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# Improved Treatment Results of Children With B-Cell Non-Hodgkin Lymphoma: A Report From the Japanese Pediatric Leukemia/Lymphoma Study Group B-NHL03 Study

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***Background.*** Previous Japanese studies of childhood B-cell non-Hodgkin lymphoma (B-NHL) have shown a favorable outcome, though the study size was too small to effectively assess the efficacy and safety of treatment for childhood B-NHL. ***Procedure.*** We performed a nation-wide prospective B-NHL03 study to assess the efficacy and safety of short-pulse intensive chemotherapy for children with B-NHL. They were stratified into four treatment groups according to disease stage, tumor resectability and bone marrow/CNS involvement: Group 1 with all resected stage I/II, Group 2 with non-resected stage I/II, Group 3 with stage III & CNS-negative stage IV, and Group 4 with CNS-positive stage IV & Burkitt leukemia. Treatment duration was 2 courses for Group 1, 4 courses for Group 2, and 6 courses for Groups 3 and 4, respectively. CNS irradiation was omitted in all patients. ***Results.*** The follow-up time ranged from 0.8 to 88 months, with a median of being 45 months. For 321 patients analyzed in this study, overall survival and event-free survival (EFS) at 4 years was 92.7% and 87.4%, respectively. The 4-year EFS according to treatment group were 94% for Group 1 (n = 17), 98% for Group 2 (n = 103), 84% for Group 3 (n = 111), and 78% for Group 4 (n = 90). There was no significant difference in outcome by histology. Therapy-related death occurred in three patients in remission. ***Conclusions.*** Our nationwide large-scale study resulted in a cure rate above 90% with <1% toxic death in childhood B-NHL. Pediatr Blood Cancer 2014;61:1215–1221.


**Key words:** B-NHL03; childhood; JPLSG; non-Hodgkin lymphoma

## INTRODUCTION

Childhood B-cell non-Hodgkin Lymphoma (B-NHL) consists mainly of two histological subtypes, namely Burkitt lymphoma (BL), which includes Burkitt leukemia (B-ALL), and diffuse large B-cell lymphoma (DLBCL). The cure rate of childhood BL has been markedly improved over the past 30 years, and long-term event-free survival (EFS) of patients has reached to approximately 90%. This is largely due to prospective studies of European and North American groups that developed a short intensive chemotherapy regimen, including a high-dose methotrexate (HDMTX), an intermediate dose of cyclophosphamide (CPA), and anthracyclines [1–6]. Although DLBCL is a distinct disease entity from BL, the treatment is the same as that for patients with Burkitt histology, and excellent outcome has been reported [1–6]. Previously most clinical experiences of childhood B-NHL were reported by European and North American study groups, and there were few data on Japanese or Asian patients with B-NHL. In the1990s, we conducted group-wide trials for childhood B-NHL [7–10]: Horibe et al. showed a 4-year EFS with 70% for 57 patients (BL 31, B-ALL 17, DLBCL 9) [8], Kikuchi et al. showed a 6-year EFS with 82% for 91 patients (BL 45, B-ALL 9, DLBCL 26, others 11) [10], and Tsurusawa et al. showed a 7-year EFS with 93% for 30 patients with DLBCL [9]. In addition, Lee et al. has recently shown a 5-year EFS with 95% for 61 patients (BL 46, DLBCL 15) [11]. However, the treatment duration of these studies was relatively long and the number of patients was small compared to the European and North American studies [1–6].

Here, we report on the results of the nation-wide large prospective study for children with B-NHL. The primary object was to evaluate the efficacy and safety of short-pulse intensive chemotherapy regimen designed by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG).

## PATIENTS AND METHODS

### Study Design and Diagnostic Criteria

The B-NHL03 study was a prospective nonrandomized trial that investigated the efficacy and safety of short-pulse intensive chemotherapy in childhood B-NHL. The chief aim was to improve the outcomes of patients enrolled in the B-NHL03 study to the level of those of European and North American studies.

Additional Supporting Information may be found in the online version of this article.

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The diagnosis of B-NHL was based on histopathology, immunocytochemistry, and cytogenetics. All histopathological specimens were first classified by the institutional pathologist and finally each of them were reviewed by a group of seven pathologists of a central pathological review committee according to WHO classification, that is, BL or Burkitt-like lymphoma (BLL), DLBCL, mediastinal large B-cell lymphoma (MLBCL), and mature B-cell neoplasm, NOS (not otherwise specified) [12]. A mature B-cell phenotype was primarily defined as positive for C20 and/(or) CD79a and negative for CD3 and terminal deoxynucleotidyl transferase. When an immunophenotype study was not available, specific translocations t(8;14)(q24;q32), t(2;8)(p11;q24), t(8;22)(q24;q11) at cytogenetic analysis were included. CNS involvement was diagnosed by the presence of one or more of the following: any blasts with FAB L3 morphology in CSF, isolated intracerebral mass, or intra-spinal extension. The clinical stage was defined by Murphy's classification [13].

## Treatments

The treatment outline is shown in Figure 1 and chemotherapy regimens are shown in Table I. They were stratified into four treatment groups according to disease stage, tumor resectability and bone marrow/CNS involvement: Group 1 with all resected stage I/II, Group 2 with non-resected stage I/II, Group 3 with stage III & CNS-negative stage IV, and Group 4 with CNS-positive stage IV & B-ALL. All groups except Group 1 received a pre-phase therapy of prednisolone (PSL), vincristine (VCR), CPA and it (intrathecal) MTX to reduce tumor volume. As shown in Figure 1, Group 1 received two courses (1A × 2), Group 2 received 4 courses (2A × 2 + 2B × 2), Group 3 received 6 courses (3A × 4 + 3B × 2), and Group 4 received 6 courses (4A1 × 2 + 4A2 × 2 + 4B × 2), respectively. No patients received prophylactic cranial irradiation. Patients with CNS involvements received HDMTX (5 g/m$^2$) plus an extended it regimen (14 times), but no therapeutic cranial irradiation. The schedule of HDMTX administration was identical

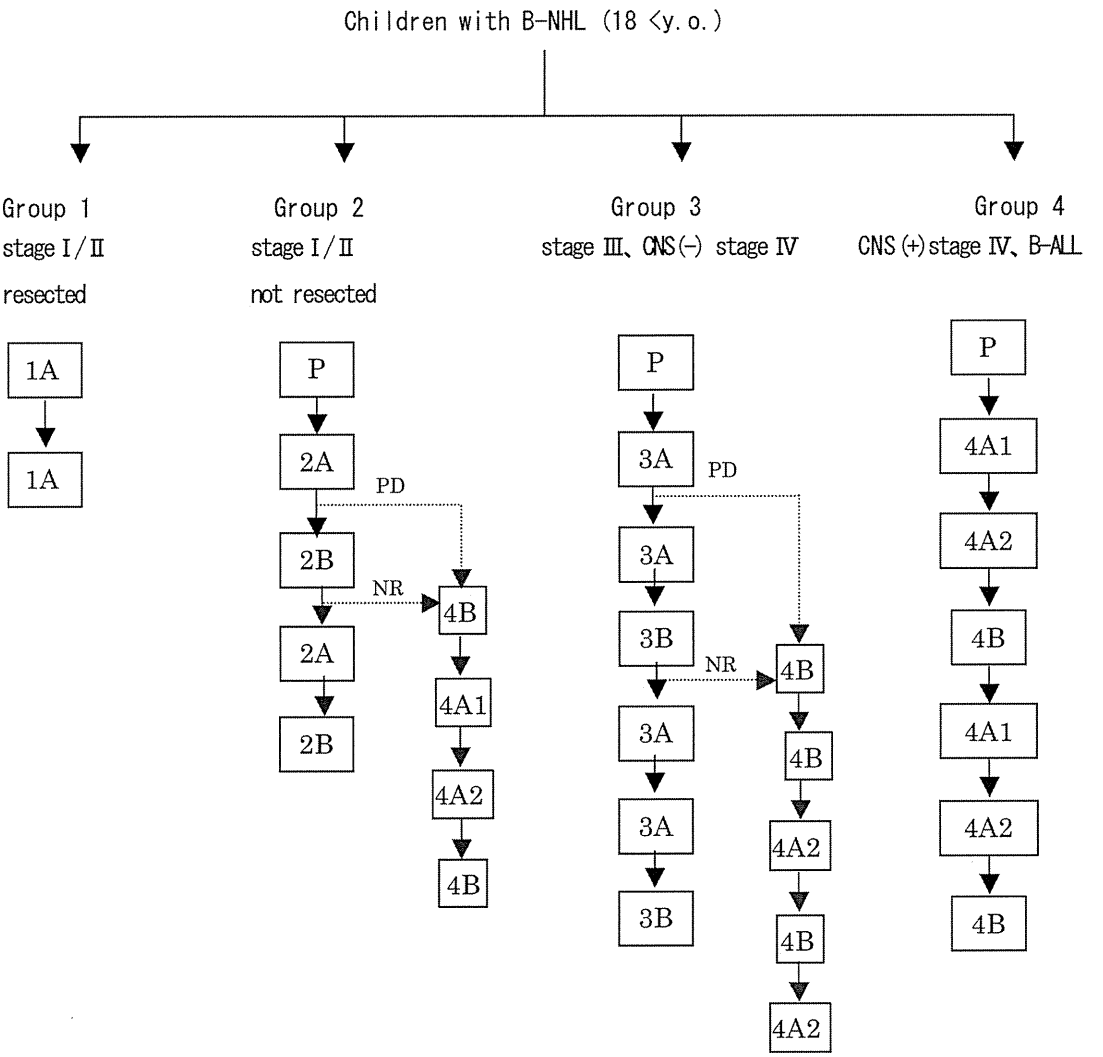


**Fig. 1.** Treatment framework of the B-NHL03 study. Patients were stratified into four treatment groups according to disease stage, tumor resectability, and BM/CNS involvement. All groups except Group 1 received pre-phase therapy. Group 1 received two courses of chemotherapy, Group 2 received 4 courses, Groups 3 and 4 received 6 courses, respectively. When patients in Group 2 or 3 did not achieve CR or CRu during the first 2 or 3 courses, they received salvage therapy consisting of 4B and 4A1/2 courses.

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**TABLE I. B-NHL03 Treatment Schedules**

| Regimen | Administration | Daily dose | Days |
|---|---|---|---|
| Pre-phase | | | |
| Prednisolone | Orally | 30 mg and 60 mg/m$^2$ | Days 1–3 and 4–7 |
| Vincristine | IV | 1 mg/m$^2$ | Day 3 |
| Cyclophosphamide | IV | 150 mg/m$^2$ | Days 4–6 |
| Methotrexate | TIT | 12 mg/m$^2$ | Day 1, (4)[a] |
| Hydrocortisone | TIT | 25 mg/m$^2$ | Day 1, (4)[a] |
| Cytarabine | TIT | 30 mg/m$^2$ | Day (4)[a] |
| Regimen 1A | | | |
| Prednisolone | Orally | 60 mg/m$^2$ | Days 1–5 |
| Methotrexate | IV | 1 g/m$^2$ | Day 1 |
| Vincristine | IV | 1.5 mg/m$^2$ | Day 2 |
| Cyclophosphamide | IV | 250 g/m$^2$ × 2 | Days 2–4 |
| THP-adriamycin | IV | 30 mg/m$^2$ | Days 3, 4 |
| Methotrexate | DIT | 12 mg/m$^2$ | Day 1 |
| Hydrocortisone | DIT | 25 mg/m$^2$ | Day 1 |
| Regimen 2A | | | |
| Same as 1A except for dexamethasone | Orally | 10 mg/m$^2$ | Days 1–7 |
| Methotrexate | IV 24 hours with LV rescue | 3 g/m$^2$ | Day 1 |
| Regimen 3A | | | |
| Same as 2A except for *t.i.t* at day 1 | | | |
| Regimen 4A1 | | | |
| Same as 3A except for methotrexate | IV 24 hours with LV rescue | 5 g/m$^2$ | Day 1 |
| Methotrexate | TIT | 12 mg/m$^2$ | Day 1, (5),[a] 8 |
| Hydrocortisone | TIT | 25 mg/m$^2$ | Day 1, (5),[a] 8 |
| Cytarabine | TIT | 30 mg/m$^2$ | Day 1, (5),[a] 8 |
| Regimen 4A2 | | | |
| Same as 4A1 except for cyclophosphamide | IV | 1 g/m$^2$ | Days 4, 5 |
| Regimen 2B | | | |
| Methotrexate | IV 6 hours | 500 mg/m$^2$ | Day 1 |
| Cytarabine | cIV | 150 mg/m$^2$ | Days 1–5 |
| Methotrexate | DIT | 12 mg/m$^2$ | Day 1 |
| Hydrocortisone | DIT | 25 mg/m$^2$ | Day 1 |
| Regimen 3B | | | |
| Same as 2B except for TIT at day 1, and cytarabine | cIV | 150 mg/m$^2$ | Days 1–6 |
| Etoposide | IV | 100 mg/m$^2$ × 2 | Days 3–5 |
| Regimen 4B | | | |
| Same as 3B except for without methotrexate, DIT at day 1 and TIT at day 8, and dexamethasone | Orally | 10 mg/m$^2$ | Days 1–7 |
| Cytarabine | IV | 2 g/m$^2$ × 2 | Days 2–4 |
| Etoposide | IV | 150 mg/m$^2$ | Days 2–5 |
| Vincristine | IV | 1.5 mg/m$^2$ | Day 1 |

LV, leucovorin; IV, intravenous; cIV, continuous intravenous; DIT, double intrathecal; TIT, triple intrathecal. [a]For CNS positive patients.

to that of the B-NHL960 study [9]: HDMTX was administered for the first 24 hours, and 12 hours later, leucovorin (LV) 15 mg/m$^2$ was given orally every 6 hours, for a total of seven doses [9]. Blood MTX concentration was measured 24, 48, and 72 hours after the MTX administration. When patients showed delayed MTX clearance ($\geqq$0.2 μM after 72 hours), LV rescue was continued until MTX concentration level decreased to less than 0.2 μM.

Induction failure (IF) was defined as patients who did not achieve complete remission (CR) or unconfirmed remission (CRu) until the last evaluation time (before the second course of 2A in Group 2, before the third course of 3A in Group 3, before the second course of 4A1 in Group 4). When patients in Group 2 or 3 were evaluated to have progressive disease or no response during the first 2 or 3 courses, they received salvage therapy consisting of regimens 4B and 4A1/2. The cumulative dose of cytotoxic drugs for treatment groups was as follows: CPA 3 g/m$^2$, THP 120 mg/m$^2$ for Group 1; CPA 3.45 g/m$^2$, THP 120 mg/m$^2$ for Group2; CPA 6.45 g/m$^2$, THP 240 mg/m$^2$, VP16 0.6 g/m$^2$ for Group 3; CPA 7.45 g/m$^2$, THP 240 mg/m$^2$, VP16 1.2 g/m$^2$ for Group 4.

## Statistical Analysis

Final statistical analyses were performed based on data obtained in June 2012. Overall survival (OS) was defined as the time between diagnosis and death from any causes, and EFS was defined as the time to first events defined as an occurrence of induction failure, relapse at any site, death from any causes, or second malignant neoplasm. For patients who did not experience an event, EFS was defined as the time to the last follow-up. Survival curves were prepared using the Kaplan–Meier method and standard errors (SEs) with the Greenwood formula. The significance of differences in survival outcomes was determined by means of the log-rank test.

STATA® statistical analysis software (version 11.0; StataCorp LP, College Station, TX) was used for all computations.

## RESULTS

### Patients

The protocol was conducted in 112 hospitals of the JPLSG after approval by each institution's review board, and written informed consent was provided by patients or legal guardians before treatment. Between November 2004 and January 2011, 346 cases of newly diagnosed B-NHL were enrolled in this study. Of these, 25 cases were excluded: 14 due to ineligible pathology, 8 for late enrollment, 2 for ineligible clinical stage, and 1 for prior chemotherapy. A total of 321 cases of four treatment groups were analyzed (Fig. 2).

Patient characteristic are shown in Table II. There were few protocol deviations: 10 patients in the Group 3/4 skipped or postponed HDMTX therapy in the A course, 5 because of retention of ascites or pleural effusion, 2 because of renal dysfunction, 2 due to septic infection, and one for stomatitis.

### EFS and OS

The follow-up time ranged from 0.8 to 88 months, with a median 47 months. For the 321 patients analyzed in this study, 4-year OS was 92.7% ± 1.4% and 4-year EFS was 87.3% ± 1.8% (Fig. 3A). There was no significant difference in outcome by gender (4-year EFS, male 87.5% ± 2.2% vs. female 87.0% ± 3.8%, $P = 0.864$). The 4-year OS and EFS according to treatment subgroup were 100% and 94.1% ± 5.7% for Group 1, 100% and 98.6% ± 1.4% for Group 2, 93.6% ± 2.3% and 83.6% ± 3.5% for Group 3, and 82.1% ± 4.1% and 77.8% ± 4.4% for Group 4 (Fig. 3B). The 4-year OS and EFS according to clinical stage were 100% and 97.7% ± 2.3% for stage I, 100% and 97.8% ± 2.0% for stage II, 92.0% ± 2.9% and 82.9% ± 4.0% for stage III, 84.6% ± 5.8% and 71.8% ± 7.2% for stage IV. The 4-year OS and EFS of B-ALL were 86.2% ± 4.0% and 83.6% ± 4.3%. The 4-year EFS by histology was 86.1% ± 2.6% for BL/BLL, 87.3% ± 3.5% for DLBCL, 92.1% ± 4.3% for others, and 100% for MLBCL ($P = 0.717$) (Fig. 3C). When we analyzed the outcome of patients who had BM or CNS disease, the 4-year EFS was 83.8% ± 4.3% for patients (n = 74) with BM involvement only (BM+/CNS−), 60.0% ± 1.5%

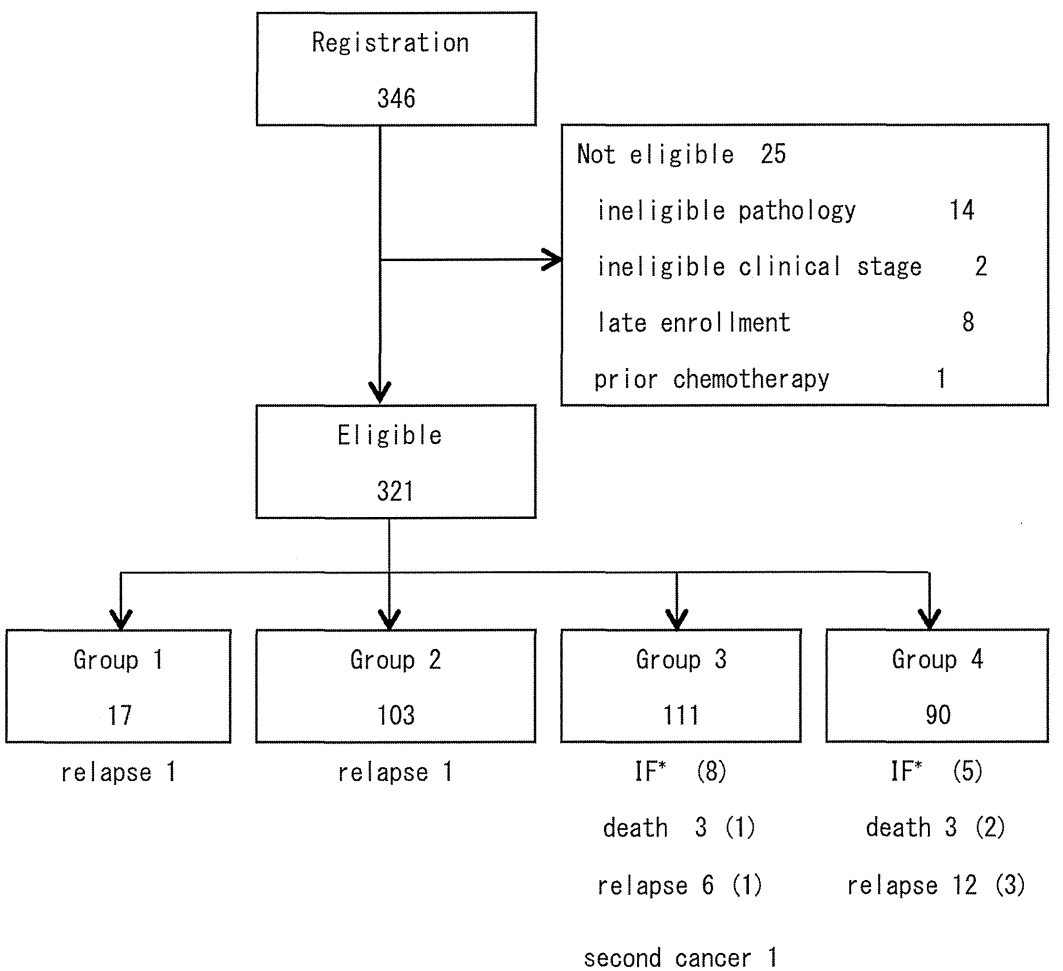


**Fig. 2.** Patient flow chart and events according to the treatment group. There were 40 events which consisted of each one in Group 1 and 2, 18 in Group 3, and 20 in Group 4. Number in parentheses indicates events occurred during protocol chemotherapy. Number in parenthesis indicates events occurred during protocol chemotherapy. *IF, induction failure defined as patients did not achieve complete remission or unconfirmed remission at the last evaluation time in group 3/4.

**TABLE II. Patients Characteristics**

| Therapy groups | G1 | G2 | G3 | G4 | Total (%) |
|---|---|---|---|---|---|
| No. of patients | 17 | 103 | 111 | 90 | 321 |
| Sex | | | | | |
| Male | 12 | 72 | 90 | 71 | 245 (76) |
| Female | 5 | 31 | 21 | 19 | 76 (24) |
| Age | | | | | |
| 0–4 | 2 | 12 | 18 | 16 | 48 (15) |
| 5–9 | 3 | 45 | 42 | 39 | 129 (40) |
| 10–14 | 8 | 42 | 42 | 27 | 119 (37) |
| 15– | 4 | 4 | 9 | 8 | 25 (8) |
| Histology | | | | | |
| BL/BLL/B-ALL | 5 | 33 | 62 | 80 | 180 (56) |
| DLBCL | 12 | 58 | 26 | 5 | 101 (31.4) |
| MLBCL | 0 | 0 | 2 | 0 | 2 (0.6) |
| Others | 0 | 12 | 21 | 5 | 38 (12) |
| Primary sites | | | | | |
| Thorax | 5 | 30 | 7 | 1 | 43 |
| Head & neck | 5 | 39 | 12 | 2 | 58 |
| Peripheral lymph nodes | 0 | 3 | 3 | 0 | 6 |
| Abdomen | 7 | 29 | 75 | 11 | 122 |
| Mediastinum | 0 | 0 | 8 | 0 | 8 |
| B-ALL | 0 | 0 | 0 | 73 | 73 |
| CNS | 0 | 0 | 0 | 2 | 2 |
| Other tumor site | 0 | 2 | 5 | 0 | 7 |
| Not specified | 0 | 0 | 1 | 1 | 2 |
| BM involvement | 0 | 0 | 22 | 80 | 102 (32) |
| CNS involvement | 0 | 0 | 0 | 38 | 38 (12) |

BL, Burkitt lymphoma; BLL, Burkitt-like lymphoma; B-ALL, Burkitt leukemia; DLBCL, diffuse large B-cell lymphoma, MLBCL, mediastinal large.

for patients (n = 10) with CNS involvement only (BM−, CNS+), and 75.0% ± 8.2% for patients (n = 28) with BM and CNS involvements (BM+/CNS+), ($P = 0.102$) (Fig. 3D). Outcome by treatment response to initial A courses were as follows: The 4-year OS and EFS for patients who achieved CR (n = 236) or CRu (n = 54) at the last evaluation time were 95.7% ± 1.6% and 93.5% ± 1.6%, and 96.1% ± 2.7% and 86.9% ± 4.6%, respectively, while the 4-year OS and EFS for patients (n = 13) who did not achieve CR/CRu was 69.2% ± 12.8% and 15.4% ± 10.1% ($P < 0.001$), respectively.

## Treatment Failure Events

Forty patients experienced an event and 25 have died (Fig. 2). The cause of death was tumor progression in 14, infection in 7, stem cell transplantation-related death in 3, and pulmonary bleeding in 1. The 40 events consisted of 13 induction failures, 6 deaths, 20 relapses, and one second cancer. Of the13 patients (6 in Group 3 and 7 in Group 4) who failed the initial treatment, 4 patients in Group 3 received salvage therapy and achieved CRu. At the time of the last analysis, 8 patients (4 in Group 3 and 4 in Group 4) were alive without tumor. Death in remission occurred in 3/321 (1%) patients: two died of infection and one died of pulmonary bleeding. The longest duration before relapse from the start of therapy was 38.9 months in DLBCL and 13.6 months in Burkitt histology. Relapse sites were 10 in local, 6 in BM, 2 in BM+CNS, one in local + CNS, and one in CNS. All CNS relapse occurred in patients with BL, but not with DLBCL. Thus, isolated CNS failure was only one among 38 patients with CNS involvement. Of the 20 relapsed patients, 11 died and 9 survived without tumor. A second cancer occurred among the patients who failed the initial treatment: a 12-year-old male with BL developed a secondary malignancy with acute myeloid leukemia (FAB M5) 17 months after the initial diagnosis.

## Toxicity

Acute toxicity of treatment courses (A and B) was evaluated by the scale of NCI-CTC version 2.0., and rates of acute toxicity Grade 3 among patients in Groups 2, 3, and 4 are shown in Supplemental Table I. Anemia and neutropenia were the most frequent hematological toxicities with grade III or IV in all groups. In particular, grade IV neutropenia occurred in almost all patients (>98%) during A courses. In nonhematologic toxicity, infection was the single most frequent occurring with grade III or IV at least once in 70% of patients although the rate of grade IV infection was very small (<1%). Stomatitis and hepatotoxicity were also frequent, occurring with grade III or IV at least once in 20–35% and 24–38% of patients, respectively. The rate of renal toxicity grade III was very low. Leukoencephalopathy was reported in two patients of Group 3, and their MRI findings disappeared within 2 months without neurological symptoms. The overall incidence of renal insufficiency associated with tumor lysis syndrome was 2 out of 96 (2%) in Group 4, and these required assisted renal support with continuous hemodiafiltration.

# DISCUSSION

During the last two decades, the survival outcome of children with B-NHL has been markedly improved through consecutive

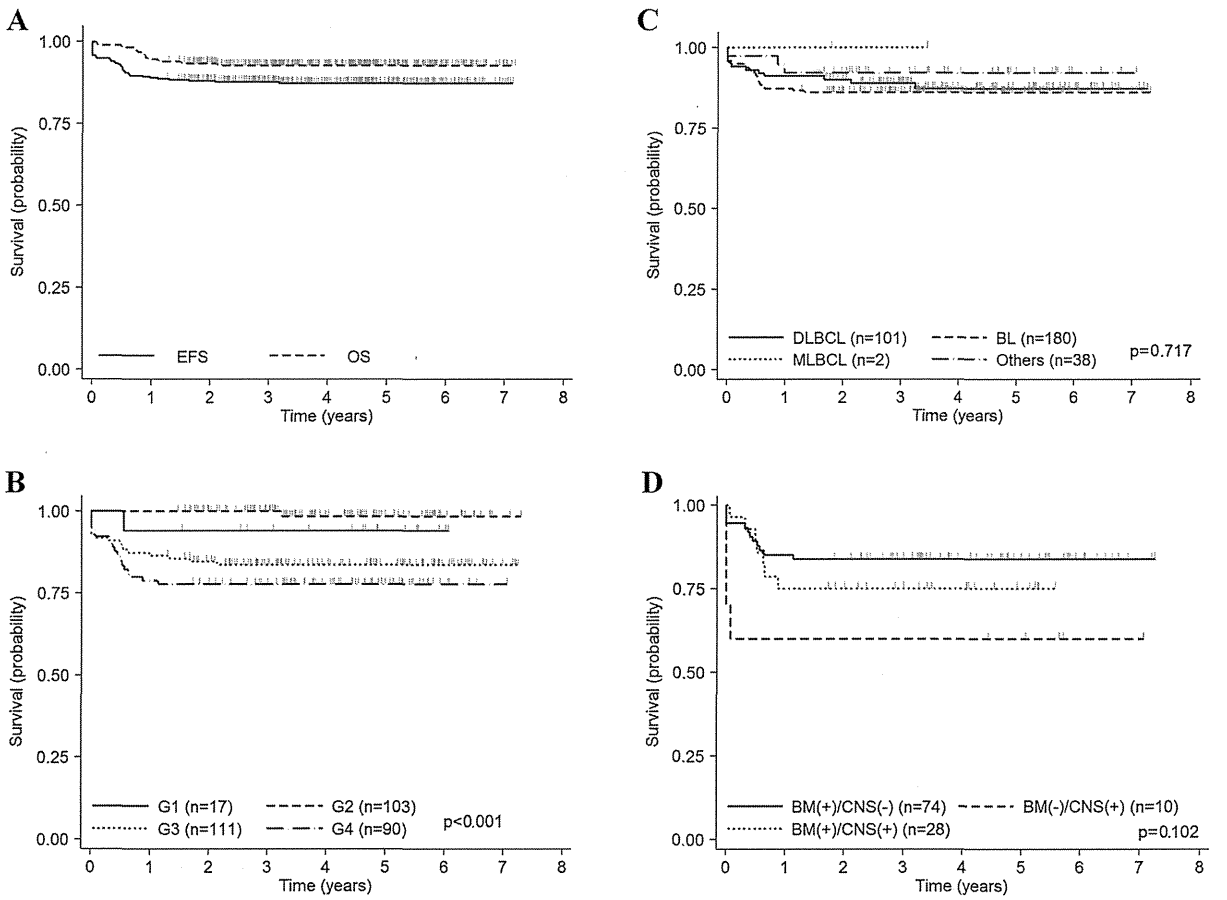


**Fig. 3.** Kaplan–Meier curves for OS and EFS of all patients (**A**). Kaplan–Meier curves for EFS according to treatment group (**B**), histology (**C**), and BM/CNS involvement (**D**).

clinical trials in large study groups, and the cure rate of childhood B-NHL has reached 90% [1–6]. In the present study, we showed an excellent survival outcome with 4-year OS 93% in children with B-NHL. In our study, the 4-year EFS 84% of Group 3 patients was considerably lower than the 4-year EFS 90% of intermediate risk group in the FAB/LMB96 study [5] or the 6-year EFS 88% of stage III patients in the BFM90 study [2], whereas, the 4-year EFS 78% of Group 4 patients compared favorably with the 4-year EFS 79% of high-risk group in the FAB/LMB96 study [5] and the 6-year EFS 74% of stage IV/B-ALL patients in the BFM90 study [2]. This outcome was obtained via the short-intensive chemotherapy regimen based on COPAD (CPM, VCR, PSL, and ADR) regimen plus the HDMTX of the lymphomas malin B (LMB) studies [3]. We omitted cranial irradiation for all patients, because recent studies have suggested the possibility of deleting radiotherapy in treating CNS diseases as well as CNS prophylaxis [2,3,5,9]. However, having no experience in administrating 8 g/m$^2$ HDMTX, we employed 5 g/m$^2$ HDMTX over 24 hour-infusion and not the 8 g/m$^2$ HDMTX over 4 hour-infusion in the LMB protocols for treating patients with CNS disease [3,5]. The treatment result for CNS disease was satisfactory, because CNS failure was only one of 38 patients with primary CNS disease in the present study.

This suggests that the 5 g/m$^2$ HDMTX over 24 hour-infusion is equally as effective to the CNS-positive disease as the aforementioned 8 g/m$^2$ HDMTX over 4 hours infusion, and reinforces the possibility that CNS irradiation could be omitted without jeopardizing the outcome of patients with CNS disease by using systemic and it MTX therapy [3,5,9].

The treatment of DLBCL as well as BL was another important focus of our study, because the incidence of DLBCL in childhood B-NHL is relatively more frequent than that of Western countries: the number of DLBCL was almost similar to that of BL (excluding B-ALL) in the present study and our recent national survey for childhood hematological malignancies has shown that the ratio of DLBCL to BL was 0.79 [14]. In our study, according to the strategy that DLBCL was treated by short-pulse chemotherapy as well as BL [15], we followed the same protocol, and achieved a favorable outcome of 4-year EFS with 87% for DLBCL which was not inferior to that of BL. This outcome can be partly explained by shared biological features, that is, that more than half of childhood DLBCL has the molecular subtypes of BL [16].

Several factors associated with poor outcome in the high-risk group in childhood B-NHL have been reported. Cairo et al. has shown a significantly inferior outcome (4-year EFS 61% ± 6%) of the subgroup of children with combined BM and CNS involvement at diagnosis as compared with children with BM or CNS only [5]. However, our results in Group 4 showed that the outcome (4-year EFS 75% ± 8%) of this subgroup with BM+/CNS+ was not significantly inferior than that of the subgroup with BM+ (83% ± 4%) or CNS+ (60% ± 1%). Failure to initial therapy is

also known to be a strong, unfavorable prognostic factor. Past studies in LMB 89/96 have shown that non-responders to pre-phase therapy (COP regimen) suffer a significantly inferior outcome as compared with responders or incomplete responders [3,5]. In our study, an appropriate evaluation of tumor regression just after pre-phase therapy was difficult for many patients, such that we compared the outcome according to response at the final evaluation time after two or three courses of therapy. These results showed that 4-year EFS of patients who did not achieve CR/CRu was only $15\% \pm 10\%$, which was as dismal as the outcome of poor-responders to COP regimen in the FAB/LMB 96 study [5]. To rescue the poor-responders in our study, we employed salvage therapy with high-dose Ara-C and VP16 to patients who did not achieve remission after 2 or 3 courses of therapy in Group 2 or 3, as in the BFM90 or FAB96 study [2,4]. As a result, 4 of 6 patients in Group 3 received salvage therapy and survived without tumor. This response rate was similar to that of FAB96 study, in which 10 out of 16 patients who received the second phase treatment intensification after the consolidation phase were alive. Thus, our results reconfirmed the efficacy of the salvage therapy.

Management of acute toxicity by short-pulse intensive chemotherapy is essential to successfully carry out the treatment protocol for childhood B-NHL. In our study, grade IV neutropenia occurred in almost all patients, but the rate of grade IV infection was quite low. Consequently, therapy-related death was less than 1% in all patients, and 2.1% in Group 4 patients. These results show the safety and feasibility of our treatment protocol. Anthracycline cardiotoxicity and secondary malignancy by alkylating agents are serious late events in pediatric cancer treatment [17,18]. To reduce the risk of cardiotoxicity, we employed THP-adriamycin (pirarubicin) instead of ADR. Pirarubicin is a derivative of ADR with reportedly less cardiotoxicity in adults [19–24]. Recently, we have reported that no significant cardiac dysfunction was detected in long-term survivors of children with acute lymphoblastic leukemia who received THP treatment [25–27]. In the present study, there were no patients with cardiac insufficiency or cardiac myopathy during the 7-year observation period. These results suggest that late-onset cardiotoxicity induced by pirarubicin is uncommon in childhood lymphoid malignancies, at least up to the cumulative dose of 240 $mg/m^2$. In our study, there was one male with a second cancer with acute myeloid leukemia, although the correlation between his second cancer and the protocol treatment is uncertain because he was resistant to the pre-phase followed by arbitrary treatment.

As shown above, chemotherapy-related toxicity of our protocol treatment was within acceptable range. However, a 6-course treatment for Group 3 seemed to be more intensive as compared with a 4-course treatment for intermediate risk group in the FAB96 study [4]. In order to reduce the total dose of cytotoxic drugs without impairing the survival outcome, new approaches including targeted monoclonal antibody therapy in combination with chemotherapy [28,29], are needed for children with an advanced or resistant disease in coming studies.

In conclusion, our nationwide study resulted in a cure rate above 90% with <1% toxic death in childhood B-NHL.

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# Statistical Analysis of Relation Between Plasma Methotrexate Concentration and Toxicity in High-Dose Methotrexate Therapy of Childhood NonHodgkin Lymphoma

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***Background.*** Plasma monitoring of Methotrexate (MTX) levels is a standard approach to predict MTX-related toxicities in a high-dose (HD) MTX monotherapy for childhood acute lymphoblastic leukemia. However, it is uncertain whether plasma MTX levels can predict MTX-related toxicity in the HDMTX plus additional chemotherapy for childhood B-cell nonHodgkin lymphoma (B-NHL). ***Procedures.*** To statistically analyze the relationship between MTX pharmacokinetic parameters and MTX-related toxicities, we collected data from patients with delayed MTX elimination ($\geq$1 μM at 48 hr and/or $\geq$0.5 μM at 72 hr) in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) BNHL 03 study. Blood MTX levels were measured at 24, 48, and 72 hr after 3 or 5 g/m$^2$ HD-MTX administration for 24 hr. ***Results.*** Three hundred and four patients received 2–4 courses of the HDMTX plus additional chemotherapy, and delayed MTX elimination was observed in 165 courses of 127 patients. In those, nephrotoxicity was significantly correlated with plasma MTX levels for each patient ($P=0.03$), and also for each course ($P=0.009$), but no other toxicities were correlated. Another analysis according to HDMTX courses showed no significant correlation between the first high plasma MTX levels and subsequent MTX levels in later course. It also showed that incidence of liver and gastrointestinal toxicities was most frequent in the first HDMTX course, and then sharply decreased in later courses ($P<0.001$). ***Conclusions.*** Our results suggest that plasma MTX level is not a reliable predictor for adverse events except for nephrotoxicity in multiple HDMTX therapy courses in childhood B-NHL. Pediatr Blood Cancer 2015;62:279–284. © 2014 Wiley Periodicals, Inc.

**Key words:** childhood; HDMTX; nonHodgkin lymphoma; toxicity

## INTRODUCTION

In the past two decades, treatment outcome of childhood B-cell nonHodgkin Lymphoma (B-NHL) has been greatly improved by using a short intensive multiagent regimen including high-dose methotrexate (HDMTX), intermediate-dose cyclophosphamide (CPA) and anthracycline [1–4]. Since this treatment rationale is based on rapid elimination of tumor cells with short cell cycle time by subsequent administration of multiple anticancer agents, imprudent prolongation of treatment intervals or dose reduction according to drug toxicity may increase the risk of treatment failure [5–8]. Therefore, the balance between efficacy and adverse events is one of the major clinical challenge to achieve a high cure rate of the disease. Among the multiple drugs, MTX-related toxicity may possibly be predicted based on plasma MTX levels in childhood acute lymphoblastic leukemia (ALL), because HDMTX is used as monotherapy in intensification and maintenance phases [9,10]. However, it might be difficult to predict what kinds of toxicities are associated with plasma MTX levels in a HDMTX plus additional chemotherapy for childhood BNHL, because CPA and anthracycline which are concomitantly used with HDMTX, also induce various toxicities similar to MTX toxicities. In addition, it is unknown whether high plasma MTX level is associated to a particular patient, in other words, the first high MTX level is likely to repeat in later HDMTX courses in a particular patient.

In this study, to answer those clinical issues, we statistically analyzed the relationship between MTX pharmacokinetic parameters and MTX-related toxicities in patients with B-NHL treated by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) B-NHL03 protocol study [4].

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## PATIENTS AND METHODS

### Patients and Protocol Treatment

The protocol was conducted in 112 hospitals of the Japanese Pediatric Leukemia/Lymphoma Study group (JPLSG) after approval by each institution's review board, and written informed consent was provided by patients or legal guardians before treatment. A total of 346 untreated B-NHL patients under 18 years of age, were registered to participate in the JPLSG B-NHL03 study (University hospital Medical Information Network Japan, UMIN ID: C000000317) between November 2004 and January 2011. Patients were stratified into four therapy groups (G1, G2, G3, G4) based on Murphy's stage, tumor resectability and BM/CNS involvement. Chemotherapy regimens are shown in supplemental Table SI. HDMTX was administered to patients in regimen A (2A for G2, 3A for G3, and 4A1 and 4A2 for G4). G2 received four courses (2A → 2B, ×2), G3 and G4 received six courses (3A → 3A → 3B, ×2; 4A1 → 4A2 → 4B, ×2) of chemotherapy regimens. Regimen A consisted of HDMTX, dexamethasone, vincristine, intermediate-dose cyclophosphamide (CPA), and pirarubicin (THP-adriamycin, THP). Patients in G2 and G3 received 3 g/m$^2$ HDMTX and those in G4 received 5 g/m$^2$ HDMTX. HDMTX was administered for 24 hr and intravenous hydration at a rate of 100 ml/m$^2$/hr with 4.3% glucose, $NaHCO_3$ 33 mEq/L, L-Lactate 20 mEq/L, NaCl 35 mEq/L, and KCL 20 mEq/L was initiated 12 hr before the MTX infusion and was maintained for 48 hr after the infusion. During this period, acetazolamide (125 mg <5 years old or 250 mg ≥5 years old) was administered every 12 hr. Urine pH was checked with each void and a bolus of $NaHCO_3$ (8.4 mEq in 20 ml) was administered if the pH was <7.0. After 12 hr of MTX infusion, leucovorin (LV) 15 mg/m$^2$ was given orally every 6 hr for a total of seven doses. When patients showed high plasma MTX levels (≥0.2 μM) at 72 hr, LV rescue was continued until MTX concentration level decreased to less than 0.2 μM.

### Measurements of Plasma MTX Concentration

Plasma MTX concentrations were determined by each institute, and the measurements were performed by a monoclonal antibody-based immunoassay (fluorescence polarization immunoassay, FPIA) in 91 institutes, or by an enzyme multiplied immunoassay technique (EMIT) in 21 institutes. Delayed MTX elimination was defined as plasma MTX concentration ≥1 μM at 48 hr and/or ≥0.5 μM at 72 hr after MTX administration. Since only one third of the data of MTX concentrations at 24 hr after MTX administration (the end of 24-hr infusion) was available and there were also no sufficient sampling points between 24 and 48 hr to calculate the pharmacokinetic parameters of MTX, we could not analyze the appropriate pharmacokinetic parameters including systemic clearance (CLSYS) based on the two-compartmental model. We therefore calculated the basic two parameters of MTX (elimination rate constant (ke) and terminal half-life ($t_{1/2}$)). The terminal slope of MTX concentration (C) versus time (t), which represents ke, was calculated as $ke = [\ln(C1) \cdot \ln(C2)]/(t2 \cdot t1)$, where C1 and C2 were concentrations at t1 (48 hr) and t2 (72 hr), respectively. The $t_{1/2}$ was calculated by dividing 0.693 by ke.

### Statistics

Plasma MTX levels and toxicity data were prospectively collected for each treatment phase and toxicity severity was graded according to National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0. Continuous variables were summarized as the mean ± standard deviation (SD) or median (minimum, maximum) and categorical variables were presented as numbers and percentages. Correlation between the two variables was estimated by Spearman's correlation coefficient. The plasma MTX concentrations in patients with an adverse event (AE) were compared to those in patients without the AE by using Wilcoxon's rank sum test. In this analysis, one observation for each patient was taken into account. The observation with the AE and the highest concentration at 48 hr was preferentially used if a patient received more than one course and had more than one observation. Furthermore, log-transformed MTX concentrations were compared between patients with and without AE using generalized estimating equations (GEE) method [11] including AE (yes vs. no) and course as factors, in order to take into account repeated measures of the same patient. The presence (≥grade 3) of toxicity (hepatic toxicity, stomatitis, and infection) were analyzed using the GEE with repeated-measures logistic regression model including nephrotoxicity (yes vs. no) and course as factors. We assumed an exchangeable covariance matrix for the repeated-measures in the GEE analyses. All tests were two-sided, and p values less than 0.05 were considered to indicate statistical significance. Statistical analyses were carried out using SAS 9.3 (SAS Institute, Inc., Cary, NC).

## RESULTS

### Pharmacokinetic Parameters

One hundred twenty seven patients out of a total of 304 patients who received HDMTX therapy showed delayed MTX elimination. MTX concentrations in patients with delayed MTX elimination are summarized in Table I. Percentages of patients with delayed MTX elimination by treatment groups were 26.2% in G2, 40.5% in G3, and 62.2% in G4, respectively. The male to female ratio in patients with delayed MTX elimination was more than double than patients without delayed MTX elimination (107/20 = 5.35 vs. 123/54 = 2.27,

**TABLE I. Summary of High Plasma MTX Concentrations at 48 and 72 hr After MTX Dosing***

| | | | MTX concentration at 48 hr | | MTX concentration at 72 hr | |
|---|---|---|---|---|---|---|
| Group | No. of patients | No. of courses | Mean ± SD | Median (Min, Max) | Mean ± SD | Median (Min, Max) |
| 2 | 26 | 27 | 2.63 ± 2.25 | 1.86 (0.99, 11.00) | 0.66 ± 0.52 | 0.61 (0.08, 1.90) |
| 3 | 45 | 53 | 3.41 ± 5.19 | 1.77 (0.80, 29.70) | 1.02 ± 1.55 | 0.44 (0.17, 8.23) |
| 4 | 56 | 85 | 3.80 ± 6.29 | 1.82 (0.93, 48.00) | 1.06 ± 1.79 | 0.54 (0.05, 11.00) |

*≥1 μM at 48 hr and/or ≥0.5 μM at 72 hr after MTX administration.

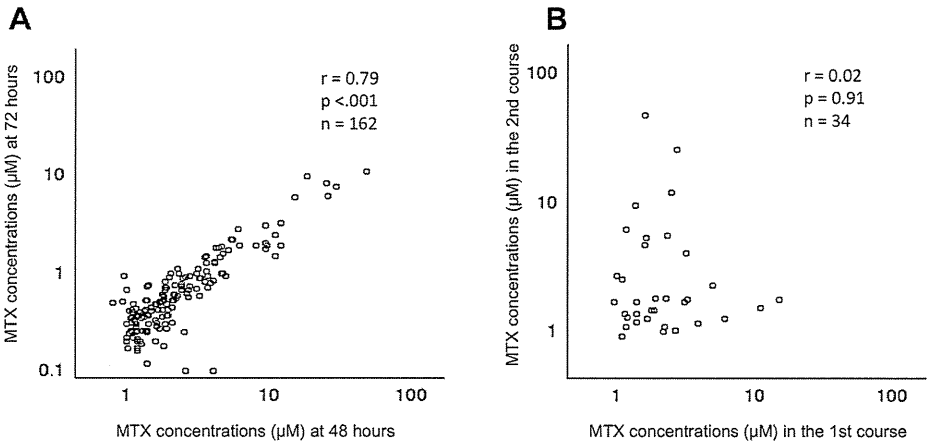


**Fig. 1.** Correlation between blood MTX concentrations at 48 and 72 hr in patients with delayed MTX clearance (**A**). Correlation between blood MTX concentrations in first and second courses at 48 hr (**B**). r denotes Spearman's rank correlation coefficient.

$P = 0.004$ by Fisher's exact test), and the ratios according to treatment group were 5.5 in G2, 10.2 in G3, and 3.6 in G4. Thus, males with G3 showed the highest risk of delayed MTX elimination. However, there was no significant difference in age between the two groups (mean of years 8.8 vs. 8.8). MTX concentrations were widely variable between patients at either dosage. MTX concentrations at 48 hr ranged from 0.99 to 29.7 μM in the 3 g/m$^2$ HDMTX group, and 0.93 to 48 μM in the 5 g/m$^2$ HDMTX group. There was a significantly positive correlation between MTX concentrations at 48 and 72 hr in each patient (Fig. 1A). On the other hand, there was no significant

**TABLE II. Relationship Between Plasma MTX Pharmacokinetics (MTX Concentration and MTX Half-Life) and Adverse Events**

| | Adverse event (number of patients base) | | | Adverse event (number of courses base) | | |
|---|---|---|---|---|---|---|
| | No, n = 118 | Yes, n = 9 | $P^a$ | No, n = 156 | Yes, n = 9 | $P^b$ |
| Nephrotoxicity ≥grade 2[c] | | | | | | |
| 48 hr | 1.93 (0.80, 25.50) | 6.17 (0.99, 48.00) | 0.0307 | 1.81 (0.80, 25.50) | 6.17 (0.99, 48.00) | 0.009 |
| 72 hr | 0.53 (0.08, 9.89) | 1.82 (0.35, 11.00) | 0.0023 | 0.50 (0.05, 9.89) | 1.82 (0.35, 11.00) | <0.001 |
| $t_{1/2}$ (hr) | 12.3 (4.5, 35.4) | 14.1 (9.9, 42.6) | 0.373 | 12.7 (4.5, 1571.7) | 14.1 (9.9, 42.6) | 0.318 |
| | Adverse event (number of patients base) | | | Adverse event (number of courses base) | | |
| | No, n = 84 | Yes, n = 42 | $P^a$ | No, n = 120 | Yes, n = 44 | $P^b$ |
| Hepatic toxicity ≥grade 3/4[c] | | | | | | |
| MTX 48 hr | 1.98 (0.80, 48.00) | 1.91 (0.95, 15.00) | 0.65 | 1.82 (0.80, 48.00) | 1.82 (0.95, 15.00) | 0.95 |
| MTX 72 hr | 0.58 (0.08, 11.00) | 0.59 (0.16, 6.00) | 0.96 | 0.51 (0.05, 11.00) | 0.53 (0.16, 6.00) | 0.58 |
| $t_{1/2}$ (hr) | 12.6 (4.5, 35.4) | 13.0 (8.0, 1,571.7) | 1.00 | 12.8 (4.5, 35.4) | 12.9 (8.0, 1,571.7) | 0.26 |
| | Adverse event (number of patients base) | | | Adverse event (number of courses base) | | |
| | No, n = 68 | Yes, n = 58 | $P^a$ | No, n = 98 | Yes, n = 66 | $P^b$ |
| Oral mucositis ≥grade 3/4[c] | | | | | | |
| MTX 48 hr | 1.86 (0.80, 48.00) | 2.28 (0.95, 25.50) | 0.38 | 1.69 (0.80, 48.00) | 2.23 (0.95, 25.50) | 0.25 |
| MTX72 hr | 0.49 (0.08, 11.00) | 0.60 (0.10, 9.89) | 0.35 | 0.45 (0.05, 11.00) | 0.59 (0.10, 9.89) | 0.23 |
| $t_{1/2}$ (hr) | 12.9 (5.7, 35.4) | 12.6 (4.5, 1,571.7) | 0.83 | 12.8 (5.4, 35.4) | 12.7 (4.5, 1,571.7) | 0.23 |
| | Adverse event (number of patients base) | | | Adverse event (number of courses base) | | |
| | No, n = 23 | Yes, n = 103 | $P^a$ | No, n = 37 | Yes, n = 127 | $P^b$ |
| Infection ≥grade 3/4[c] | | | | | | |
| MTX 48 hr | 2.03 (0.80, 48.00) | 1.90 (0.93, 29.70) | 0.49 | 1.90 (0.80, 48.00) | 1.81 (0.93, 29.70) | 0.56 |
| MTX 72 hr | 0.70 (0.22, 11.00) | 0.56 (0.08, 9.89) | 0.31 | 0.50 (0.22, 11.00) | 0.52 (0.05, 9.89) | 0.25 |
| $t_{1/2}$ (hr) | 13.0 (10.0, 35.4) | 12.4 (4.5, 27.7) | 0.48 | 12.9 (8.5, 35.4) | 12.6 (4.5, 1,571.7) | 0.74 |

Data are presented as median (min, max). n: number of patients or courses. [a]Wilcoxon's rank sum test. [b]Generalized estimating equations method for repeated log-transformed MTX concentrations. [c]NCI-CTC version 2.0.

correlation between MTX concentrations at 48 hr in the first and next HDMTX courses in each patient (Fig. 1B).

## Toxicities

In order to clarify what kinds of MTX toxicities are closely associated with MTX pharmacokinetic parameters, we statistically analyzed the correlation between the parameters (plasma MTX levels and half-life ($t_{1/2}$)) and MTX-related toxicities (stomatitis, nephrotoxicity, hepatic toxicity, and infection). In this study, we excluded hematological toxicity and CNS toxicity from the analysis, because neutropenia ≥grade 3 was observed in almost all (>98%) patients regardless of MTX levels, and CNS toxicity ≥grade 3 occurred in only one case. In general, adverse events (AEs) ≥grade 3 were collected for analysis, but serum creatinine and proteinuria ≥grade 2 were used for nephrotoxicity because the number of nephrotoxic AEs ≥grade 3 was very few (n = 4) and proteinuria has been shown to be a HDMTX-related nephrotoxicity [12]. The number of patients with nephrotoxicity ≥grade 2 was nine: five in grade 2, one in grade 3 and one in grade 4 with high serum creatinine levels, and two in grade 2 with proteinuria. As shown in Table II, only nephrotoxicity was significantly correlated with higher MTX levels for each patient, and also for each course, but other toxicities had no correlations to MTX levels. MTX half-life showed no significant relation to any of the MTX-related toxicities. We also analyzed statistical difference in the frequency of other toxicities, such as hepatic toxicity, stomatitis and infection between patients with nephrotoxicity and patients without (Table III). These results showed that patients with nephrotoxicity tended to have higher frequencies of hepatic toxicity, although the difference did not reach significant levels.

Lastly, we studied the difference in incidences of severe toxicities according to HDMTX courses in all patients of group 3 and group 4 (Fig. 2). Incidences of hematological toxicities did not vary widely during the four courses. However, incidences of non-hematological toxicities such as liver and gastrointestinal toxicities showed a large variation during the courses: the incidence was the greatest in the first course, and then sharply decreased in later courses in both groups ($P < 0.001$). In addition, the incidences seemed to be unrelated with plasma MTX levels.

**TABLE III. Incidence of MTX-Related Toxicities According to Nephrotoxicity**

| | | Nephrotoxicity ≥grade 2 | | |
|---|---|---|---|---|
| Group | | No | Yes | $P^a$ |
| Hepatic toxicity | | | | |
| Total | Grade 3 | 36 (23.2) | 3 (33.3) | |
| | Grade 4 | 2 (1.3) | 2 (22.2) | |
| | ≥grade 3/4 | 38 (24.5) | 5 (55.6) | 0.051 |
| 2 | Grade 3 | 5 (20.0) | 1 (50.0) | |
| | Grade 4 | 0 (0.0) | 0 (0.0) | |
| | ≥grade 3/4 | 5 (20.0) | 1 (50.0) | 0.34 |
| 3 | Grade 3 | 10 (20.4) | 0 (0.0) | |
| | Grade 4 | 1 (2.0) | 1 (33.3) | |
| | ≥grade 3/4 | 11 (22.4) | 1 (33.3) | 0.79 |
| 4 | Grade 3 | 21 (25.9) | 2 (50.0) | |
| | Grade 4 | 1 (1.2) | 1 (25.0) | |
| | ≥grade 3/4 | 22 (27.2) | 3 (75.0) | 0.10 |
| Oral mucositis | | | | |
| Total | Grade 3 | 57 (36.8) | 3 (33.3) | |
| | Grade 4 | 5 (3.2) | 1 (11.1) | |
| | ≥grade 3/4 | 62 (40.0) | 4 (44.4) | 0.84 |
| 2 | Grade 3 | 5 (20.0) | 1 (50.0) | |
| | Grade 4 | 1 (4.0) | 0 (0.0) | |
| | ≥grade 3/4 | 6 (24.0) | 1 (50.0) | 0.42 |
| 3 | Grade 3 | 20 (40.8) | 1 (33.3) | |
| | Grade 4 | 0 (0.0) | 1 (33.3) | |
| | ≥grade 3/4 | 20 (40.8) | 2 (66.7) | 0.46 |
| 4 | Grade 3 | 32 (39.5) | 1 (25.0) | |
| | Grade 4 | 4 (4.9) | 0 (0.0) | |
| | ≥grade 3/4 | 36 (44.4) | 1 (25.0) | 0.39 |
| Infection | | | | |
| Total | Grade 3 | 119 (76.8) | 7 (77.8) | |
| | Grade 4 | 1 (0.6) | 0 (0.0) | |
| | ≥grade 3/4 | 120 (77.4) | 7 (77.8) | 0.87 |
| 2 | Grade 3 | 20 (80.0) | 1 (50.0) | |
| | Grade 4 | 0 (0.0) | 0 (0.0) | |
| | ≥grade 3/4 | 20 (80.0) | 1 (50.0) | 0.28 |
| 3 | Grade 3 | 38 (77.6) | 3 (100.0) | |
| | Grade 4 | 0 (0.0) | 0 (0.0) | |
| | ≥grade 3/4 | 38 (77.6) | 3 (100.0) | NC |
| 4 | Grade 3 | 61 (75.3) | 3 (75.0) | |
| | Grade 4 | 1 (1.2) | 0 (0.0) | |
| | ≥grade 3/4 | 62 (76.5) | 3 (75.0) | 0.75 |

[a]Generalized estimating equations method for repeated adverse event (≥grade 3/4). NC: not calculated. Data are n (%).

## Modification of Protocol Treatments

In our study, treatment modifications according to delayed MTX elimination were reported in 15 patients (2 in group 2, 4 in group 3, and 9 in group 4). Eleven of which had suffered from MTX-induced nephrotoxicity with high creatinine levels (6 in grade 1, 3 in grade 3, 1 in grade 3 and 1 in grade 4). The modifications were as follows: dose reduction or prolongation of treatment intervals of CPA and THP in 8, withdrawal of CPA and THP in 2, reduction of HDMTX dose (from 5 to 3 g/m$^2$) in the next HDMTX course in 3 (2 between 1st and 2nd course, one between 2nd and 3rd course), and exchanging course 4A with course 4B without HDMTX in 2. Of the 15 patients, 14 patients except one, who had CNS involvement, survived without diseases.

## DISCUSSION

Recent pharmacokinetic and pharmacogenetic studies of HDMTX treatment in childhood lymphoid malignancies have shown significant relations between polymorphisms in genes coding for enzymes involved in folate metabolisms and MTX-related toxicities. However, individual prediction of MTX toxicity and dose adjustment of HDMTX based on pretreatment genotyping do not reach a practical use [13–15] and routine monitoring of plasma MTX concentrations still has an important role to predict MTX toxicities in clinical practice.

In the present study, we analyzed the relation between MTX pharmacokinetics and MTX-related toxicities in the HDMTX plus additional chemotherapy for childhood B-NHL. We found that plasma MTX levels were significantly correlated with nephrotoxicity (creatinine and/or proteinuria ≥grade 2), but not other toxicities. MTX half-life was not associated with any toxicity. These results suggest that MTX-induced nephrotoxicity could be

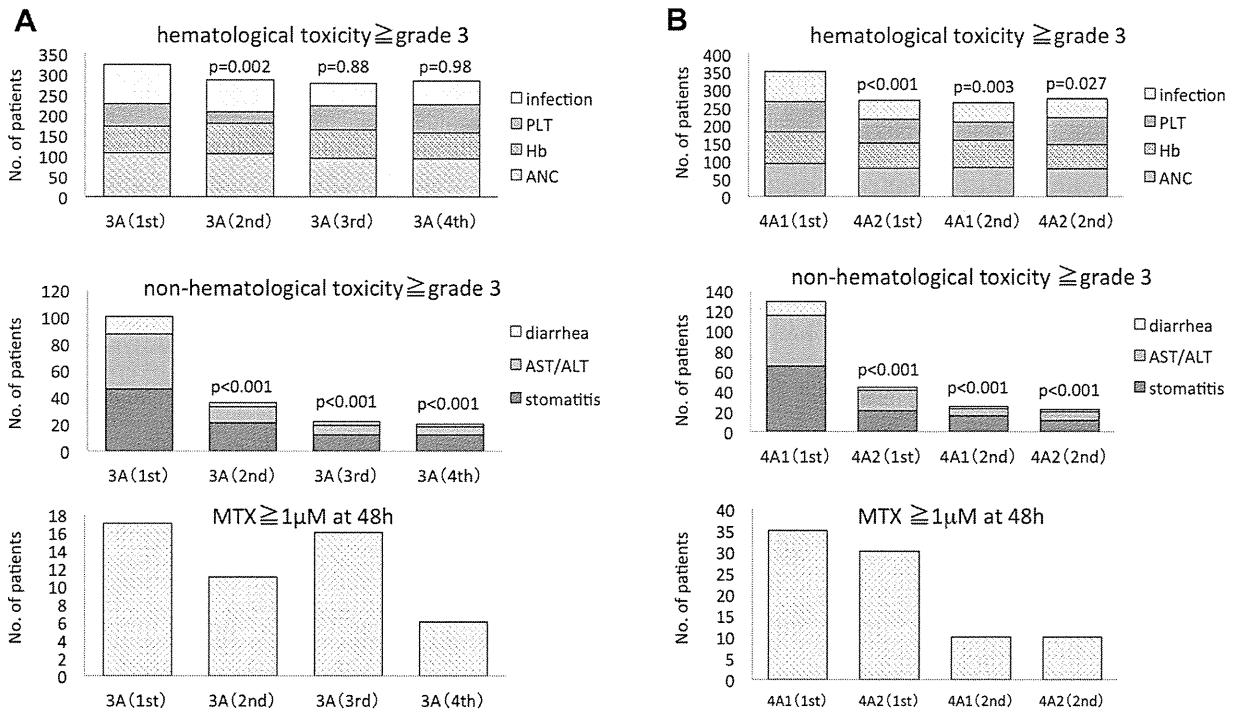


**Fig. 2.** Incidence of MTX-related toxicities and delayed MTX elimination according to HDMTX course. Left panel (**A**) for group 3; Right panel (**B**) for group 4. Number in vertical axis shows number of patients with hematological toxicities (**upper panel**), non-hematological toxicities (**middle panel**), and delayed MTX elimination (≥1 μM at 48 hr) (**lower panel**). Number of patients who received HDMTX therapy of each course was 108 in 1st 3A1, 106 in 2nd 3A1, 95 in 3rd 3A1, and 94 in 4th 3A1 in group 3, and 94 in 1st 4A1, 83 in 1st 4A2, 79 in 2nd 4A1, and 78 in 2nd 4A2 in group 4, respectively. *P* values reported from Dunnett's test based on the generalized estimating equation method comparing the toxicity count by course (reference group is 3A (1st) or 4 A1 (1st)).

caused by the long-time exposure to high plasma MTX levels during 48–72 hr, but is not related with MTX half-life determined in the elimination phase in our study.

Very few studies have been reported on nephrotoxicity of HDMTX in lymphoma patients [12,16]. May et al. [16] retrospectively studied the incidence of nephrotoxicity in adults with lymphoma, and reported a 21% (37/179 courses) incidence of nephrotoxicity with creatinine ≥grade 2 in patients associated with delayed MTX elimination. This was five times higher than 4% (7/165 courses) incidence of nephrotoxicity in our study. This discrepancy may be due to the difference in age of patients between the two studies. They also suggested that renal toxicity was not related to delayed MTX elimination, because the ratio (20%) of nephrotoxicity of patients who do not have was the almost same as patients with delayed MTX elimination. However, this is not consistent with our findings, because the incidence of nephrotoxicity ≥grade 2 in patients without delayed MTX elimination was 0% in our study (data not shown). Lack of correlation between delayed MTX elimination and other toxicities was rather unexpected. This finding suggests that MTX-related toxicities such as stomatitis, hepatic toxicity and infection are affected by CPA and THP as well as MTX in the HDMTX courses in childhood B-NHL treatment.

In our study, delayed MTX elimination was significantly associated with male sex. This finding is inconsistent with some HDMTX studies in childhood ALL, in which female sex has been reported to be associated with high MTX concentrations or low MTX clearance [17,18], whereas other studies have shown that gender is not significantly associated with MTX concentrations or pharmacokinetic polymorphism in childhood ALL [19,20]. Thus, the role of gender in MTX pharmacokinetics still remains to be elucidated in childhood ALL. In childhood NHL, our results may provide actionable observation that male sex has two times higher risk than female to suffer delayed elimination of MTX in HDMTX therapy, although male sex was not an unfavorable prognostic factor in outcome [4].

There was no significant relation between the first high plasma MTX levels and subsequent MTX levels in the later HDMTX course. This finding showed that there was a wide intra-individual variability of blood MTX levels as previously described by others [21]. Since MTX is primarily eliminated by kidney, creatinine clearance may reflect blood MTX levels. However, there have been controversial studies for relation between creatinine concentrations and plasma MTX levels. One study of children who received 3 or 5 g/m$^2$ of HDMTX has shown a positive correlation between serum creatinine concentrations and blood MTX levels [22] whereas, another study for children who received 1 or 2 g/m$^2$ failed to show the positive association [21]. Although creatinine clearance is not steady, it is unlikely that creatinine clearance may change during administration of HDMTX, since all patients were strictly monitored and maintained a high urine output and urinary alkalization during HDMTX administration in our study. From the point of view of clinical practice, we

infer that the first episode of delayed MTX elimination does not predict subsequent high MTX levels in later HDMTX courses. This is also supported by the study of Hempel et al., in which they showed that glomerular toxicity at the end of HDMTX can be completely reversed until the next HDMTX course [12].

The last finding was that the first HDMTX courses had a great incidence of liver and gastrointestinal toxicities followed by a sharp reduction of the incidence in later courses. These results may be explained by the plasma folate concentrations in HDMTX courses. Valik et al. [23] reported a severe encephalopathy occurred at the first HDMTX course but not the second course in a male with acute leukemia, where pretreatment plasma folate concentrations were low before the first HDMTX course and then 10-fold higher before the second course. In addition, Sterba et al. [24] showed the plasma folate concentrations increase significantly with increasing number of HDMTX courses in children with ALL and NHL, and they suggested that the increasing folate baseline concentration could be caused by repetitive LV administration. Similar result was reported in osteosarcoma patients [25]. Consequently, low frequencies of gastrointestinal and liver toxicity in later HDMTX courses in our study may be explained by the difference of pretreatment folate levels according to HDMTX courses, although plasma folate levels were not available in our study. In contrast to the non-hematological toxicities, incidence of hematological toxicity showed few changes by the HDMTX courses and plasma MTX levels, suggesting that hematological toxicity was more affected by CPA and THP than HDMTX. This finding shows the need of prophylaxis and countermeasure for patients with neutropenia to prevent developing severe infections throughout the HDMTX courses.

In this study we employed a 24-hr infusion of HDMTX. However, recent studies have shown the efficacy of 4-hr infusion of HDMTX for childhood B-NHL. Woessmann et al. [26] compared the 4-hr infusion and 24-hr infusion of HDMTX in the NHL-BFM95 study and concluded that a 4-hr infusion is not inferior to, but less toxic than, a 24-hr infusion for low- and intermediate-risk patients. In addition, Cairo et al. [27] have reported that a 4-hr infusion of HDMTX resulted in a favorable outcome for high-risk BNHL patients in the FAB/LMB96 study. Consequently, 4-hr infusion of HDMTX should be considered in our next studies.

In summary, we did not find evidence for relation between plasma MTX levels and MTX-related toxicities except nephrotoxicity. This suggests that when high blood MTX levels are associated with nephrotoxicity, the occurrence of other developing toxicities should be taken into consideration. In addition, the first HDMTX administration was associated with a great incidence of gastrointestinal and liver toxicities followed by a reduction of the incidence in later courses. Hence, these findings suggest that the first episode of severe non-hematological toxicity does not predict the recurrence of severe toxicities in later courses.

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# Hematopoietic Stem Cell Transplantation for Patients With Acute Lymphoblastic Leukemia and Down Syndrome

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***Background.*** Hematopoietic stem cell transplantation (HSCT) is one curable option for high-risk acute lymphoblastic leukemia (ALL); however, transplant-related toxicities might be severe in patients with Down syndrome and ALL (DS-ALL). ***Procedure.*** HSCTs performed in patients with DS-ALL were identified in the Japan Society for Hematopoietic Cell Transplantation registry. ***Results.*** In the registry data, 11 patients with DS-ALL were identified. The median age at HSCT was 9 years (range: 6–22 years). Six patients underwent HSCT at non-remission status. Allogeneic grafts were utilized in all patients, including eight patients who received HSCT from unrelated donors. Reduced intensity conditioning regimens were used in three patients. All patients achieved neutrophil engraftment by a median of day 18 (range: day 11–61). Ten patients experienced grade 3 or more infectious episodes. Six patients experienced complications of the respiratory system. The incidences of II–IV or III–IV acute GVHD were nine (81.8%) or seven patients (63.6%), respectively. Chronic GVHD was observed in five (55.6%) out of nine evaluable patients. Seven patients died at a median of 6 months (range: 0–24 months) after HSCT. Two-year relapse-free and overall survival were 33.3% (95% CI: 2.5–64.1%) or 37.5% (95% CI: 5.9–69.1%), respectively. The causes of death were relapse (n = 2), infection (n = 2), bleeding (n = 1), thrombotic microangiopathy (n = 1), and chronic GVHD (n = 1). ***Conclusions.*** Therapy-related mortality accounted for five out of seven deceased patients in this case series. Attempts to reduce toxicities should be considered in HSCT for patients with DS-ALL. Pediatr Blood Cancer 2015;62:148–152. 

**Key words:** acute lymphoblastic leukemia; Down syndrome; GVHD; relapse; transplantation

## INTRODUCTION

Patients with Down syndrome (DS) are known to be at high risk of developing acute leukemia [1,2]. Different from acute myeloid leukemia (AML) associated with DS which is known to have excellent prognosis [3,4], treatment results of patients with DS and acute lymphoblastic leukemia (ALL) have been reported to be worse compared with those in patients without DS [5–7]. The poor prognosis of patients with DS-ALL has been suggested to be attributed to the biology of ALL cells [8,9], higher toxicity of chemotherapy [10,11], and less intensification of treatment [12,13].

Hematopoietic stem cell transplantation (HSCT) is an option for cure of high-risk or relapsed ALL. In patients with DS-ALL, however, the role of HSCT has not been established. Earlier studies reported high therapy-related mortality (TRM) after HSCT [14,15]. In contrast, more recent studies identified ALL relapse rather than TRM as the main cause of treatment failure [16,17].

In this study, we accessed the national HSCT registry data to obtain further information to assess the risks and benefits of HSCT for patients with DS-ALL.

## PATIENTS AND METHODS

In the Japanese Society of Hematopoietic Cell Transplantation (JSHCT) registry from April 1977 to December 2011, 13 patients with DS were identified to have received HSCT against ALL. Two patients were excluded from the study because of the guardians' refusal to allow participation in the clinical study (n = 1) and insufficient data to confirm that the patient had DS (n = 1). In total, the clinical courses of 11 patients with DS-ALL who underwent HSCT were studied. The missing data in the JSHCT registry and additional clinical information such as doses of methotrexate (MTX) in GVHD prophylaxis, grades of transplant-related toxicities (TRTs), and comorbidities at the time of HSCT were obtained by direct contact with transplant institutions. Grading of TRT was scored according to Common Terminology Criteria for Adverse Events version 4.0. Survival after HSCT was calculated using the Kaplan–Meier method. This study was approved by the

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## RESULTS

### Characteristics of Patients and HSCT

The characteristics of the patients with DS-ALL are shown in Table I. Five were female and six were male. The median age at diagnosis of ALL was 7 years (range: 4–21 years). Median age at HSCT was 9 years (range: 6–22 years). The immunophenotype of ALL was B cell precursor in all patients, including the case of secondary leukemia, who developed ALL after the treatment for AML (case 2). Data on cytogenetics were available in 10 out of the 11 patients, and two had additional chromosomal aberrations (der[16]t[1;16][q2;q2.1]; case 2, del [9]; case 5) besides trisomy 21. Disease status at the time of HSCT was first complete remission (CR) in three patients (case 5 and 11 failed to achieve CR after the first-line induction therapy), second or later CR in two, relapse in five, and induction failure in one. Comorbidities included three patients with diabetes mellitus, two with thyroid dysfunction, and one with mild aortic regurgitation.

These patients underwent HSCT between April 2001 and April 2011. All patients received allogeneic grafts: five unrelated bone marrow (BM), two related BM, one related peripheral blood, and three unrelated cord blood. Total body irradiation at a dose of 8–12 Gy was used as part of the conditioning regimen in six patients. Two patients received high-dose busulfan regimens, and three received fludarabine and melphalan-based reduced intensity regimens. GVHD prophylaxis regimens are summarized in Table II.

### Outcome

Neutrophil engraftment was reported in all patients at median day 18 (range: day 11–61). Platelet recovery over $2 \times 10^4/\mu l$ was achieved in all but one patient (case 9) at median day 37 (range: day 21–58; the recovery date was unavailable in case 6).

Among six patients who received HSCT at non-remission status, one patient did not achieve complete remission even after HSCT (case 1). Among 10 patients who achieved complete remission before or after HSCT, ALL recurrence was observed in four patients 4–11 months after HSCT (case 3, 5, 6, and 10). At the time of this study, four patients are alive 16–77 months after HSCT (case 2, 3, 7, and 11). One patient who received HSCT at ALL relapse has survived over 2 years after HSCT (case 7). In total, the 2-year overall survival or leukemia-free survival rate was 37.5% (95% CI: 5.9–69.1%) or 33.3% (95% CI: 2.5–64.1%), respectively. Main causes of death were reported as ALL (n = 2), infection (n = 2), chronic GVHD (n = 1), thrombotic microangiopathy associated with chronic GVHD (n = 1), or bleeding (n = 1). Thus, in this case series, TRT was considered as the major cause of death rather than ALL relapse. Three patients died of TRT without ALL recurrence.

### Transplant-Related Toxicities

More than grade 3 non-hematological toxicity was observed in all but one patient. The most frequent TRTs were infection (10/11 patients: 90.9%) and mucositis (7/11 patients: 63.6%). As shown in Table II, all seven patients with more than grade 3 mucositis developed infection such as febrile neutropenia (n = 3), sepsis (n = 3), or pneumonia (n = 1). TRT in the respiratory system was also common, including acute respiratory distress syndrome (ARDS, n = 2), pleural effusion (n = 2), pneumonia (n = 2), and airway obstruction (n = 1).

### GVHD

The incidence of acute GVHD was relatively high in this case series (10/11 patients: 90.9%). Greater than grade II acute GVHD was observed in nine patients (81.8%), and grade III acute GVHD was seen in seven (63.6%). Five (55.6%) out of nine evaluable patients developed chronic GVHD, which was related to transplant-related mortality in two patients.

Patients with DS are known to be susceptible to MTX-toxicity [18] and the optimal dose of MTX as GVHD prophylaxis is not standardized in patients with DS. Therefore, besides the JSHCT registry data, doses of MTX used in GVHD prophylaxis were additionally investigated in this study. As shown in Table II, the doses were considered to be relatively low, especially in four patients, in whom the doses were as follows: 10 $mg/m^2$ followed by a single dose of 7 $mg/m^2$; 7.5 $mg/m^2$ followed by three doses of 5 $mg/m^2$; four doses of 5 $mg/m^2$; and three doses of 2.5 $mg/m^2$, respectively. These four patients developed grade II–III acute GVHD, although a clear association between MTX doses and the occurrence of GVHD was not observed in this case series.

## DISCUSSION

In previous studies, survival after HSCT in patients with acute leukemia and DS was 19–48% [14,17,19,20], which is comparable with our results. In this study, six out of 11 patients with DS-ALL received HSCT at non-remission status. Uncontrolled disease status at the time of HSCT might have affected the treatment results. However, contrary to recent reports that indicated that leukemia relapse was the major cause of death in patients with DS-ALL after HSCT, three patients in this case series (27.3% of total and 42.9% of deceased patients) died of TRT without ALL recurrence. Because patients' backgrounds such as disease status, donor sources or transplant procedures were highly heterogeneous in this study, we could not specify the single factor associating with mortality after HSCT. However, our results suggest TRT associated with HSCT is still a major problem in patients with DS-ALL.

DS is frequently complicated by congenital and acquired diseases such as heart defects or metabolic disorders. The prevalence of congenital heart defects or thyroid dysfunction in patients with DS is about 44–58% or 28–40% [21], respectively. Considering these incidences, comorbidity at the time of HSCT in this case series was not significantly frequent so that it could explain the high rate of therapy-related mortality. The relatively low frequency of severe congenital disease in this study suggests that the indication of HSCT has been restricted in patients with DS-ALL because of their physical condition.

Infection and mucositis were frequent TRTs associated with HSCT in patients with DS-ALL. The mucocutaneous complication is prevalent even during conventional-dose chemotherapy in patients with DS and acute leukemia, and this is possibly related to the high incidence of infection [10]. Mucositis caused by the conditioning regimen may have resulted in the high incidence of infection after HSCT as well. In this study, all seven patients who had more than grade 3 mucositis developed severe infection. Complications in the respiratory system were also frequent,

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**TABLE I. Characteristics of DS-ALL Patients**

| Case | Age at Diag.*1 (yr) | Age at HSCT (yr) | Gender | Diag. | karyotype | ALL status at HSCT | Complications at HSCT | Donor type | HLA match*2 | Conditioning | Relapse after HSCT | Outcomes (mo after HSCT) | Cause of death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 4 | 6 | Male | BCP | +21 | Rel | | uBM | 6/8 allele | TBI 8 Gy(4)*3, Flu 150 mg/m$^2$, CY 120 mg/kg | no CR*4 | Dead, 6 mo | cGVHD |
| 2 | 8 | 8 | Female | BCP, secondary | +21, der(16)t (1;16) (q2;q2.1) | 1st CR | | uBM | 7/8 allele | CY 120 mg/kg, ETOP 60 mg/kg, Bu 16 mg/kg | no | Alive, 24 mo+ | |
| 3 | 7 | 12 | Male | BCP | +21 | 2nd CR | DM (steroid induced) | rPB | 8/8 allele | Flu 180 mg/m$^2$, Mel 180 mg/m$^2$, ATG 1.25 mg/kg | yes | Alive, 16 mo+ | |
| 4 | 7 | 12 | Male | BCP | +21 | Rel | | uBM | 5/6 allele | Flu 125 mg/m$^2$, Mel 210 mg/m$^2$, TBI 3 Gy(1) | no | Dead, 24 mo | TMA |
| 5 | 8 | 9 | Male | BCP | +21, del(9) | 1st CR | | uBM | 6/6 allele | TBI 12 Gy (6), CY 2800 mg/m$^2$ | yes | Dead, 4 mo | ALL |
| 6 | 9 | 9 | Male | BCP | +21 | IF | | uCB | 5/6 antigen | TBI 8 Gy (4), CY, Tepa | yes | Dead, 11 mo | ALL |
| 7 | 6 | 9 | Male | BCP | +21 | Rel | DM | uCB | 5/6 allele | Flu 125 mg/m$^2$, Mel 140 mg/m$^2$, TBI 6 Gy (3) | no | Alive, 55 mo+ | |
| 8 | 21 | 22 | Female | BCP | NE | Rel | epilepsy hypothyroidism | uBM | 6/6 allele | TBI 12 Gy (6), Mel 180 mg/m$^2$, ETOP 50 mg/kg, Bu 6.4 mg/kg | no | Dead, 5 mo | Bleeding |
| 9 | 5 | 12 | Female | BCP | +21 | >3rd CR | | rBM | 4/8 allele | TBI 12 Gy(6), CY 120 mg/m$^2$ | — | Dead, 0 mo | Infection |
| 10 | 6 | 7 | Female | BCP | +21 | Rel | | rBM | 7/8 antigen | Bu 16 mg/kg, Mel 180 mg/m$^2$, ETOP 50 mg/kg | yes | Dead, 8 mo | Infection |
| 11 | 18 | 19 | Female | BCP | +21 | 1st CR | aortic regurgitation hyperthyroidism DM | uCB | 6/6 allele | TBI 10 Gy(6), CA 12 g/m$^2$, CY 120 mg/m$^2$ | no | Alive, 77 mo+ | |

Diag., diagnosis; mo: months; BCP, B cell precursor; ALL: acute lymphoblastic leukemia; Rel, relapse; CR, complete remission; IF, induction failure; DM, diabetes melitus; uBM, unrelated bone marrow; rPB, related peripheral blood; uCB, unrelated cord blood; rBM, related bone marrow; TBI, total body irradiation; Flu, fludarabine; CY, cyclophosphamide; Bu, busulfan; Mel, melphalan; ETOP, etoposide; CA, cytarabine; NE, data not evaluable; TMA, thrombotic microangiopathy; *1: diagnosis of ALL; *2: 6 allele/antigen = HLA-A, B, DR loci, 8 allele/antigen = HLA-A, B, C, DR loci; *3: fractions of irradiation; *4: the patient did not achieve CR.

**TABLE II. GVHD Prophylaxis Regimens and Transplant-Related Toxicities**

| Case | GVHD prophylaxis | MTX (mg/m$^2$) | acute GVHD | chronic GVHD | Transplant-related toxicities > grade 3 |
|---|---|---|---|---|---|
| 1 | FK, MTX, mPSL | 10 × 1, 7 × 2 | grade III (skin 2, gut 4) | extensive | none |
| 2 | FK, MTX | 10 × 1, 7 × 1 | grade III (skin 3, gut 2) | limited | ARDS, FN, mucositis |
| 3 | CsA, MTX | 10 × 1, 7 × 3 | none | limited | candidemia, pleural effusion, edema, hypokalemia |
| 4 | FK, MTX | 15 × 1, 10 × 2 | grade III (skin 2, gut 3) | extensive | FN, airway obstruction (mucosal damage), mucositis |
| 5 | FK, MTX | 7.5 × 1, 5 × 3 | grade II (skin3) | none | FN, mucositis |
| 6 | CsA, MTX, PSL | NA | grade III | none | aspergillosis, bacterial pneumonia |
| 7 | FK, MTX | 15 × 1, 10 × 3 | grade I (skin 1) | none | FN |
| 8 | FK, MTX | 15 × 1, 10 × 3 | grade III (skin 2, gut 2) | NE | sepsis (pseudomonas aeruginosa), radiation dermatitis, pleural effusion, mucositis |
| 9 | CsA | none | grade III (skin 3, liver 2) | NE | ARDS, VOD, pneumonia, mucositis |
| 10 | CsA, MTX | 5 × 4 | grade III (skin 1, gut 2) | none | sepsis (pseudomonas aeruginosa), mucositis |
| 11 | FK, MTX | 2.5 × 3 | grade II (skin 3, liver 1) | extensive | sepsis (staphylococcus epidermidis), mucositis |

FK, taclorimus; MTX, methotrexate; mPSL, methyl prednisolone; CsA, cyclosporine A; PSL, prednisolone; ARDS, acute respiratory distress syndrome; FN, febrile neutropenia; VOD, veno-occlusive disease; NA, data not available; NE, not evaluable.

consistent with the results in previous studies [14,15]. Patients with DS are vulnerable by nature to respiratory complications, and have been reported to develop ARDS and acute lung injury more frequently compared with children without DS after lung and airway distress such as mechanical ventilation [22]. Such inherited condition of patients with DS could be associated with the high incidence of lung complications after HSCT.

Reducing the intensity of the conditioning regimen is a possible consideration to improve survival after HSCT in patients with DS-ALL. In patients with DS-AML, survival after HSCT was better when a reduced intensity regimen was employed [20]. In patients with ALL, however, the efficacy of a reduced intensity conditioning regimen for HSCT is a matter of debate. Several retrospective studies reported that reduced intensity HSCT for ALL resulted in a higher relapse rate, but comparable survival with myeloablative HSCT [23,24]. Reduction of the conditioning intensity might be beneficial in patients who are vulnerable to toxicities associated with high-dose chemo-radiotherapy, such as children with DS, although further studies are required to evaluate if reduced intensity stem cell transplantation improves survival of patients with DS and high risk ALL.

In this study, the incidence of acute or chronic GVHD was relatively high, consistent with an earlier study [16]. Theoretically, severe mucositis or cutaneous damage after the conditioning regimen might increase the risk of developing GVHD [25]. Impaired thymic function in children with DS which is indicated by low TCR excision circle levels in blood might lead to the high incidence of GVHD [26,27]. GVHD prophylaxis is also a factor associated with the development of GVHD. Most patients in this study received the standard prophylaxis regimen consisting of a calcineurin inhibitor and MTX. However, doses of MTX were rather low in some patients compared to the standard doses [28,29]. Severe MTX-related toxicity in patients with DS has been well characterized. However, it is due to tissue sensitivity and the pharmacokinetics of MTX are not different between DS and patients without DS [18]. Low-dose MTX is possibly not sufficient to repress donor lymphocytes which are more tolerant to MTX than tissues of patients with DS.

Due to the high TRM rate, it is not acceptable to consider HSCT as the standard treatment option for patients with DS-ALL. Discovering appropriate GVHD prophylaxis is one of the solutions to improve survival, such as the use of folinic acid after administering the MTX dose which might allow the use of standard doses of MTX [30,31]. Reducing the intensity of the conditioning regimens is another conceivable option, although it might be associated with an increased risk of ALL relapse. To develop the optimal HSCT procedure for patients with DS-ALL, a study in a larger cohort, which could be achieved by international collaboration, is necessary.

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Original Article

# Pain management during bone marrow aspiration and biopsy in pediatric cancer patients

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**Abstract** ***Background***: The pain associated with bone marrow aspiration and biopsy (BMAB) has an enormous impact on pediatric cancer patients and their families, but no specific reference standards for sedation and analgesia have been developed in Japan. To determine the problems associated with pain management during BMAB, a cross-sectional investigation was conducted.

***Methods***: A survey was sent in October 2011 to data managers in institutions belonging to the Tokyo Children's Cancer Study Group, addressing the non-pharmacological and pharmacological pain management for BMAB performed on pediatric cancer inpatients between January 2010 and December 2010.

***Results***: The eligible response rate was 41 of 57 institutions (71.9%). Non-pharmacological pain intervention was provided in 68% of surveyed institutions. All institutions provided pharmacological pain management. In most institutions, sedation/analgesia was performed by pediatric oncologists in a treatment room in the ward. Standards for pain management were developed and utilized in only four institutions. Other means of pain management were provided in various settings. Twelve institutions reported insufficient sedation/analgesia. In total, 80% of institutions reported some adverse events. Two serious adverse events were reported in cases of underlying or complicated conditions. No serious long-term consequences were reported.

***Conclusions***: Significant issues were identified regarding the efficacy and safety of pain management. Adverse events can occur in any institution. Children with underlying or complicated conditions are at high risk for serious adverse events. Therefore, adequate and systematic assessment, patient monitoring, preparation and treatment for adverse events, and cooperation with skilled specialists of pediatric oncology, anesthesiology, and intensive care are essential.

**Key words** adverse events, bone marrow aspiration and biopsy, procedural analgesia, procedural pain, procedural sedation.

Bone marrow aspiration and biopsy (BMAB) in pediatric cancer patients are described as "moderate to severe painful procedures" by the World Health Organization (WHO).[1] These procedures are performed repeatedly throughout the clinical course of illness. Children have difficulty understanding the necessity of these procedures. The pain associated with BMAB has an enormous impact on children and their families, not only physically but also psychologically. Both short- and long-term negative consequences of insufficient pain management have been reported.[1] The importance of non-pharmacological professional approaches to pain has been emphasized in studies based in Western countries. To improve the safety and efficacy of procedural pain management in patients requiring BMAB, some guidelines have been established by WHO and Western countries, including the USA,[2] Scotland,[3] Australia[4] and Italy.[5] In these guidelines, adequate sedation and analgesia are recommended as part of strict systemic management, but no specific references are made to sedation and analgesia in the clinical guidelines for cancer pain management developed by the Japanese Society for Palliative Medicine,[6] or the guidelines for palliative care of pediatric cancer patients developed by the Children's Cancer Association of

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