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High Number of Memory T Cells Is Associated with Higher Risk of Acute Graft-versus-Host Disease after Allogeneic Stem Cell Transplantation

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A B S T R A C T

The pathophysiology of acute graft-versus-host disease (GVHD) remains poorly understood in humans. Although T cell subsets have been identified to play a major role in disease initiation in rodents, clinical data on the effect of these different subsets are scarce and conflicting. To address this question, immunophenotyping analyses were performed on the graft in 210 patients. The onset of acute GVHD was retrospectively correlated with these subpopulations. In an adjusted analysis, only the absolute count of CD45lo/CD62Llo $CD8^+$ T cells (effector memory T cells) was significantly associated with the onset of grade 2 to 4 acute GVHD. Thus, in contrast to experimental data, we found that the number of effector memory but not of naïve T cells was associated with the onset of GVHD. These results should be kept in mind while clinical trials, which aim to deplete naïve T cells, are underway in several institutions.

INTRODUCTION

Acute graft-versus-host disease (aGVHD), the leading cause of transplantation-related mortality [1,2], still occurs in 40% to 70% of transplantations [3,4]. Donor T cells are major effectors of both graft-versus-host disease (GVHD) and graft-versus-leukemia effects [5,6]. During the last decade. T cells subsets have been investigated to better understand the chronology of and to predict onset of aGVHD. They are divided into 4 main subsets: naïve, central memory, effector memory, and terminally differentiated T cells. Naïve and central memory T cells strongly expressed CD62L. Naïve and terminally differentiated T cells expressed CD45RA, whereas other memory T cells do not. Naïve T cells after activation in lymphoid organs have been implicated in the genesis of GVHD in experimental models [7,8]. Conversely, alloreactive effector memory T cells are immediate effectors [9-11]. In humans, in vitro studies demonstrated an increased capacity to induce alloreactivity in naïve CD4 T cells [12,13]. A prospective and a retrospective study in patients tend to confirm this hypothesis, showing that naïve CD4 T cells represent a significant biomarker of onset aGVHD [14,15]. Nevertheless, these studies included a small number of patients. As graft engineering now allows efficiently removing the naïve T cell subset [16], with the aim of

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ORIGINAL ARTICLE

Folinic acid after MTX as prophylaxis for GVHD in pediatric bone marrow transplantation

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Abstract The effect of folinic acid (FA) on toxicity secondary to the use of methotrexate (MTX) for the prevention of graft-versus-host disease (GVHD) has not been determined. We retrospectively analyzed data from 111 patients who received allogeneic bone marrow transplantation (allo-BMT) in our institution. Fifty patients did not receive FA (non-FA), 37 received FA four times (low dose, LD-FA), and 24 received FA 25 times (high dose, HD-FA) in BMT. No significant differences were observed in the severity of stomatitis after allo-BMT among the three groups while the median of peak value of ALT in HD-FA was significantly lower ($P = 0.031$). The median time to neutrophil engraftment after allo-BMT in the HD-FA group was significantly shorter than that in the non-FA group ($P = 0.034$). No significant difference in the median time to neutrophil engraftment was observed between the LD-FA and non-FA groups ($P = 0.44$). Stepwise multiple regression analysis revealed that the determinants of the shorter duration of neutropenia were transfused total nucleated cell dose ($P = 0.001$) and the administration of HD-FA ($P = 0.036$). There was no significant difference in 3-year overall survival among the three groups. Frequent administration of FA may reduce the time to neutrophil engraftment after hematopoietic stem cell transplantation.

Keywords Folinic acid · Methotrexate · Graft-versus-host disease · Children

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Introduction

Methotrexate (MTX) is a highly effective agent for the prevention of graft-versus-host disease (GVHD) in hematopoietic stem cell transplantation (HSCT), and the combination of calcineurin inhibitors (CIs) and MTX is widely used [1]. However, use of MTX may be associated with considerable toxicity, including delayed engraftment, stomatitis, and hepatotoxicity. Folinic acid (FA) is often used to minimize the side effects of antifolate chemotherapeutic agents administered to patients suffering from hematological and oncological disorders. Therefore, FA might improve the tolerability of MTX following HSCT.

In the 1990s, some reports showed that FA administration following MTX for the prophylaxis of GVHD in HSCT could improve tolerance to the regimen without inhibiting its ability to prevent GVHD [2, 3]. In 2000, the European Group for Blood and Marrow Transplantation (EBMT) proposed a uniform pediatric GVHD prophylaxis policy to include 15 mg/m^2/day FA administration 24 h after MTX [4]. However, administration of FA after MTX in HSCT was used in only 37 (45.7 %) of 81 EBMT centers and eight (44 %) of 18 centers in Australia and New Zealand [5, 6]. Based on these data, the usefulness of FA in HSCT has been controversial for a long time among most clinicians. Recently, Sugita et al. [7] reported that FA administration reduced the incidence of severe stomatitis after MTX in HSCT. Hudspeth et al. [8] also reported that administration of FA ameliorated MTX toxicity in children. In contrast, Lindqvist et al. [9] reported that FA at a high dose was associated with graft rejection in pediatric HSCT. These recent data suggest that the role of FA in HSCT is still unclear. The inconsistent results regarding FA in recent reports might be due to the various methods of FA administration, including drug dosage, schedules, and route of

administration. As with many other aspects of HSCT, there appears to be considerable variance among transplant centers with regard to the use of FA following MTX.

In our institution, FA was not routinely used after MTX administration for the prophylaxis of GVHD until February 2007. From March 2007 to March 2009, FA was routinely used a total of 25 times following MTX administration according to a report by Nevill [2]. From April 2009 to November 2012, FA was administered a total of four times according to EBMT recommendations [4]. After this time, we ceased FA administration because of institutional conditions. To determine whether the number of FA administrations is associated with the effect of FA on prevention of adverse events related to MTX, we retrospectively reviewed the charts of patients who underwent HSCT at our institution.

Methods

Patients

We retrospectively analyzed data for 120 patients who had undergone HSCT and received MTX and a CI for GVHD prophylaxis between January 2003 and May 2013 at the Department of Pediatrics, National Kyushu Cancer Center. None of the patients received peripheral blood stem cell transplantation in our cohorts. Patients who received cord blood transplantation (CBT, $n = 7$) with insufficient data ($n = 2$) on the administration of FA were excluded. MTX was administered at a dose of 15 mg/m^2 i.v. on day 1 and 10 mg/m^2 on days 3, 6, and 11. Data for remaining 111 patients who received bone marrow transplantation (BMT) were analyzed in this study. All patients or their parents provided written informed consent for HSCT. This retrospective study and the use of patient data were approved by the institutional review board of the National Kyushu Cancer Center.

Administration of FA

Fifty patients did not receive FA administration (non-FA). Thirty-seven patients received FA a total of four times (LD-FA). Patients in the LD-FA group received i.v. FA 15 mg/m^2 24 h after each administration of MTX according to EBMT recommendations (Fig. 1) [4]. Twenty-four patients received FA a total of 25 times (HD-FA) according to a previous report (Fig. 1) [2]. FA after first and second MTX started at 12 h after the administration of MTX and was administered three times and six times after first and second MTX, respectively. And then, FA after third and fourth MTX started at 24 h after the administration of MTX and was administered eight times. The total dose of FA after MTX for the prevention of GVHD in LD-FA and HD-FA was 60 and 265 mg/m^2, respectively. Of six patients who were not received the fourth administration of MTX because of toxicities, including renal failure and edema, two patients did not receive FA, three patients received FA a total of 17 times, and one patient received FA a total of three times.

Definition and evaluation of toxicity

Neutrophil engraftment was defined as an absolute neutrophil count >0.5 $\times$ 10^9/L for three consecutive measurements. Platelet engraftment after conditioning regimens was defined as an increase in platelet counts to $\geq$20 $\times$ 10^9/L for seven consecutive measurements without transfusion support. Graft rejection was defined as failure of donor engraftment accompanied by return of host lymphocytes. Acute GVHD was graded according to the consensus criteria [10].

The durations of stomatitis, trismus, inability to eat, and administration of opioids were used as indicators of the severity of stomatitis. The duration of stomatitis was defined as the period between the appearance of ulcer, aphtha, hemorrhagic lesion and erythema in oral cavity and the disappearance of those symptoms. The rate of engraftment, and days to neutrophil and platelet engraftment were used as indicators of myelotoxicity. The peak values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (T-bil) (within 30 days after HSCT) were used as indicators of hepatotoxicity due to MTX.

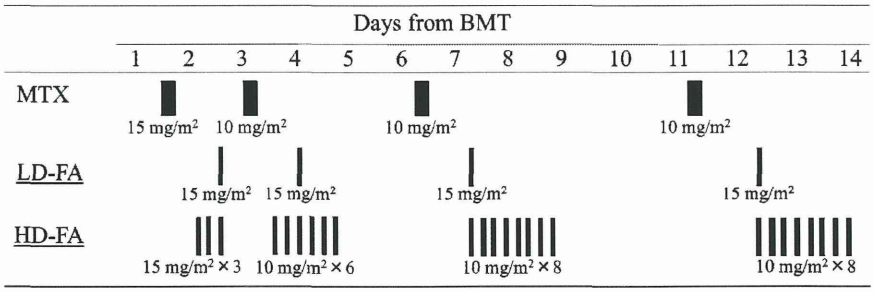

Fig. 1 Schematic representation of FA administration

Conditioning regimens, GVHD prophylaxis and stem cell donor

Of 111 patients, 36 patients received total body irradiation (TBI, 12.0–13.2 Gy) and cyclophosphamide (CPA) with or without etoposide or cytarabine, 22 patients received TBI and melphalan (L-PAM), 17 patients received busulfan (BU) and CPA with or without etoposide or cytarabine, 16 patients received BU and L-PAM, 13 patients received low-dose TBI and CPA, and 7 patients received fludalabine and L-PAM. Ninety-eight patients received tacrolimus and MTX as the prophylaxis of GVHD and 14 patients did cyclosporine and MTX. Twenty-three patients received BM from related donors and 88 patients from unrelated donors.

Statistical analysis

To compare characteristics for categorical variables, the number of patients and their respective percentages were calculated. The Chi-square test was performed to compare the three groups. For continuous variables, the median and range were calculated, and the Kruskal–Wallis test was used to analyze differences among these groups. The probability of overall survival (OS) was calculated using the Kaplan–Meier estimator. Comparison of survival was carried out with the log-rank test. Cumulative incidence curves were used in competing risks setting, with death without engraftment, in remission and from disease treated as a competing event, to calculate the provability of neutrophil engraftment rate, relapse and treatment-related mortality (TRM), respectively. Gray test was used for comparisons. Multiple linear regression analyses using the stepwise method were performed to assess the independent variables; transfused total nucleated cell dose (TTNCD), the administration of HD-FA, stem cell source, gender and conditioning regimen affecting the dependent variable the duration of neutropenia. $P < 0.05$ was used to determine statistical significance. All analyses were performed by SPSS 17 (SPSS Co., Tokyo, Japan) and EZR (Saitama Medical Center, Jichi Medical University).

Results

Patient characteristics are shown in Table 1. Except for gender, there were no significant differences in baseline characteristics, including age, diagnosis, disease status at HSCT, conditioning regimen, prophylaxis for GVHD, stem cell source, and total nucleated cell dose among patients in the non-FA, LD-FA, and HD-FA groups.

Four (8 %) patients in the non-FA group, 3 (8.3 %) patients in the LD-FA group and one (4.2 %) patient in the HD-FA did not achieve neutrophil engraftment. There was no significant difference in the engraftment rate among the three groups ($P = 1.000$). The median times to neutrophil engraftment in the non-FA, LD-FA, and HD-FA groups were 18 days (range 13–23 days), 16 days (range 12–41 days), and 14 days (range 11–28 days), respectively (Fig. 2). The median time to neutrophil engraftment in the HD-FA group was significantly shorter than that in the non-FA group ($P = 0.034$). However, the median time to neutrophil engraftment in the LD-FA group was not significantly different to that in the non-FA group ($P = 0.44$). No significant difference was observed in the median time to platelet engraftment among the groups (data not shown). A stepwise multiple regression analysis revealed that the determinants of the shorter duration of neutropenia were TTNCD ($P = 0.001$) and the administration HD-FA ($P = 0.036$) (Table 2).

No significant differences were observed in the severity of stomatitis among the three groups (Table 3). There were no significant difference in the peak value of AST and T-bil among the three groups while significant difference was observed in the peak value of ALT among the three groups ($P = 0.031$) (Table 3). The incidence of patients with grade II–IV acute GVHD was 667, 654, and 542 % in the non-FA, LD-FA, and HD-FA, groups, respectively ($P = 0.707$).

The median follow-up time for the surviving patients was 36 months (range 1–130 months) after BMT. Three-year OS in the non-FA, LD-FA, and HD-FA groups was 60.6 % [95 % confidence interval (CI) 45.3–72.8], 76.0 % (95 % CI 56.8–87.6), and 70.8 % (95 % CI 48.4–84.9), respectively (Fig. 3a, $P = 0.127$). The cumulative incidence of treatment-related mortality in the non-FA, LD-FA, and HD-FA groups was 26.8 % (95 % CI 15.1–39.8), 10.8 % (95 %CI 3.4–23.3), and 12.5 % (95 %CI 3.0–29.1), respectively, ($P = 0.121$). Thirty-seven (74 %) patients in the non-FA group, 31 (84 %) patients in the LD-FA group and 22 (92 %) patients in the HD-FA group had malignant diseases, including leukemia, myelodysplastic syndrome, and malignant lymphoma. The cumulative incidence of malignant disease relapse in the non-FA, LD-FA, and HD-FA groups was 25.8 % (95 % CI 12.5–41.3), 27.5 % (95 % CI 12.7–44.6), and 22.7 % (95 % CI 8.0–42.0) (Fig. 3b, $P = 0.881$).

Discussion

The effect of FA on toxicity due to MTX for the prevention of GVHD in HSCT is controversial. Additionally, a standard method of FA administration after MTX in HSCT, including drug dosage, schedules and route of administration, has not been determined. Therefore, we retrospectively analyzed data on toxicity due to MTX among three groups, including patients in the non-FA, LD-FA, and HD-FA groups. In

Table 1 Patient characteristics

	Total $n = 111$ (%)	non-FA $n = 50$ (%)	LD-FA $n = 37$ (%)	HD-FA $n = 24$ (%)	*P* value
Age					0.52
Median		9	9	9	
Range		1–23	0–19	0–24	
Gender					0.023
Male	67 (60.4)	25 (50.0)	22 (59.5)	20 (83.3)	
Female	44 (39.6)	25 (40.0)	15 (40.5)	4 (16.7)	
Disease					0.282
ALL	39 (35.1)	10 (20.0)	16 (43.2)	13 (54.2)	
AML	24 (21.6)	11 (22.0)	8 (21.6)	5 (20.8)	
MDS	5 (4.5)	4 (8.0)	1 (2.7)	0 (0.0)	
JMML	12 (10.8)	7 (14.0)	4 (10.8)	1 (4.2)	
CML	6 (5.4)	3 (6.0)	1 (2.7)	2 (8.3)	
ML	4 (3.6)	2 (4.0)	1 (2.7)	1 (4.2)	
AA	14 (12.6)	7 (14.0)	5 (13.5)	2 (8.3)	
Benign	7 (6.3)	6 (12.0)	1 (2.7)	0 (0.0)	
Disease status at transplantation					0.081
CR	57 (51.4)	21 (42.0)	21 (56.8)	15 (62.5)	
Non-CR	22 (19.8)	8 (16.0)	8 (21.6)	6 (25.0)	
CP/SD	32 (28.8)	21 (42.0)	8 (21.6)	3 (12.5)	
Conditioning					0.134
TBI + CPA ± (VP-16 or Ara-C)	36 (32.4)	14 (28.0)	17 (45.9)	5 (20.80)	
TBI + L-PAM	22 (19.8)	6 (12.0)	6 (16.2)	10 (41.7)	
Bu + CPA ± (VP-16 or Ara-C)	17 (15.3)	11 (22.0)	4 (10.8)	2 (8.3)	
Bu + L-PAM	16 (14.4)	7 (14.0)	5 (13.5)	4 (16.7)	
LD-TBI + CPA	13 (11.7)	8 (16.0)	3 (8.1)	2 (8.3)	
FLU + L-PAM	7 (6.3)	4 (8.0)	2 (5.4)	1 (4.2)	
GVHD prophylaxis					0.223
Tac + MTX	97 (87.4)	42 (84.0)	35 (94.6)	20 (83.3)	
CsA + MTX	14 (12.6)	8 (16.0)	2 (5.4)	4 (16.7)	
Stem cell source					0.469
RBM	23 (20.7)	10 (20.0)	6 (16.2)	7 (29.2)	
URBM	88 (79.3)	40 (80.0)	31 (83.8)	17 (70.8)	
Total nucleated Cell dose (×108/kg)					0.472
Median		2.91	2.79	2.76	
Range		0.53–7.36	0.77–7.17	0.5–8.2	

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *MDS* myelodysplastic syndrome, *JMML* juvenile myelomonocytic leukemia, *CML* chronic myelocytic leukemia, *ML* malignant lymphoma, *AA* aplastic anemia, *CR* complete remission, *CP* chronic phase, *SD* stable disease, *TBI* total body irradiation, *Bu* busulfan, *CPA* cyclophosphamide, *VP-16* etoposide, *Ara-C* cytarabine, *L-PAM* melphalan, *LD-TBI* low-dose TBI, *GVHD* graft-versus-host disease, *Tac* tacrolimus, *MTX* methotrexate, *CsA* cyclosporine, *RBM* related bone marrow, *URBM* unrelated bone marrow

the present study, stomatitis, engraftment rate, the incidence of grade II–IV acute GVHD, and OS in HSCT were not affected by the administration of FA. There were no significant differences in the peak value of AST and T-bil among three groups while the value of ALT in HD-FA group was significantly lower than that in Non-FA group. Therefore, the effect of the administration of FA on hepatotoxicity after BMT was not determined in this study. In contrast, the time to neutrophil engraftment in patients receiving frequent FA was shorter than that in those without FA.

In the present study, we used the duration of stomatitis, trismus, inability to eat, and administration of opioids as indicators of the severity of stomatitis. We did not find any difference in the severity of stomatitis among the three groups. In contrast, Sugita et al. [7] reported that administration of FA reduces the incidence of severe stomatitis, as well as the duration of opioid administration and inability to eat. Additionally, Nevill et al. [2] described that administration of FA prevents the incidence of stomatitis. The cause of discrepancy between those studies and our study might be the following. First, most of our patients were children, while most of the patients in the two previous studies were adults. Because MTX clearance decreases with aging according to recent data in the Children's

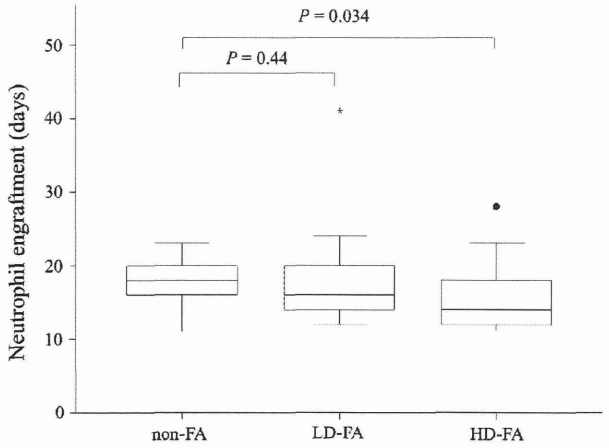

Fig. 2 Neutrophil engraftment among patients in the non-FA, LD-FA, and HD-FA groups

Table 2 Stepwise multiple linear regression analysis for the duration of neutropenia

Independent value	β	*P* value
TTNCD	−0.319	0.001
HD-FA	−0.205	0.036
Stem cell source	−0.082	0.386
Gender	0.128	0.184
TBI	0.016	0.896
BU	−0.058	0.638

R2 = 0.157 adjusted R2 = 0.107 $p = 0.008$

TTNCD transfused total nucleated cell dose, *HD-FA* high-dose folinic acid, *TBI* total body irradiation, *BU* busulfan, *β* standardized regression coefficient

Table 3 Effect of FA on toxicities related to MTX

	Non-FA	LD-FA	HD-FA	*P* value
Stomatitis (days)				0.376
Median	12	15	10	
Range	0–34	0–30	0–21	
Trismus (days)				0.825
Median	11	10	12	
Range	0–34	0–30	0–20	
Inability to eat (days)				0.913
Median	16	16	13	
Range	0–37	0–51	0–41	
Opioid (days)				0.282
Median	7	11	11	
Range	0–25	0–36	0–30	
AST (IU/l)				0.139
Median	110	84	76	
Range	26–2,470	17–296	16–237	
ALT (IU/l)				0.031
Median	194	173	88	
Range	22–1,149	31–899	35–893	
T-bil (mg/dl)				0.606
Median	0.9	1	0.9	
Range	0.5–7.7	0.4–7.3	0.4–7.1	

Durations of stomatitis, trismus, inability to eat and opioid were shown as days. The peak values of AST, ALT and T-bil were shown

FA folinic acid, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *T-bil* total bilirubin

Oncology Group [11], the effect of FA on stomatitis in pediatric patients might be small. Second, Sugita et al. described that FA mouthwash is effective for the prevention of severe oral stomatitis, but no patients in the present study received FA mouthwash. Although there is no recommendation to support the use of FA mouthwash for prevention of stomatitis, FA mouthwash could be useful for the prevention of stomatitis.

To evaluate the difference in the median time to achieve neutrophil engraftment after HSCT among three groups, we excluded seven patients who received CBT in our study. In the HD-FA group, the time to achieve neutrophil engraftment was significantly reduced compared with that in the non-FA group (14 vs 18 days). This result is compatible with a previous report by Nevill [2]. However, in the LD-FA group, it was not significantly different compared with that in the non-FA group (16 vs 18 days). Sugita et al. [7] also reported that the use of FA did not reduce the duration of neutropenia in their study. This difference in results could be due to the dosage of FA administration. Patients in the HD-FA group in our study received FA administration 25 times based on Nevill's report [2], while patients in Sugita et al. 's [7] study received FA a total of nine times. Therefore, frequent administration of FA might reduce the time to achieve neutrophil engraftment after BMT. However, previous studies and our study were retrospective and had a small sample size. To clarify the association between the frequency of FA administration and the duration of neutropenia after HSCT, prospective randomized studies are warranted.

We showed that administration of FA was not associated with graft rejection in the current study. Recently, Lindqvist et al. [9] reported that FA at a higher dose is associated with graft rejection in pediatric HSCT. In their study, the median dose of FA until day 21 post-HSCT in patients with graft rejection was 207 mg/m^2. This dose was higher than that in patients without graft rejection. However, in our study, all patients in the HD-FA group, who received FA at a total dose of 265 mg/m^2 until day 13 post-BMT, achieved neutrophil engraftment. Only one patient in HD-FA, who did not receive the fourth administration of MTX and received FA a total of 17 times (185 mg/m^2), did not achieve

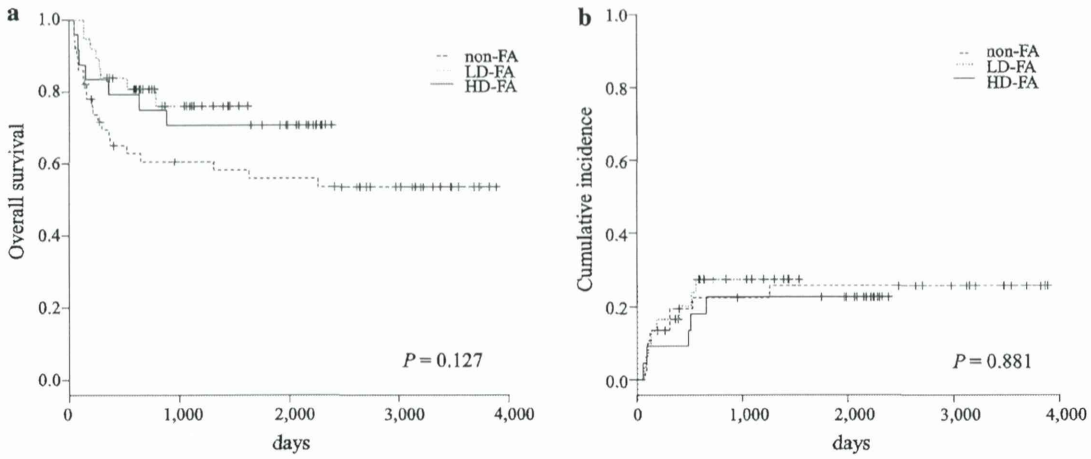

Fig. 3 Outcome of all patients. **a** Overall survival following allo-BMT and **b** the cumulative incidence of relapse

neutrophil engraftment. This discrepancy between studies might be due to differences in patients' characteristics. Lindqvist et al.'s [9] study consisted of many nonmalignant patients (45 %). However, most of our patients (80 %) had malignant disorders. Lindqvist et al. [9] described how residual recipient marrow cells, especially in patients with nonmalignant diseases, might have a better chance to survive because of FA, resulting in subsequent rejection. The association between graft rejection and FA might be small in patients with malignant diseases.

There are concerns that FA might increase the incidence of acute GVHD because of reducing the effect of MTX. Actually, in institutions that did not use FA in HSCT, half of them did not use FA because of the possibility that it may increase the risk of acute GVHD [6]. However, our study showed that the incidence of grade II–IV acute GVHD was not significantly different among patients in the non-FA, LD-FA, and HD-FA groups. In 1978, Gratwohl et al. [12] showed that the effect of MTX on the prevention of acute GVHD was maintained in a prospective randomized comparative study in dogs with dog leukocyte antigen-mismatched allogeneic BMT when FA was given 6 h after each dose of MTX. After this study, other reports showed that even various administration methods of FA could not increase the incidence of acute GVHD in humans [2, 3, 7]. To the best of our knowledge, no reports have shown an association between administration of FA after MTX and an increase in the incidence of acute GVHD.

In summary, the present study indicates that the administration of FA could not reduce the toxicity due to MTX for the prophylaxis of GVHD while frequent administration might shorten the time to achieve neutrophil engraftment after BMT. However, to determine the role of FA in HSCT, prospective randomized studies are warranted.

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Conflict of interest The authors declare no conflict of interest.

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Meralgia Paresthetica as a Presentation of Acute Appendicitis in a Girl With Acute Lymphoblastic Leukemia

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Summary: A 7-year-old girl with Philadelphia chromosome-positive acute lymphoblastic leukemia developed recurrent fever and meralgia paresthetica (MP) during chemotherapy, which resolved after administration of antibiotics. Five months after the onset of these symptoms, enhanced computed tomography showed a periappendiceal abscess extending into the psoas muscle. The cause of her fever and MP was thought to be appendicitis, which probably developed during induction chemotherapy but did not result in typical abdominal pain. Patients with recurrent fever and MP should be evaluated by imaging examinations including computed tomography to search for appendicitis.

Key Words: appendicitis, meralgia paresthetica, leukemia

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The incidence of appendicitis as a complication of treatment among the patients with hematologic malignancies has been reported to be 0.5% to 4.4%.[1 4] It was similar to the incidence of appendicitis in the general pediatric population. In children with leukemia, the abdominal tenderness and signs of peritoneal irritation (rebound tenderness, involuntary guarding, and abdominal wall rigidity), usually caused by appendicitis, are frequently blunted by the administration of chemotherapeutic agents including corticosteroids.[5] Neutropenia may also play a role in the atypical clinical presentation of appendicitis in this population, especially the vague nature of symptoms and the lack of localized pain.[6] The atypical presentation may result in delayed diagnosis of appendicitis in children with leukemia, which can lead to severe complications such as abscess formation, peritonitis, sepsis, and death.[1]

Meralgia paresthetica (MP) is caused by mononeuropathy of the lateral femoral cutaneous nerve. Patients with MP have disabling or distracting pain, burning, numbness, and decreased sensation over the anterolateral thigh.[7] MP is usually caused by mechanical compression such as tight clothing, obesity, pregnancy, or an abdominal mass.[8] Here we report an unusual case of MP caused by appendicitis in a girl with acute lymphoblastic leukemia (ALL).

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CASE REPORT

A 7-year-old girl was diagnosed with Philadelphia chromosome-positive (Ph +) ALL based on bone marrow and chromosome analysis. She had no family history of malignancy and no significant previous medical history.

The patient received multidrug induction chemotherapy with prednisolone, vincristine, cyclophosphamide, daunorubicin, and L-asparaginase. A bone marrow aspirate smear on day 15 demonstrated no blasts. On day 17 of induction chemotherapy, she developed vague pain in her right leg. Her white blood cell (WBC) count was 1.36×10^9/L with an absolute neutrophil count of 0.65×10^9/L. On day 23, she developed fever and hematochezia. Her WBC count was 9.09×10^9/L (absolute neutrophil count 8.1×10^9/L) and C-reactive protein level was 14 mg/L. She did not complain of abdominal pain. She was diagnosed with acute enterocolitis and received tazobactam/piperacillin. Bacterial and fungal cultures of the blood and feces were negative. The following day, her C-reactive protein level was elevated to 78.8 mg/L. Her fever and other symptoms resolved 5 days after the initiation of antibiotic therapy, and postinduction chemotherapy for Ph + ALL was continued.

During the first consolidation therapy, she became febrile and complained of pain, numbness, and tingling of the anterior and lateral aspects of her right thigh. She had no abdominal pain, abdominal tenderness, rebound tenderness, or muscular guarding. Her WBC count was 0.1×10^9/L and C-reactive protein level was 49 mg/L. She was diagnosed with febrile neutropenia. After administration of tazobactam/piperacillin and neutrophil recovery, her fever and thigh pain resolved. The peak value of C-reactive protein was 150.3 mg/L 5 days after the onset of febrile neutropenia. Bacterial blood cultures were negative. The same pattern of symptoms recurred during each cycle of chemotherapy, with resolution after administration of antibiotics and neutrophil recovery. However, she developed increasing difficulty with extension of her right knee and hip. Magnetic resonance imaging of her right knee did not show any abnormalities.

Five months after the initiation of chemotherapy, the patient underwent abdominal computed tomography (CT) to search for a focus of infection before allogeneic bone marrow transplantation (allo-BMT), which showed a periappendiceal abscess extending into the psoas muscle (Fig. 1). Because of the local abscess formation, appendiceal perforation was highly suspected. She underwent laparoscopic appendectomy and drainage of the abscess. Intraoperative exploration found appendicitis with a retroperitoneal abscess that extended from the appendix to the perirectal part of the abdominal wall. Histopathologic examination showed chronic inflammation of the appendix, with lymphocytic infiltration but no leukemic infiltration.

Cultures of the abscess fluid grew *Enterococcus faecium* and *Bacillus* species. Antibiotic therapy was administered for 19 days. The pain, numbness, and tingling of her anterolateral right thigh and the difficulty with extension of her right knee and hip resolved before she underwent allo-BMT. On the basis of her clinical course, the recurrent pain in her right thigh is thought to have been a symptom of MP. One month after her appendectomy surgery, she underwent allo-BMT for Ph + ALL on schedule. One year after allo-BMT, she remained in remission with no further MP.

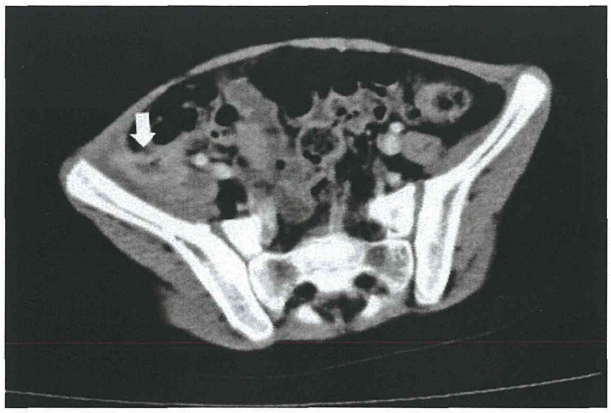

FIGURE 1. Enhanced abdominal computed tomography image showed a low-attenuation mass with enhancing rim at the back of the ascending colon (white arrow). It invaded the right psoas muscle and iliac muscle, and appeared to continue to the distal end of the appendix. These findings suggested retroperitoneal abscess due to appendiceal perforation.

DISCUSSION

Diagnosis of appendicitis is often difficult in patients with hematologic malignancies because the typical clinical signs and symptoms may be masked. Localized right lower quadrant abdominal tenderness was reported to occur in only 3 of 7 patients with appendicitis and hematologic malignancy, and 2 of the 7 patients did not have abdominal pain.[3] Our patient also did not complain of abdominal pain.

The difficulty of diagnosis may result in delayed treatment of appendicitis in children with leukemia. Hobson et al[3] reported that in patients with hematologic malignancy and appendicitis, the median time from the onset of symptoms to surgery was 9 days (range, 0.3 to 24 d). Kim et al[9] reported that in patients with leukemia and appendicitis, the median time from the onset of symptoms to diagnosis was 4 days (range, 1 to 9 d). In our patient, it took 5 months to obtain a definitive diagnosis of appendicitis, probably because she did not have distinctive features of appendicitis, and the symptoms of MP always improved soon after administration of antibiotics. To the best of our knowledge, only 1 case of MP due to appendicitis has previously been reported, in an otherwise healthy patient. In that case, the patient had occasional episodes of low-grade fever in addition to MP, and appendicitis was diagnosed by abdominal ultrasonography after 10 months.[10] The clinical courses of that patient and of our patient indicate that patients with MP and prolonged fever should undergo imaging examinations including enhanced CT to search for appendicitis.

MP is an entrapment syndrome of the lateral femoral cutaneous nerve that manifests as paresthesia, pain, numbness, or sensory loss in the distribution of the nerve.[8] MP occurs when the lateral femoral cutaneous nerve is compressed by mechanical causes such as tight clothes, obesity, pregnancy, or an abdominal mass.[8] In the previously reported patient described above, MP may have been caused by inflammation of the appendix, because no adhesions were detected between the appendix and adjacent structures.[10] In our patient, MP resolved with antibiotic therapy, suggesting that the symptoms were caused by acute inflammation rather than the periappendiceal abscess. The symptoms recurred when she developed neutropenia secondary to chemotherapy, and was therefore more prone to severe infection. We consider that her MP was caused by inflammation, although this cannot be definitively determined.

It is not always easy to identify the cause of leg pain in children with hematologic malignancies because leg pain can be caused by the primary disease, drug toxicity, bone fracture due to reduced bone mineral density, and muscle atrophy because of prolonged bed rest. However, appendicitis may also cause leg pain. Patients who present with recurrent fever and MP should be investigated with imaging examinations including enhanced CT to search for appendicitis, even if there are no abdominal symptoms.

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Efficacy of prophylactic additional cranial irradiation and intrathecal chemotherapy for the prevention of CNS relapse after allogeneic hematopoietic SCT for childhood ALL

Fukano R, Nishimura M, Ito N, Nakashima K, Kodama Y, Okamura J, Inagaki J. Efficacy of prophylactic additional cranial irradiation and intrathecal chemotherapy for prevention of CNS relapse after allogeneic hematopoietic SCT for childhood ALL.

Abstract: We evaluated the efficacy of CRT and IT chemotherapy, in addition to conditioning including TBI, for the prevention of CNS relapse, in allogeneic HSCT for childhood ALL. From January 1999 to December 2009, a total of 48 patients, without previous or presenting CNS involvement, underwent HSCT for ALL. All patients received myeloablative conditioning including TBI of 12 or 13.2 Gy and IT chemotherapy twice between days −10 and −2 prior to HSCT. Twenty-five patients received CRT prior to TBI (CRT+), and 23 patients did not (CRT−). CRT+ and CRT− patients had a seven-yr EFS rate of 40.0 ± 9.8% and 41.7 ± 10.6%, respectively (p = 0.8252). The seven-yr relapse rates for CRT+ and CRT− patients were 45.0 ± 11.2% and 38.4 ± 11.6%, respectively (p = 0.7460). CNS relapses were evident in 1 (4.0%) CRT+ patient and 1 (4.4%) CRT− patient (p = 1.000). There were no significant differences in EFS and the probability of CNS relapse between CRT+ and CRT− patients. These results demonstrate that CRT and IT chemotherapy, in addition to conditioning chemotherapy, may not be necessary in childhood ALL patients without previous or presenting CNS involvement.

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Key words: acute lymphoblastic leukemia – children – stem cell transplantation – cranial irradiation – intrathecal chemotherapy – central nervous system relapse

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Introduction

Childhood ALL has been proven to be curable in most patients. Although approximately 80–85% of childhood ALL can be cured with risk-based chemotherapy treatment, approximately 15–20% of patients experience disease relapse. This occurs in the testicles and CNS in 2–3% of these cases at first-line chemotherapy (1, 2). With intensive chemotherapy and allogeneic HSCT, 30–50% of relapsed childhood ALL can be cured. The probability of further relapse after HSCT for relapsed ALL in children has been estimated to be 20–30% (1, 3–5). In addition, a few studies have focused on the probability of CNS relapse in ALL after HSCT, and it is thought to range from 2 to 5.5% (6–9).

To prevent CNS relapse after HSCT, investigators have added prophylactic CRT to boost the conditioning regimen or IT chemotherapy before and after HSCT (6, 10–13). However, the role of these treatment strategies in preventing CNS relapse remains uncertain. To reduce CNS relapse and improve the prognosis for ALL, we

Abbreviations: ALL, acute lymphoblastic leukemia; ANC, absolute neutrophil count; BCP, B-cell precursor; BM, bone marrow; BU, busulfan; CA, cytarabine; CB, cord blood; CNS, central nervous system; CR, complete remission; CRT, cranial irradiation; CSF, cerebrospinal fluid; EFS, event-free survival; GVHD, graft-versus-host disease; HDC, hydrocortisone; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; IT, intrathecal; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; OS, overall survival; PB, peripheral blood; TBI, total body irradiation; VOD, veno-occlusive disease.

employed a pre-HSCT CRT boost, in addition to TBI and IT chemotherapy. The purpose of this retrospective analysis was to evaluate the efficacy and safety of these treatment modalities for the prevention of CNS relapse after allogeneic HSCT for childhood ALL.

Materials and methods

Patient characteristics and transplant procedure

From January 1999 to December 2009, a total of 72 patients with ALL underwent HSCT at the Department of Pediatrics, National Kyushu Cancer Center. The indications for allogeneic HSCT for ALL were history of first or subsequent relapse, unfavorable cytogenetics including *t*(9;22) or *t*(4;11), and induction failure. Infant ALL was excluded from this analysis as children aged <1 yr did not receive CRT or TBI. Patients who had previous or presenting CNS involvement and a history of prior HSCT were also excluded; therefore, 48 patients were analyzed retrospectively. The patient characteristics are shown in Table 1. The median age at HSCT was 8.6 (range: 2–24 yr) yr. The ALL immunophenotypes were BCP, T cell, and mature B cell. Forty-one of the 48 (85%) patients were BCP-ALL, and 11 of these patients were Philadelphia-positive ALL. Thirty-two patients had achieved CR at HSCT, and 16 patients had active disease. The sources of stem cells were BM in 36 patients, CB in nine patients, and PB in three patients. HLA-MRDs were used for 14 patients, and 19 patients received stem cells from HLA-MUDs. HLA-mismatched donors were used in 15 patients.

Table 1. Patient characteristics

	CRT+ patients	CRT− patients	p-Value
Number of patients	25	23	
Age at HSCT (yr)			0.933
Median	7.8	9.3	
Range	2.1–24.3	2.2–18.1	
Sex			0.040
Male	18	9	
Female	7	14	
Phenotype			0.401
BCP (Ph+)	22 (7)	19 (4)	
T cell	3	2	
Mature B	0	2	
WBC count at diagnosis			0.971
≥50 000	9	9	
<50 000	15	13	
Unknown	1	1	
Disease status at HSCT			1.000
CR1/CR2/CR3	5/11/1	3/9/3	
Non-CR	8	8	
Stem cell source			0.835
BM	18	18	
CB/PB	5/2	4/1	
Donor			0.893
MRD	7	7	
MUD	10	9	
MMRD	3	4	
MMUD	5	3	
Conditioning			0.162
TBI ± others	22	16	
TBI/BU ± others	3	7	

Ph+, Philadelphia chromosome positive; WBC, white blood cell; MMRD, mismatched related donor; MMUD, mismatched unrelated donor.

Conditioning regimens

All 48 patients received myeloablative conditioning including TBI. The conditioning regimens were TBI ± others in 38 patients and TBI/BU ± others in 10 patients (Table 1). Up until 2004, TBI of 13.2 Gy was administered twice or thrice daily in 1.2 Gy fractions for a total of four days. From 2005, because of institutional changes, a new protocol was adopted of 12 Gy TBI in twice daily fractions of 2 Gy, for a total of three days. As a result, 29 patients received TBI of 13.2 Gy, and 19 patients received TBI of 12 Gy. All patients received TBI at a dose rate of 8 cGy/min using a 10-MV X-ray with laterally opposed fields. Lung shielding was used to limit the dose to 90% of the total, and eye shielding was also used to reduce the risk of cataracts.

CRT boost and IT chemotherapy

As shown in Table 1, 25 patients received a CRT boost (CRT+), and 23 patients did not (CRT−) because of prior CRT in their first-line treatment, history of leukoencephalopathy, other neurological complications, and poor performance status. The CRT boost was administered to the whole brain, not including the spine; a total of 6 Gy as once daily 2 Gy fractions or 4.8 Gy as once daily 1.6-Gy fractions for three days prior to TBI was administered. The CRT dose rate was 2 Gy/min using a 6-MV X-ray. Of the 25 CRT+ patients, 11 patients with a TBI of 13.2 Gy-containing conditioning received a 4.8-Gy CRT boost and 14 patients with a TBI of 12 Gy received a 6-Gy CRT boost; thus, the cumulative irradiation dose to the whole brain was 18 Gy. Fifteen of 48 patients (31%) had a history of prior cranial radiotherapy for prophylaxis. Although there were more male patients in the CRT+ group than in the CRT− group, there was no significant difference among the other patient characteristics between the CRT+ and CRT− groups (Table 1).

All of the 48 patients received double (CA and HDC) or triple (MTX, CA and HDC) IT chemotherapy twice between days −10 and −2 prior to HSCT. MTX dose was 12 mg/m^2 or 12.5 mg/body, CA dose was 30 mg/m^2 or 25 mg/body and HDC dose was 50 mg/m^2 or 25 mg/body. Ten patients received double IT chemotherapy, and 38 patients received triple IT chemotherapy. We did not adopt post-HSCT IT chemotherapy in any patient to avoid the risk of complications such as bleeding into the CSF and CNS infection following HSCT.

Assessments of engraftment, CNS relapse and GVHD

Engraftment was defined as the first day the ANC reached 0.5×10^9/L for three consecutive days within 28 days after transplantation. CNS relapse was defined as the presence of 0.005×10^9/L leukemic cells in the CSF or isolated cranial nerve palsy. The diagnosis and grade of acute GVHD were made on the basis of established clinical criteria (14). The onset forms and types of chronic GVHD were differentiated according to the published classification (15).

Statistical analysis

OS, EFS, and cumulative incidents of relapse were estimated by the Kaplan–Meier method. The *t*-test, χ^2-test, and

Fisher's exact test were used to test for differences in patient characteristics, probability of acute and chronic GVHD, second malignancy, treatment-related death, and relapse. The level of statistical significance was set at $p < 0.05$.

Results

Engraftment

Engraftment was observed in 25 (100%) of CRT + patients and 20 (87%) of CRT−. Three of 23 CRT− patients died of infection before engraftment. The median engraftment days of CRT+ and CRT− patients were 17 and 19 days, respectively.

GVHD and toxicity

Acute GVHD of grade II–IV and chronic GVHD occurred in 17 (68%) and 15 (60%) of CRT+ patients, respectively, and 10 (43%) and nine (39%) of the CRT− patients, respectively (Table 2). There were no significant differences in the incidence of acute GVHD of grade II–IV and chronic GVHD between the CRT+ ($p = 0.145$) and CRT− patients ($p = 0.248$).

There was no acute neurological complication such as leukoencephalopathy, cerebrovascular disease, or CNS infection in CRT+ patients and CRT− patients. Second malignancies occurred in two of the CRT− patients: a brain tumor (primitive neuroectodermal tumor) and a soft tissue tumor of the femur (malignant peripheral nerve sheath tumor). Treatment-related death occurred in seven CRT+ patients and in seven CRT− patients. Among CRT+ patients, five patients died of infectious complications and two patients died of VOD. Of the CRT− patients, six patients died of infectious complications and one patient died of a second malignancy.

Relapse

Overall relapse including BM and extramedullary relapse occurred in nine of 25 (36%) CRT+ patients and seven of 23 (30%) CRT− patients (Table 2), and median intervals from HSCT to relapse were 12 (range: 3–24 months) months and eight (range: 4–54 months) months, respectively. The seven-yr relapse rate for CRT+ and CRT− patients was 45.0 ± 11.2% and 38.4 ± 11.6%, respectively (Fig. 1). There was no significant difference in the cumulative incidence of relapse between CRT+ and CRT− patients ($p = 0.7460$).

CNS relapse was observed in one of the 25 (4.0%) CRT+ patients and in one of the 23 (4.4%) CRT− patients ($p = 1.000$). Both of these patients had active disease at HSCT and also had BM relapse (Table 2). One of these patients received a 6-Gy CRT boost and triple IT chemotherapy and relapsed in the CNS, BM and skin at 11 months after HSCT. The other patient received triple IT chemotherapy without CRT and relapsed in the CNS, BM and bone at seven months after HSCT.

Table 2. GVHD, toxicity, and relapse

	CRT+	CRT−	p-Value
Number of patients	25	23	
Acute GVHD	21 (84%)	14 (61%)	0.106
Grade II–IV	17	10	0.145
Chronic GVHD	15 (60%)	9 (39%)	0.248
Second malignancy	0 (0%)	2 (9%)	0.224
Treatment-related death	7 (28%)	7 (30%)	1.000
Infection	5	6	0.736
VOD	2	0	0.490
Relapse	9 (36%)	7 (30%)	0.765
BM	8	6	0.756
CNS	1	1	1.000

OS and EFS

The median follow-up period from HSCT to the last follow-up for all survivors was 73 (range: 25–142 months) months. CRT+ and CRT− patients had a seven-yr OS rate of 31.5 ± 11.9% and 46.3 ± 10.7%, respectively. The seven-yr EFS rate was 40.0 ± 9.8% and 41.7 ± 10.6% for CRT+ and CRT− patients, respectively (Fig. 2). There were no significant differences in OS ($p = 0.5563$) and EFS ($p = 0.8252$) between CRT+ and CRT− patients.

Discussion

Over the last two decades, the prognosis for childhood ALL has improved owing to the use of CRT as a first-line treatment, and it is consid-

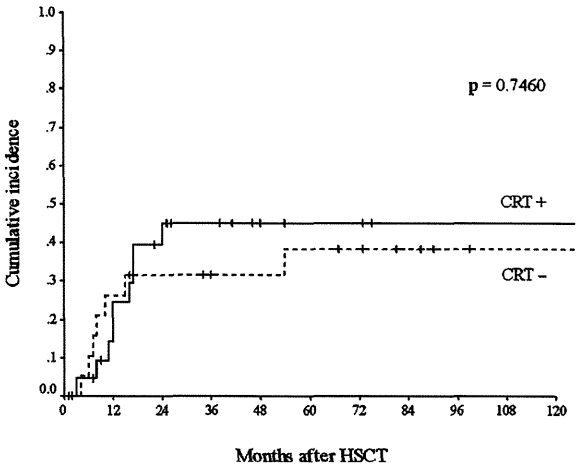

Fig 1. Cumulative incidence of relapse in CRT+ and CRT− patients. HSCT, hematopoietic SCT.

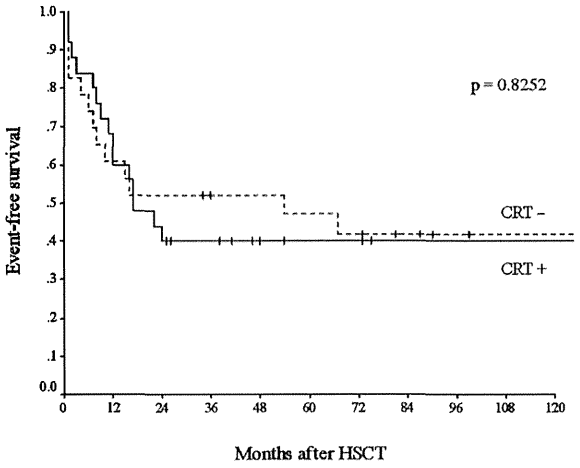

Fig 2. EFS of CRT+ and CRT− patients. HSCT, hematopoietic SCT.

ered necessary for high-risk patients, such as those with T-ALL and for Philadelphia-positive or CNS-positive cases (16–18). However, complications such as the risk of growth deficiency (19, 20), neuropsychological toxicity (21), and secondary neoplasms of the brain (22) are significantly higher in patients who receive CRT than in those who do not receive CRT. Systemic chemotherapy including high-dose MTX with IT chemotherapy instead of CRT has reduced the CNS relapse rate and improved the prognosis of standard-risk patients, and the CRT dose used in first-line treatment has been reduced. Increased use of both high-dose chemotherapy and IT chemotherapy that cross the blood–brain barrier has successfully replaced CRT, and CRT can be eliminated from first-line treatment entirely in standard-risk patients (16, 23–26).

The efficacy of CRT in addition to conditioning chemotherapy is uncertain in patients who have undergone HSCT for ALL. There are very few reports of prophylactic CRT for HSCT patients. In the present study, CRT in addition to TBI-containing myeloablative conditioning did not improve OS, EFS, or the probability of CNS relapse when compared to myeloablative conditioning without CRT. CNS relapse was observed in one (3.9%) CRT+ patient, and there was no significant difference compared with CRT− patients. This result supports the outcome of previous report (10) in which CRT in addition to TBI for ALL patients was not associated with reduction in CNS relapse especially in patients without presenting CNS involvement at HSCT. In addition, the probability of CNS relapse in CRT+ patients of our study was similar to that of previous reports which CRT boost was not administered before HSCT (8, 9, 13). Therefore, prophylactic CRT prior to TBI may not be necessary for ALL patients without previous or presenting CNS involvement. Because of the small number of patients in our study and isolated CNS relapse was not observed in either group, we need a larger patient cohort to evaluate the efficacy of prophylactic CRT for the prevention of CNS relapse after HSCT.

Seattle group (7) reported that the risk of post-HSCT CNS relapse in patients with ALL who received post-HSCT IT chemotherapy was lower than that in patients who did not receive this therapy. Recently, we (11) also reported the use of post-HSCT IT chemotherapy for CNS-involved ALL and non-Hodgkin's lymphoma and showed that allogeneic BM transplantation combined with post-HSCT IT chemotherapy was an effective treatment for patients with lymphoid malignancy and with CNS relapse. Conversely, the efficacy of pre-HSCT IT chemotherapy in patients without previous CNS involvement is unclear. Some reports suggest that there is no benefit from pre- or post-HSCT IT chemotherapy for ALL patients, especially in those without prior CNS involvement (6, 12, 13). However, a high risk of leukoencephalopathy after post-HSCT IT chemotherapy has been documented, and the risks of bleeding into the CSF and CNS infection should also be considered (7). To avoid the risk of these complications for post-HSCT IT chemotherapy and to prevent CNS relapse after HSCT, double or triple IT chemotherapy was given to ALL patients twice between days −10 and −2 prior to HSCT in our institution, and CNS relapse was only observed in one CRT− patient. Therefore, it would seem that pre-HSCT IT chemotherapy is not essential to prevent CNS relapse, particularly in patients without previous or presenting CNS involvement.

In the present study, there was no acute neurotoxicity, including leukoencephalopathy, cerebrovascular disease, and CNS infection, in CRT+ and CRT− patients. Additional CRT and two rounds of IT chemotherapy before HSCT did not increase the risk of these complications and was well tolerated by the patients in this study. Generally, acute neurotoxicity in patients receiving HSCT is a rare event. For example, a previous report revealed that the incidence of leukoencephalopathy after HSCT with either prophylactic or therapeutic CRT and/or IT chemotherapy was 3.8% (7). Therefore, a larger number of patients are necessary to compare the difference in acute neurotoxicity between the two groups with and without a CRT boost. In CRT− patients, two cases of second malignancy were observed. Although there was no second

malignancy in CRT+ patients, long and careful follow-up is required as its incidence will continue to increase, even 10–20 yr after HSCT. Moreover, neurodevelopmental outcomes and endocrine function are the most important considerations for the evaluation of late toxicities of HSCT with additional CRT and pre-HSCT IT chemotherapy, but we did not have sufficient data to evaluate these factors in this retrospective analysis. Further observations are needed to evaluate the late toxicity of additional CRT.

Finally, there were no significant differences in EFS and the probability of CNS relapse between CRT+ and CRT− patients. Because of the small number of patients in this retrospective analysis and the lack of statistical power in each subgroup, our data do not allow any definite conclusions regarding the efficacy of additional CRT at HSCT and pre-HSCT IT chemotherapy. However, this study suggests that CRT in addition to conditioning chemotherapy and pre-HSCT IT chemotherapy may not be necessary for patients without previous or presenting CNS involvement. Further evaluation of these treatment strategies with a larger number of patients is required, and multicenter analysis may be helpful in providing a more definitive answer to this question.

Conflicts of interest

There are no conflicts of interest to declare.

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