concentrations more strictly to not exceed 10.0 mg/L since 2008 in this study period. The average maximum value of VCM trough levels was lowered to 8.7 ± 2.1 mg/L from 12.2 ± 4.6 mg/L in the previous study, and proportion of patients with trough levels >10.0 mg/L was also decreased from 57% to 24%. Although baseline sCr levels and foscarnet use were associated with the incidence of AKI, VCM trough levels were not associated with AKI in univariate analysis. No factor was associated with AKI in multivariate analysis. Parikh et al. (11) reported AKI significantly affects survival after myeloablative allogeneic SCT in their meta-analysis, and more recently, Kagoya et al. (7) as well as Gooley et al. (12) reported the association of severity of AKI classification and non-relapse mortality within 100 days after transplantation. Although cumulative incidence of AKI was 34% in this study, no patients required hemodialysis or died of transplant-related causes. Recently, Yazaki et al. (13) reported the association of overall mortality and early bacterial infection of CBT in adults. They reported that cumulative incidence of early bacterial infection at day 100 was 21%, early bacterial infection had a negative effect on survival for adults, and the median day of development was 10 days after transplant, suggesting that prevention of bacterial infection in the very early post-CBT phase is important. Recently, a shift has occurred in the type of infecting organisms that cause bacteremia from predominantly gram-negative organisms to gram-positive cocci. The same trend is confirmed in the CBT (13, 14). VCM has an important role for infection control of gram-positive bacteremia, and was given to almost all the patients in this study. The reduced susceptibility of staphylococci for VCM has been reported since the mid 1990s, and prolonged exposure to lower VCM concentration has been associated with resistance (15). Although very few studies about pharmacokinetics and pharmacodynamics of VCM are available, several studies revealed area under the curve/minimum inhibitory concentration (AUC/MIC) as a preferred parameter, and AUC/MIC >400 associated with successful outcome and prevention of resistance (15, 16). Because of the difficulty of determining multiple concentrations for calculating AUC in the clinical setting, VCM trough concentrations have been recommended as the best surrogate marker for AUC/MIC, and concentrations of 15–20 mg/L – higher than the 5–15 mg/L previously recommended – is recommended as the target range (16). However, because an increased risk of nephrotoxicity with elevated VCM trough concentrations has been reported, and no appropriate pharmacokinetic/pharmacodynamic parameters for VCM have been determined (15, 17, 18), careful assessments are needed for using VCM at high target concentrations. Although we controlled VCM levels to not exceed 10.0 mg/L in this study, no patient died of bacterial infections. Further studies are required to determine the optimal VCM trough concentrations. Few reports are available about monitoring VCM trough concentrations for preventing AKI in allogeneic SCT in adults. Despite the limitations associated with this retrospective review of a small number of patients, our results suggest that strict monitoring of VCM serum trough concentrations has a beneficial effect on outcomes of CBT.

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