シクロビル投与期間の21日以内にCMV抗原血症は完全に陰性化した。投与終了後に4例においてCMV抗原血症の再活性化を認めた。CMV 感染症を発症した症例は認めなかった。10 例中 1 例において、grade4 の好中球減少をみとめた他は、有害事象は認めなかった。

D. 考察

バルガンシクロビルを同種移植後に経口投与することによって、CMV 再活性化に有効であることが示され、その有効性は静注ガンシクロビルと同等であった。有害事象は軽微で、安全に投与可能であった。21 日間の経口バルガンシクロビル投与後に 40%において CMV 再活性化を認めたことより、投与終了後も CMV 抗原血症のモニタリングの継続が必要である。

E. 結論

本研究の結果から、同種移植後の高度な免疫抑制状態の患者においては経口バルガンシクロビルの投与によって、CMV の発症を抑制できることがわかった。特に合併症のない外来患者に有用であると考えられる。今後の保険適応拡大を含めて、対応を検討する必要がある。

F. 健康危険情報

なし。

G. 研究発表

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H. 知的財産権の出願・登録状況

なし。

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分担研究報告書

「フィラデルフィア染色体陽性急性リンパ性白血病(Ph+ALL)の造血幹細胞移植における微少残存病変(MRD)の解析」

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研究要旨：フィラデルフィア染色体陽性急性リンパ性白血病(Ph+ALL)は造血幹細胞移植が唯一の根治療法であるが、移植後の再発が問題である。我々は移植後早期よりイマチニブを投与する群（予防投与群）と移植後 MRD+になってからイマチニブを投与する群（治療投与群）において最終的には両群とも血液学的再発をおこすことを確認した。すなわち移植後のイマチニブが生存期間を延ばすものの、必ずしも治癒をもたらすものではないことより、移植前の MRD を陰性にすることが最も大事であることが確認された。今後 Ph+ALL の MRD を計測する RQ-PCR の保険適応が望まれる。

A. 研究目的

Ph+ALL は最も予後不良の白血病であるが、イマチニブにより多くの患者に寛解が得られ生存期間は延長したが造血幹細胞移植が依然として唯一の根治療法である。しかし移植前の MRD の状態が移植成績に大きな影響を与えるとともに、移植後早期に MRD を見つけることにより、その後の治療を適正に行える。しかし本邦においては MRD をモニターする定量的 PCR 法（RQ-PCR）検査の保険適応は限られており、多くの病院では、施設負担をしているのが現状である。このことは他の白血病でも同じことである。本研究においては、MRD 検出の有用性を明らかにし、この保険適応を得ることを目的とする。今年度は、移植時に MRD＋の症例は再発をしやすいという我々の知見に基づき、これらの症例に対し移植後早期よりイマチニブを投与する群（予防投与群）と移植後 MRD+になってからイマチニブを投与する群（治療投与群）において、予後に差があるか検討した。

B. 研究方法

MRD 陽性の状態で造血幹細胞移植を受けた 34 例の Ph+ALL を対象とした。年齢中央値 40 歳、移植時血液学的寛解にあった症例 18 例、血縁者ドナー20 例、非血縁者ドナー18 例であった。7 人が移植後にイマチニブ投与を受けた。予防投与群 3 例、治療群 4 例であった。

C. 研究結果

3yOS はイマチニブ投与を受けた群が 66.7%、受けなかった群が 29.6%と有意差を認めた(p=0.03)。しかし EFS は 0%と 29.6%と逆の結果であり、有意差は認めなかった。予防投与群では MRD 陰性期間が中央値で 12 か月と治療群の 6 か月と比較して長かった。しかし両グループにおいて、全例 MRD 陽性となり、最終的には血液学的再発をみた。

D. 考察

イマチニブを利用することにより再発後の生存期間を延ばした。移植時の MRD 陽性であることが移植後の再発と結びつくという我々のデータより、移植時 MRD 陽性の症例に対し移植後早期よりイマチニブを投与する群（予防投与群）と移植後 MRD+になってからイマチニブを投与する群（治療投与群）において、予後に差があるか検討したが、予想に反し予防投与群も最終的には血液学的再発を認めた。このことは、移植後に RQ-PCR 感度以下で残存している白血病細胞は、イマチニブに対して抵抗性であり、最終的には血液学的再発をきたす症例もあることを示している。一般的には移植後

の再発は移植前処置前の腫瘍量、前処置によっても残存する腫瘍量、その後の GVL 効果によりコントロールされていると考えられる。移植時に MRD+の症例においては、移植後のイマチニブが生存期間を延ばすものの、必ずしも治癒をもたらすものではないことより、移植前の MRD を陰性にすることが最も大事であることが確認された。今後は移植前に MRD 陽性の症例に対し新規 TKI などを用い MRD 陰性にすることが望まれる。

E. 結論

MRD を計測する RQ-PCR の一日も早い保険適応が求められる。

F. 健康危篤情報

該当なし

G. 研究発表

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Ⅲ．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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Ⅳ．研究成果の刊行物・別刷

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Umbilical Cord Blood Transplantation after Reduced-Intensity Conditioning for Elderly Patients with Hematologic Diseases

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ABSTRACT

Although allogeneic hematopoietic stem cell transplantation is a potentially curative approach for advanced hematologic diseases, its application to elderly people is limited because of their comorbid physical conditions and lower chance of finding suitable related donors. Umbilical cord blood transplantation with reduced-intensity pretransplant conditioning (RI-UCBT) is 1 way to avoid these obstacles. We analyzed elderly patients aged 55 years and older with hematologic diseases who underwent RI-UCBT at our institute to assess feasibility and effectiveness of this treatment approach. Among the 70 patients included, 50 died, 74% of them from nonrelapse causes. Infection was the primary cause of death. Estimated overall survival and progression-free survival at 2 years were both 23%. In multivariate analyses, standard-risk diseases, age younger than 61 years, grade 0-II acute graft-versus-host disease, and the absence of preengraftment immune reaction were significantly associated with better overall survival. RI-UCBT is a potentially curative and applicable approach for elderly patients. Higher mortality, especially from nonrelapse causes, is the biggest problem to be solved to increase the feasibility of this approach.

KEY WORDS

Cord blood transplantation • Reduced intensity • Elderly patients • Hematologic diseases

INTRODUCTION

Although morbidity associated with hematologic malignant diseases in elderly patients is higher than that in younger patients [1], elderly patients are less likely to be candidates for allogeneic stem cell transplantation, because of the fact that they are more likely to have comorbid organ conditions, either clinically or subclinically, which result in a higher rate of procedure-related mortality [2], and that they are less likely to have HLA-matched related donors available, as siblings also tend to be elderly.

The development of reduced-intensity conditioning (RIC) for transplants, which results in less toxicity and depends largely on graft-versus-tumor effects rather than high-dose therapy to eliminate malignant cells, has been shown to allow elderly patients to undergo allogeneic transplants [3-5]. The use of umbilical cord blood transplantation (UCBT) has been increasing because of the potential advantage of rapid availability and the lower risk of graft-versus-host disease (GVHD), thus permitting less stringent HLA matching [6,7]. The outcome of UCBT has been reported to be similar to unrelated bone marrow in the myeloablative setting [8-10]. UCBT with reduced-intensity pretransplant conditioning (RI-UCBT) for adults, mostly younger than 55 years old, has been increasingly reported, and has been shown to be applicable even in patients with a relatively low number of nucleated cells for their body weight [11-16]. However, little information has been available on whether elderly patients can tolerate slower engraftment, more infectious complications [17], and the unique preengraftment immune reaction (PIR) associated with UCBT [18,19]. PIR has been described by us and others [18,19], characterized

by the symptoms induced possibly by hypercytokinemia, which sometimes cause severe organ damage and fatal outcome. We therefore retrospectively evaluated the use of the RI-UCBT in patients aged 55 and older by analyzing engraftment, nonrelapse mortality (NRM), GVHD, progression-free (PFS), and overall survival (OS) to address the feasibility and effectiveness of this method in older patients.

PATIENTS, MATERIALS, AND METHODS

Patients

This study included patients aged 55 and older who underwent RI-UCBT at our institute from July 18, 2002 through October 28, 2005. Patients were eligible for this study if they had any hematologic malignancies at high risk for relapse or severe aplastic anemia (AA) refractory to standard immunosuppressive therapy, as well as if they were unable to find suitable related or unrelated bone marrow (BM)/peripheral blood (PB) donors within reasonable time periods relative to their disease conditions. Patients with acute leukemia could be at first remission but at high risk for relapse because of adverse cytogenetic abnormalities, have a prior hematologic disorder, or be at any status beyond first remission. Patients with myelodysplastic syndrome (MDS) had to be refractory anemia with excess of blasts or chronic myelomonocytic leukemia, or have refractory anemia with transfusion dependency and/or severe neutropenia. Patients with chronic myeloid leukemia (CML) had to be beyond the first chronic phase. Lymphoma patients had to be beyond the first remission except those with acute or lymphoma type adult T cell leukemia. Patients who had end-stage organ dysfunction (DLco <30% predicted or LVEF <35%), or active serious infection at the time of transplantation were not eligible. All patients gave written informed consent, and the study was approved by the appropriate institutional review boards.

Donor Selection

UCB units were obtained from Japanese Cord Blood Bank Network. HLA-A and HLA-B antigens were identified by serologic typing. HLA-DRB1 alleles were determined by high-resolution molecular typing using polymerase chain reaction (PCR) sequence-specific primers. UCB grafts had at least 4 of 6 HLA-A, B antigens, and DRB1 alleles that were matched to the recipient and had a cryopreserved cell dose of at least 1.8×10^7 nucleated cells per kg of recipient body weight. The median total nucleated cell number and median $CD34^+$ cell number were 2.8 (range: 1.8–5.2) $\times$ 10^7/kg and 0.84 (0.11-3.28) $\times$ 10^5/kg, respectively.

Patient Characteristics

Seventy consecutive patients were included in this study. Their characteristics are shown in Table 1.

Table 1. *Patient and Donor Umbilical Cord Blood Characteristics*

Characteristic	No. (%) of Patients
Sex	
Male	45 (64)
Female	25 (36)
Age (years)	
Median (range)	61 (55-79)
Age distribution (years)	
55 to 59	31 (44)
60 to 64	16 (23)
65 to 69	17 (24)
At least 70	6 (9)
Diagnosis	
AML	28 (40)
MDS	3 (4)
CML	4 (6)
ALL	11 (16)
NHL	8 (11)
ATL	12 (17)
MM	1 (1)
PCL	1 (1)
AA	2 (3)
HCT-CI	
0	24 (34)
1	25 (36)
2	11 (16)
3 or greater	10 (14)
History of prior chemotherapy	
Yes	59 (84)
No	11 (16)
History of prior documented infections	
Yes	15 (21)
No	55 (79)
Disease status	
Standard risk	15 (21)
High risk	55 (79)
Conditioning regimen	
Flu/Mel/TBI	65 (93)
Flu/Bu/TBI	4 (6)
Others	1 (1)
GVHD prophylaxis	
Cyclosporine A alone	37 (53)
Tacrolimus alone	33 (47)
HLA disparity to UCB	
5/6	9 (13)
4/6	61 (87)
Sex mismatch to UCB	
Yes	51 (73)
No	19 (27)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; ATL, adult T cell leukemia; MM, multiple myeloma; PCL, plasma cell leukemia; AA, aplastic anemia; Flu, fludarabine; Mel, melphalan; TBI, total body irradiation; Bu, busulfan; UCB, umbilical cord blood; HCT-CI, hematopoietic cell transplantation-specific comorbidity index.

Of these 70 patients, 25 were women and 45 were men. Their median age was 61 years (range: 55-79 years). The patients' diagnoses included acute myeloid leukemia (AML; n = 28), acute lymphoblastic leukemia (ALL; n = 11), MDS (n = 3), CML (n = 4), non-Hodgkin lymphoma (NHL; n = 8), adult T cell

leukemia (n = 12), plasma cell leukemia (n = 1), multiple myeloma (n = 1), and AA (n = 2). Three patients had previous autologous hematopoietic cell transplantation. For disease status, those with hematologic malignancies in first or second complete remission at the time of transplant, those in the chronic phase or accelerated phase of CML, those with refractory anemia of MDS, and those with nonmalignant diseases were defined as being at standard risk (n = 15), whereas those in other situations were defined as being at high risk (n = 55). Patients were assessed for their comorbidity by the previously reported scoring system [20].

Conditioning Regimens and Postgrafting Immunosuppression

Pretransplant conditioning varied, and was determined by each attending physician according to the patient's disease, disease status, and history of prior therapy. Sixty-five patients underwent conditioning regimens with 125-180 mg/m^2 of fludarabine (Flu; 25 mg/m^2 for 5 days or 30 mg/m^2 for 6 days), along with 80 mg/m^2 of melphalan (Mel; 40 mg/m^2 for 2 days) and total-body irradiation (TBI) at a total dose of 4 Gy for 63 and 2 Gy for 2. Four patients in relatively poor performance status were conditioned with busulfan to avoid severe gastrointestinal tract toxicity induced by the use of Mel. One patient underwent a conditioning regimen with thiotepa (5 mg/kg for 2 days) in addition to 125 mg/m^2 of Flu and 80 mg/m^2 of Mel, because of the urgent transplant schedule that did not allow access to TBI. Valproate sodium (300 mg/day) was administered to all patients who received Bu. Immunosuppressive therapy with cyclosporine A (CsA, 3 mg/kg continuous infusion, aiming for a serum concentration of 250-400 ng/mL) or tacrolimus (Tac, 0.03 mg/kg continuous infusion, aiming for 12-17 ng/mL) was started on day −1. CsA was used for patients in the early phase of this study, and, based on our early experience of high early mortality related to PIR in the patients with CsA prophylaxis, Tac was subsequently used to substitute for CsA.

Supportive Care

Prophylactic antibiotics, including fluorquinolone, fluconazole, and acyclovir, were used routinely. Patients received ganciclovir or foscarnet for any sign of a cytomegalovirus reactivation, such as isolation of CMV or detection of viral proteins (pp65) or nucleic acid in any body fluid or tissue specimen. *Pneumocystis jiroveci* prophylaxis included trimethoprim-sulfamethoxazole as first-line therapy.

Definition of Engraftment, Preengraftment Immune Reaction, and End Points

OS ands PFS were computed from the date of transplantation. Engraftment was defined as absolute neutrophil count >0.5 × 10^9/L for 3 consecutive days. Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-mismatched pairs, PCR for variable number of tandem repeats was used with donor cells detected at a sensitivity of 10%. Whole blood or BM cells were assessed at the time of granulocyte engraftment. PIR was characterized by the presence of at least 2 of the following symptoms with no direct consequences of infection or adverse effects of medication 6 or more days before engraftment, as described previously [12,18]; a high fever (>38.5°C), skin eruptions, diarrhea, jaundice (serum levels of total bilirubin >2.0 mg/dL), or body weight gain >10% of baseline. NRM was defined as death in the absence of disease progression. Deaths occurring after disease progression were categorized as relapse regardless of the cause of death. Infection was considered the cause of death when bacterial, viral, or fungal infection was determined to be the proximate cause of death in patients who had not relapsed. Patients underwent BM aspiration at the time of engraftment or if clinically indicated. Relapse for AML, ALL, CML, or MDS was determined by flow cytometric, morphologic, or cytogenetic evidence of malignant or dysplastic cells with clonal markers similar to those observed before transplantation. Relapse for NHL was defined as progressive adenopathy or BM involvement. Acute and chronic GVHD (aGVHD, cGVHD) were defined and graded by standard criteria [21]. The following factors were considered potential predictors of outcomes: recipient's age, disease risk (standard versus high), ECOG performance status, HCT-specific comorbidity index score, history of prior chemotherapy (all cytoreductive chemotherapy excluding hydroxyurea and imatinib mesylate), history of prior documented infections (infectious episode with positive culture results for bacterial or yeast infections, and at least probable diagnosis of mold infection by EORTC/NIH-MSG criteria [22]), number of total nucleated cord blood cells, number of CD34$^+$ cells, HLA disparity, conditioning regimen, GVHD prophylaxis, grade of aGVHD, and the presence or absence of PIR.

Statistical Methods

OS was calculated from the day of transplantation until death from any cause or last follow-up. Disease-free survival (DFS) was calculated from the day of transplantation until relapse or death from any cause or last follow-up. The probabilities of survival and DFS were estimated and plotted using the Kaplan-Meier method [23]. Relapse and NRM rates were estimated using cumulative incidence analysis and were considered competing risks [24]. Similarly, in the analysis of GVHD rates, death because of other causes or relapse leading to early withdrawal of immune suppression were considered competing risks. The effect

of various patient and disease categoric variables on survival probabilities was studied with the log-rank test. A Cox proportional hazard model with limited variables because of small sample was used to determine the significance of multiple variables in determining these outcomes. Cumulative incidence curves were drawn using Gray's method [25].

RESULTS

Engraftment

Ten of the 70 patients were not evaluable for donor engraftment because of early death (before 28 days posttransplant) from disease progression (n = 1), infection (n = 7), and complications of central nervous system (n = 2). Of the 60 evaluable patients, the cumulative incidence of primary donor engraftment was 92% at a median of 18 days after transplantation (range: 11-53 days). Platelet recovery $>20 \times 10^9$/L was observed in 38 patients (63%), at a median of 35 days (range: 25-95 days). All patients required transfusions of platelets and red blood cells. Recovery of neutrophil counts $>0.5 \times 10^9$/L did not occur in 5 patients who survived beyond 28 days posttransplant; these patients were classified as primary graft failures. Two of these patients received secondary RI-UCBT and died of infection. The remaining 3 patients died of infection. All engrafting patients without BM relapse were complete donor chimeras beyond 1 month after transplantation (data not shown). Remarkably, all 3 evaluated patients of 10 who died before day 28 showed complete donor chimerism (94%, 100%, and 94.6% on days 12, 15, and 20 posttransplant, respectively).

PIR and GVHD

Forty-three patients experienced clinical symptoms defined as PIR, as described previously [12,18]. Patients who received Tac as GVHD prophylaxis tended to have a lower chance of experiencing PIR compared with those who received CsA, although differences were not statistically significant (53% versus 72%, respectively; $P = .1$).

Among 54 evaluable patients, 33 patients (61%) developed aGVHD of grade II or higher, including 23 patients (43%) who developed that of grade III or IV. Of the 30 patients who survived longer than 100 days posttransplant, 12 (40%) developed cGVHD, including 7 with limited and 5 with extensive form (Table 2).

Survival, Disease Progression, and NRM

At the time of analysis, 20 of 70 patients survived a median of 512 days (range: 103-1213 days) after transplantation. The Kaplan-Meier estimates of OS and PFS at 2 years were both 23% (Figure 1). The median OS time was 114 days (range: 7–1213 days), and the median PFS time was 92 days (range: 7–1213 days).

Table 2. *The Incidence and Severity of Graft-versus-Host Disease (GVHD)*

	Patients (n = 54)
	No. (%)
Acute GVHD	45 (83)
Grade II-IV	33 (61)
Grade III-IV	23 (43)
	Patients (n = 30)
	No. (%)
Chronic GVHD	12 (40)
Limited	7 (23)
Extensive	5 (17)

Eighteen patients (26%) showed progression of the underlying disease at a median of 134 days (range: 13–785 days) after transplantation, and 15 of these patients died of their disease.

Thirty-seven patients died of nonrelapse causes (Table 3). Nineteen of them were from infections, which was the leading cause of NRM. Among 33 deaths observed before day 100 posttransplant, 30 were from nonrelapse causes and 3 from disease progression. The cumulative incidences curves of NRM and disease progression are shown in Figure 2.

Factors Contributing to OS and NRM

In univariate analyses, survival was associated with recipient's age ($P = .01$), disease risk ($P < .01$), aGVHD ($P < .01$), and PIR ($P < .01$), with favorable outcomes in younger recipients (<61 years), those with standard risk, those with lower grade aGVHD (grade 0-II), and those without PIR (Figure 3A-D). Potential risk factors such as ECOG performance status, HCT-specific comorbidity index score, history of prior documented infection, history of prior chemotherapy, HLA disparity,

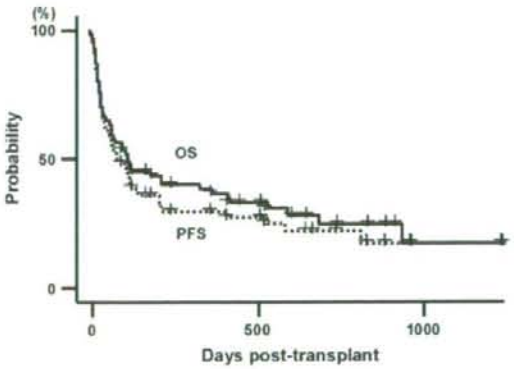

Figure 1. OS and PFS estimates for 70 patients with hematologic diseases treated with RI-UCBT.

Table 3. *Causes of Death*

	Patients (n = 70)
	No. (%)
NRM	37 (53)
Infection	19 (27)
GVHD	9 (12)
IP	4 (6)
TMA	3 (4)
Others	2 (3)
Relapse	13 (19)
Total	50 (71)

NRM indicates nonrelapse mortality; GVHD, graft-versus-host disease; IP, interstitial pneumonia; TMA, thrombotic microangiopathy.

sex mismatch, number of infused cells, number of infused $CD34^+$ cells, and cGVHD did not reach statistical significance.

In the Cox regression analyses, recipient's age equal to or older than 61 (hazard ratio [HR] = 3.33; 95% confidence interval [CI] = 1.39-7.14; $P = .006$), high risk disease (HR = 3.33; 95% CI = 1.01; 8.33 $P = .049$), grade III-IV aGVHD (HR = 2.5; 95% CI = 1.28; 5.88 $P = .0002$), and the presence of PIR (HR = 2.5; 95% CI = 1.14; 6.25 $P = .023$) were associated with statistically worse OS (Table 4). No other factors were significantly or suggestively associated with OS.

Regarding toxicity, multivariate analyses revealed that GVHD prophylaxis (HR = 3.9, 95% CI = 1.3-11.6 for CsA versus Tac; $P = .01$) and aGVHD (HR = 5.7, 95% CI = 2.1-15.7 for grade III–IV versus 0-II; $P = .001$) were associated with NRM.

DISCUSSION

This study was undertaken to evaluate engraftment and toxicities in elderly patients with advanced hematologic diseases who received UCBT matched for at least 4 loci of HLA-A, -B, and -DRB1 using a nonmyeloablative regimen.

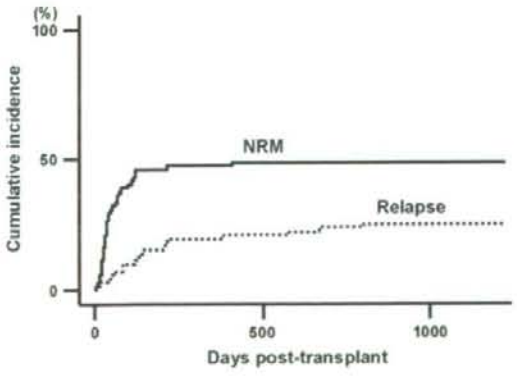

Figure 2. NRM and disease progression. Cumulative incidence estimates of NRM and disease progression for all 70 patients.

Several observations were made. First and foremost, RI-UCBT was a feasible treatment strategy for elderly patients with a successful engraftment rate of 92% without secondary graft failure except disease progression. The average interval between transplant and neurtophil recovery to 500/μL was 18 days, which is comparable to previously reported in RIC [11,12]. The chimerism study confirmed rapid engraftment of donor cells in all engrafted patients. Together with the fact that all 3 evaluated patients who died before day 28 already achieved complete donor chimerism, these data indicate that our pretransplant conditioning regimens, mainly consisting of Flu, Mel, and TBI, along with single calcineurin inhibitors for GVHD prophylaxis, can exert sufficient immunosuppressive effects that allow engraftment of CB cells. Compared to the conditioning regimen containing cyclophosphamide reported from Minnesota group [11], which allow mixed chimeric state especially for myeloid lineages during the early period of posttransplant, our conditioning is more powerful in eradicating host myeloid cells as well, which may have beneficial effect for rapid control of myeloid malignancies. The OS and PFS were estimated as both 23% at 2 years posttransplant, almost comparable to or slightly less than the data reported previously [15,16,26], which can be reasonably explained by higher age range and poor disease status before transplant in this study cohort, which can be further supported by the result of subgroup analysis indicating those with standard disease status showed much better outcome (Figure 3B).

UCBT has been associated with lower incidence of aGVHD, possibly because of the immunologic naïvety of transplanted lymphocytes; however, this naïvety raises a concern about whether transplanted cells will have sufficient antimalignant activity. Several reports indicate the in vivo antimalignancy effect of cord blood cells [27-30]. Cumulative incidence of disease progression at 2 years posttransplant in our series was 24%, which is comparable to those previously reported [15,16,26]. It plateaued later than 795 days, indicating that our RI-UCBT treatment protocol offered fairly good disease control.

The incidence of GVHD was higher than previous reports in RIC [11,12], and was almost comparable to those of BM transplants, PB cell transplants, or UCBT with conventional conditioning [8-10,31-36]. Because of the poor disease status of the majority of patients included in this study, GVHD prophylaxis was initially planned to be less intensive with single calcineurin inhibitors. Older patients' age [37] or high incidence of infectious complications, which possibly induced excessive inflammatory cytokine secretions, could have been relevant to this result [38].

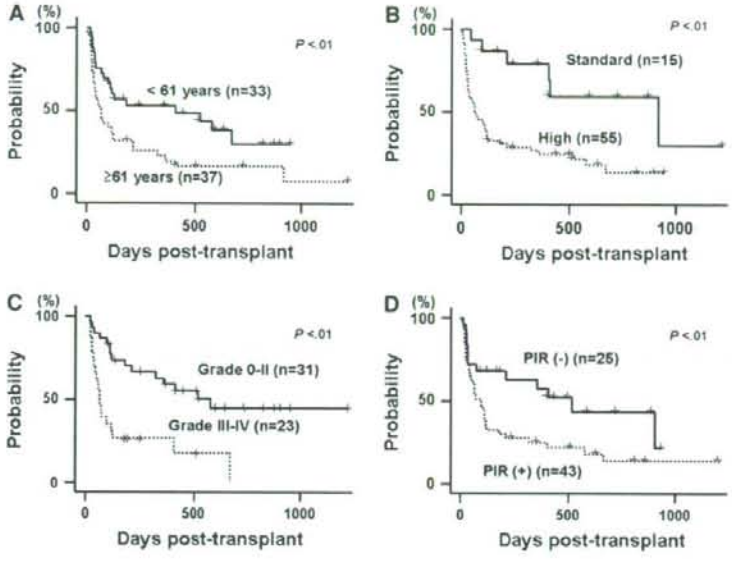

Figure 3. OS estimates after RI-UCBT (n = 70). (A) Effect of age. (B) Effect of disease status. (C) Effect of severity of aGVHD. (D) Effect of PIR.

Although RI-UCBT has been a feasible approach in terms of engraftment, a significant number of patients died from treatment-related complications. NRM was close to 3 times higher than mortality from relapse or disease progression, and most NRM occurred within 100 days posttransplant. Of 37 deaths because of NRM, 19 were from infection. Delayed engraftment relative to other stem cell sources such as BM or PB cells has been suggested to account for the higher rate of infectious complications after UCBT [32,39,40], but the time to engraftment in our series of patients was not delayed. Higher grade of aGVHD and the presence of PIR were found to be significantly associated with poor OS in multivariate analysis, indicating that immune-mediated events have strong impact on patients' outcome (Table 4). PIR is the syndrome observed in our setting of RI-UCBT. Although the mechanism behind PIR has not been investigated extensively yet, it is assumed to be reflecting alloimmune event, given our experience that more intensive GVHD prophylaxis with Tac had tendency to decrease the incidence of PIR. Moreover, development of PIR may have been suppressed in reported cases from other institutes that utilized additional agents to calcineurin inhibitors, such as methotrexate [10,19], antithymocyte globulin [31], or mycophenolate mofetil [16]. There has been a similar early immune reaction-like syndrome reported as "hyperacute GVHD" observed following BM or PBSC transplant, and responded poorly to corticosteroids compared to traditional aGVHD [41,42]. The incidence of PIR was higher than that of hyperacute GVHD, and further investigation on biologic mechanisms may help us define PIR more precisely relative to other immune-mediated diagnosis and develop optimal treatment approach. The presence of PIR was shown to cause more NRM than the absence in univariate analysis (P = .02), although it did not reach statistical significance in multivariate analysis. Thus, better management of immune-mediated complications will be the key to reduce NRM and improve OS. Based on our early experience of high early mortality related to PIR in the patients with CsA prophylaxis, Tac was subsequently used to substitute for CsA, because Tac was shown to be more potent than CsA in BM transplant [43-45]. Patients who received Tac as GVHD prophylaxis had less chance of experiencing PIR compared with those who received CsA and had less NRM, indicating the potential benefit of using Tac as a standard agent for GVHD prophylaxis. Adding methotrexate, mycophenolate mofetile, or sirolimus to the calcineurin inhibitor may further improve the final outcome [10,11,46,47]. Older age was another factor that influenced OS with statistical significance, even within the age range studied (Figure 3A and Table 4). Patients aged 61 years and older experienced more NRM than patients younger than 61 years (65% versus 39%), whereas their death rate because of disease progression was comparable (19% versus 18%), suggesting the vulnerability of higher aged population to procedure toxicity. Although the possible impact of slight variation in conditioning regimen to the outcome cannot be excluded, it is unlikely, because the great majority (93%) were conditioned with Flu/Mel/TBI regimen fairly uniformly, and comparison between Flu/Mel/TBI and others did not reach statistical significance.

Table 4. *Cox Regression Analyses of Factors Potentially Associated with OS and NRM after RI-UCBT*

Variables	HR	95% CI	P
OS			
Age			
Less than 61 years (n = 33)	0.3	0.14-0.72	.006
At least 61 years (n = 37)	1.0		
Disease risk			
Standard (n = 15)	0.3	0.12-0.995	.049
High (n = 55)	1.0		
PIR			
No (n = 25)	0.4	0.16-0.88	.023
Yes (n = 43)	1.0		
Acute GVHD			
Grade 0-II (n = 31)	0.4	0.17-0.78	.0002
Grade III-IV (n = 23)	1.0		
NRM			
GVHD prophylaxis			
CsA (n = 37)	3.9	1.3-11.6	.01
Tac (n = 33)	1.0		
Acute GVHD			
Grade 0-II (n = 31)	1.0		
Grade III-IV (n = 43)	5.7	2.1-15.7	.001

GVHD indicates graft-versus-host disease; CsA, cyclosporine A; Tac, tacrolimus; NRM, nonrelapse mortality; CI, confidence interval; HR, hazard ratio; OS, overall survival.

In conclusion, this is the first study specifically focusing on elderly patients aged 55 years and older with advanced hematologic diseases to show the feasibility of RI-UCBT. Older age per se cannot be considered to be contraindication to RI-UCBT, although a high NRM has been observed. Further optimization of the treatment protocol, such as immunosuppressive therapy for GVHD prophylaxis, is warranted to establish the safety of this promising treatment strategy for elderly patients with advanced hematologic diseases.

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ORIGINAL ARTICLE

Postmortem examination of the kidney in allogeneic hematopoietic stem cell transplantation recipients: possible involvement of graft-versus-host disease

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Abstract To investigate the association between graft-versus-host disease (GVHD) and renal injury after allogeneic stem cell transplantation (allo-SCT), we compared autopsy findings of 26 consecutive allo-SCT recipients with two control groups: patients with hematologic malignancies who received cytotoxic chemotherapy alone (Control 1, $n = 21$) and those with non-hematologic diseases (Control 2, $n = 12$). We evaluated the following renal pathology; renal tubulitis, allograft glomerulitis, intimal arteritis, allograft nephropathy, and peritubular capillaritis. These changes were found in 11 allo-SCT recipients and 10 patients in Control 1, but none in Control 2. While overall frequency of renal impairments was similar between allo-SCT recipients and Control 1 (3/26 vs. 1/21), allo-SCT recipients were more likely to have renal tubulitis and peritubular capillaritis compared to Control 1 (5/26 vs. 1/21), but less likely to present with glomerulitis (1/26 vs. 6/21). Grade III–IV acute or extensive-type chronic GVHD were seen in all of the three patients with renal tubulitis and four of the five patients with peritubular capillaritis. Allo-SCT recipients with severe GVHD tended to have tubulitis and peritubular capillaritis. These findings have implications of some renal impairment attributable to GVHD.

Keywords Tubulitis · Capillaritis · Autopsy · Glomerulitis · Bone marrow transplantation

1 Introduction

Renal dysfunction is a common problem after allogeneic hematopoietic stem cell transplantation (allo-SCT) [1–3] and is associated with many factors. Patients may have preexisting renal injury from their underlying diseases or cytotoxic chemotherapy prior to allo-SCT. Preparative

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regimens before transplantation may directly cause renal damages. Post-transplant infections complicated by renal hypoperfusion sometimes lead to acute renal insufficiency. Antimicrobials used for prophylaxis and treatment of infections can be nephrotoxic.

Calcineurin-inhibitors (CNIs) are well known to cause renal dysfunction in allo-SCT recipients [4]. CNIs can lead to arteriolar hyalinosis, resulting in glomerular sclerosis [5, 6]. Chronic GVHD involving the kidney was reported in several case series [7, 8], while it is unclear whether there is an association between acute GVHD and renal damages [9]. Renal impairments are generally not considered a part of acute GVHD; however, *in vivo* imaging of GVHD in mice showed that several non-classical organs are massively infiltrated by cytotoxic T-cell (CTL) during GVHD, including the brain, the kidneys, and the connective tissues [10]. Infiltration of CTL into the kidney may explain the high response rates following allo-SCT for metastatic renal cell carcinoma [11], which derives from the proximal renal tubules.

Since allo-SCT recipients frequently suffer from thrombocytopenia and neutropenia, renal biopsy carries a considerable risk of hemorrhage and infections, although it is essential for making an accurate diagnosis of post-transplant renal dysfunction. Little is known about the pathology of renal injury following allo-SCT. To investigate the association between GVHD and renal injury after allo-SCT, we compared postmortem renal pathology of 26 consecutive allo-SCT recipients at Toranomon Hospital with two control groups: patients with hematologic malignancies who received cytotoxic chemotherapy alone and those with non-hematologic diseases.

2 Patients and methods

2.1 Patient and data collection

Of 284 patients with hematologic diseases who received allo-SCT at Toranomon Hospital between May 2000 and January 2005, 34 patients died and underwent autopsy. Eight patients with multiple myeloma were excluded from the study, since the primary disease can affect kidney [12]. The remaining 26 allo-SCT recipients were enrolled in the study (Case). The control groups comprised 21 consecutive patients with hematologic diseases other than multiple myeloma who underwent autopsy after repeated chemotherapy (Control 1), and 12 patients with non-hematologic diseases who were randomly selected from autopsy records (Control 2).

Complete clinical data were obtained from the autopsy request forms and the patients' records. Acute and chronic GVHD were assessed according to established criteria [13, 14]. Abnormal serum creatinine level was defined as 2.0 mg/dL and above. Clinical diagnosis of renal dysfunction was judged to be present by an experienced nephrologist (J.H.) based on the clinical presentation, laboratory results, and comorbid events such as sepsis, hypotension, and nephrotoxic drug exposure.

2.2 Renal pathology

Renal pathology was assessed based on the Banff 97 workshop [15] criteria by two independent renal pathologists (S.H. and Y.Y.) blinded to the clinical courses of patients enrolled in this study. Microscopic specimens stained with hematoxylin and eosin, periodic acid-Schiff, Masson trichrome, and silver methenamine were examined. Acute-type alloreaction consisted of tubulitis, interstitial infiltration of mononuclear cells, an early type of allograft glomerulitis, arterial hyaline thickening and intimal arteritis. Chronic-type alloreactions were allograft glomerulopathy, interstitial fibrosis, tubular atrophy and fibrous intimal thickening [15]. Interstitial damages such as mononuclear atrophy, interstitial fibrosis and tubular atrophy can be associated with conditions other than renal transplantation and were not included in the present study [16]. Arterial hyaline thickening may be caused by CNI and were also excluded from the analysis [6]. Thus, we investigated the following pathology of the Banff 97 workshop criteria [15]: renal tubulitis, allograft glomerulitis, intimal arteritis, allograft nephropathy.

An animal study showed that GVHD may target a vascular endothelium [17]. In renal transplantation, vascular involvement, which is focal process, reflects more severe rejection [15]. We therefore investigated the presence of peritubular capillaritis in this study.

2.3 Statistical analysis

A univariate analysis using Fisher's exact test and the Mann–Whitney test was performed to compare clinical features of renal damages between allo-SCT recipients and either Control 1 or Control 2 patients. The level of significance was set at $P < 0.05$.

3 Results

3.1 Patient characteristics

Patient characteristics are shown in Table 1. Allo-SCT recipients were significantly younger than Control 1 and Control 2 patients ($P < 0.0001$ and $P = 0.0005$,

Table 1 Patient characteristics

Variables	Allo-SCT recipients ($n = 26$)	Control 1[a] ($n = 21$)	Control 2[b] ($n = 12$)
Age			
Median (range)	55 (16–70)	70 (54–93)	67 (59–93)
Sex			
Male/female	16/10	16/5	7/5
Primary disease			
Leukemia	16	10	0
Myelodysplastic syndrome	0	4	0
Malignant lymphoma	8	7	0
Solid tumors	1	0	8
Others	1	0	4
History of autologous stem cell transplant	6	1	0
Stem cell source			
Unrelated bone marrow	4		
Related bone marrow	3		
Related peripheral blood stem cell	6		
Unrelated cord blood	13		
Conditioning			
Myeloablative/reduced-intensity[c]	7/19		
GVHD prophylaxis			
Cyclosporine/tacrolimus	22/4		
GVHD			
Acute GVHD: grade 0–I/II–IV	18/8		
Chronic GVHD: none/limited/extensive[d]	5/2/4		
Abnormal serum creatinine level	12	8	2
Primary cause of death			
Disease progression	7	9	5
Infection	9	9	4
Interstitial pneumonitis	3	0	0
GVHD	5	0	0
Others	2	3	3

GVHD graft-versus-host disease

[a] Patients who received chemotherapy alone for the treatment of hematologic malignancies

[b] Patients with non-hematologic diseases

[c] Myeloablative regimens comprised cyclophosphamide 120 mg/kg and either total body irradiation 12 Gy ($n = 4$) or busulfan 16 mg/kg ($n = 2$). Reduced-intensity regimens were fludarabine 150 mg/m^2 and either melphalan 140 mg/m^2 ($n = 3$) or 80 mg/m^2 ($n = 12$), busulfan 8 mg/kg ($n = 4$) or cyclophosphamide 120 mg/kg ($n = 1$). Total body irradiation 4 Gy was added to reduced-intensity transplantation using unrelated bone marrow ($n = 3$) or cord blood transplantation ($n = 12$)

[d] Eleven patients who survived longer than 100 days were evaluated for chronic GVHD

respectively). Twelve of 26 allo-SCT recipients developed acute ($n = 8$) or chronic ($n = 4$) GVHD in their clinical courses, and GVHD was fatal in five.

3.2 Histological findings

Histopathological changes were found in 11 of the 26 allo-SCT recipients and 10 of the 21 Control 1 patients, while none in the 12 Control 2 patients (Table 2). The difference was statistically significant between allo-SCT recipients and Control 1 ($P = 0.0075$), as well as between allo-SCT recipients and Control 2 ($P = 0.0042$). While overall frequency of renal impairments was similar between allo-SCT recipients and Control 1 (Table 2), allo-SCT recipients were more likely to have renal tubulitis (Fig. 1) and peritubular capillaritis (Fig. 2) compared to Control 1. Allo-SCT recipients were less likely to develop glomerulitis compared to Control 1. Four of the five patients with peritubular capillaritis and all of the three patients with renal tubulitis developed severe GVHD; grade II–IV acute GVHD or extensive chronic GVHD (Table 3).

Table 2 Histopathological findings

	Allo-SCT recipients ($n = 26$)	Control 1 ($n = 21$)	Control 2 ($n = 12$)
Total[a]	11	10	0
Renal tubulitis	3	1	0
Glomerulitis	1	6	0
Peritubular capillaritis	5	1	0
Intimal arteritis	0	0	0
Glomerulopathy	2	2	0

allo-SCT allogeneic hematopoietic stem cell transplantation

[a] Patients with any histopathological finding were counted

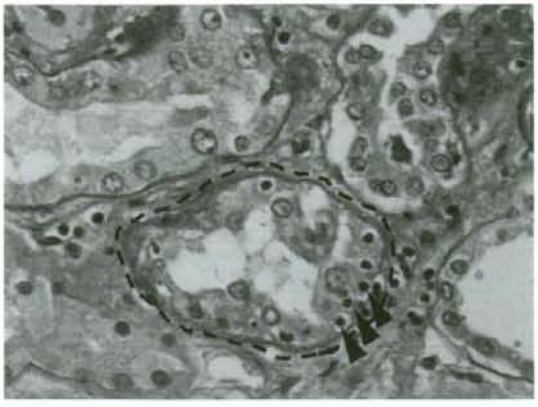

Fig. 1 Peritubular capillaritis: mononuclear leukocytes/neutorophil (*arrowheads*) within peritubular capillary (surrounded by *dotted line*)

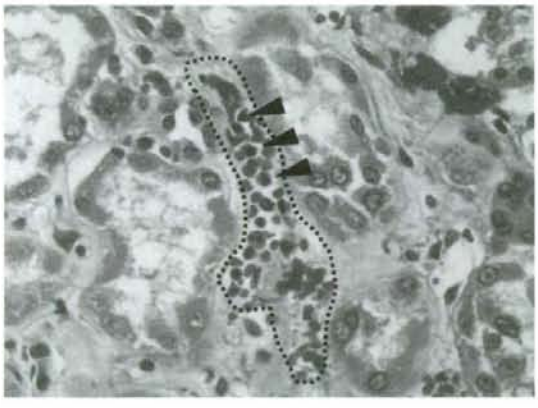

Fig. 2 Tubulitis: mononuclear leukocytes (*arrowheads*) infiltrating into renal tubular epithelium (surrounded by *broken line*)

Table 3 Histological findings according to the severity of GVHD

	No GVHD ($n = 13$)	Mild GVHD ($n = 1$)	Severe GVHD ($n = 12$)
Renal tubulitis	0	0	3
Glomerulitis	0	0	1
Peritubular capillaritis	0	0	4
Intimal arteritis	0	0	0
Double contour	1	0	1

Mild GVHD: grade I acute GVHD or limited chronic GVHD; severe GVHD: grade II–IV acute GVHD or extensive chronic GVHD

4 Discussion

Renal damage at autopsy was more frequently found in patients with hematologic malignancies compared to subjects with non-hematologic diseases. Our results support the previous studies for hematologic diseases and all-SCT recipients [18, 19]. Since patients with hematologic malignancy receive higher dose of chemotherapy and more frequent infection during neutropenia compared to other neoplasms or benign conditions, renal dysfunction may be attributable to them as well as infiltration of underlying diseases.

Our study showed that all-SCT patients tend to develop more tubulitis and most of them had GVHD. A finding is consistent with earlier clinical and animal studies [9, 10], suggesting that tubules may be targeted in the GVHD. However, our finding showed lower rates of tubulitis compared to previous study reported by El-Seisi et al. (12 vs. 60%) [18]. This is likely due to the difference in diagnostic criteria of tubulitis. Patients who developed GVHD have higher rates of infection as well as antibiotic use and other medications. Degenerative changes, atrophy and necrosis of renal tubules can result from infection and medication use [20]. Therefore, atrophic and degenerative changes of the tubules are not considered tubulitis in the Banff criteria [15]. If we were to diagnose tubulitis based on degeneration, atrophy and necrosis of tubules, we would overestimate the effect of GVHD. The fact that 12% of all-SCT recipients had tubulitis using the strict criteria suggests the association between tubulitis and GVHD. In contrast, glomerulitis was more frequently observed in patients with hematologic disease without transplantation in this study. While its exact pathogenesis remains unknown, we can speculate some possibilities concerning it. First, patients with hematologic disease without transplantation tended to be old, and drugs such as anti-neoplastic, anti-bacterial, and anti-fungal agents might have injured their kidney [21, 22]. Second, glomerulitis sometimes develops in patients with hematologic diseases, since monoclonal gammaglobulinemia or cryoglobulinemia associated with hematologic diseases cause immune-mediated glomerulitis [23, 24]. It usually disappears with the remission of the underlying diseases. The primary disease is usually controlled in transplanted patients; therefore, glomerulitis might have disappeared in transplanted patients. Since hematologic diseases are rarely cured with chemotherapy alone in elderly patients, kidney injury might have persisted until autopsy in these patients.

Vascular changes in glomeruli are important components of Banff criteria [15]. A recent study indicated that C4d deposit in peritubular capillary is a part of humoral rejection [25]. The relationship between GVHD and intravascular damages was not reported in the previous studies [26, 27]. Our results revealed that all-SCT recipients had higher rates of peritubular capillaritis, but similar frequency of glomerulitis compared to the control groups.