[19] Welte K, Reiter A, Mempel K, et al. A randomized phase III study of the efficacy of granulocyte colony-stimulating factor in children with high-risk acute lymphoblastic leukemia. Blood 1996; 87: 3143–3150.

[20] Laver J, Amylon M, Desai S, et al. Randomized trial of r-metHu granurocyte colony-stimulating factor in an intensive treatment for T-cell leukemia and advanced-stage lymphoblastic lymphoma of childhood: a Pediatric Oncology Group pilot study. J Clin Oncol 1998; 16: 522–526.

[21] Clarke V, Dunstan FD, Webb DK. Granulocyte colony-stimulating factor ameliorates toxicity of intensification chemotherapy for acute lymphoblastic leukemia. Med Pediatr Oncol 1999; 32: 331–335.

[22] Michel G, Landman-Parker J, Auclerc MF, et al. Use of recombinant human granulocyte colony-stimulating factor to increase chemotherapy dose intensity: a randomized trial in very high-risk childhood acute lymphoblastic leukemia. J Clin Oncol 2000; 18: 1517–1524.

[23] Saarinen-Pihkala UM, Lanning M, Perkkiö M, et al. Granulocyte-macrophage colony-stimulating factor support in therapy of high-risk acute lymphoblastic leukemia in children. Med Pediatr Oncol 2000; 34: 319–327.

[24] Heath JA, Steinherz PG, Altman A, et al. Human granulocyte colony-stimulating factor in children with high-risk acute lymphoblastic leukemia: a Children's Cancer Group study. J Clin Oncol 2003; 21: 1612–1617.

[25] Pui CH, Boyett JM, Hughes WT, et al. Human granulocyte colony-stimulating factor after induction chemotherapy in children with acute lymphoblastic leukemia. N Engl J Med 1997; 336: 1781–1787.

[26] Bennett CL, Stinson TJ, Lane D, et al. Cost analysis of filgrastim for the prevention of neutropenia in pediatric T-cell leukemia and advanced lymphoblastic lymphoma: a case for prospective economic analysis in cooperative group trials. Med Pediatr Oncol 2000; 34: 92–96.

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ORIGINAL ARTICLE

Respiratory syncytial virus infection in infants with acute leukemia: a retrospective survey of the Japanese Pediatric Leukemia/Lymphoma Study Group

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Abstract Respiratory syncytial virus (RSV) can cause life-threatening complications of lower respiratory tract infection (LRTI) in young children with malignancies, but reports remain limited. We performed a retrospective nationwide survey to clarify the current status of RSV disease among infants with hematological malignancies. Clinical course, treatment, and outcome of patients with hematological malignancies who suffered from RSV infections at the age of <24 months during anti-tumor therapy from April 2006 to March 2009 were investigated by sending a questionnaire to all member institutions of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Twelve patients with acute leukemia were identified as having experienced RSV disease. The primary diseases were acute myeloid leukemia ($n = 8$) and acute lymphoblastic leukemia ($n = 4$). RSV infection occurred pre- or during induction therapy ($n = 8$) and during consolidation therapy ($n = 4$). Eight patients developed LRTI, four of whom had severe pneumonia or acute respiratory distress syndrome; these four patients died despite receiving intensive care. In our survey, the prognosis of RSV disease in pediatric hematological malignancies was poor, and progression of LRTI in particular was associated with high mortality. In the absence of RSV-specific therapy, effective prevention and treatment strategies for severe RSV disease must be investigated.

Keywords Respiratory syncytial virus · Acute leukemia · Infants

Introduction

Survival rate of children with pediatric hematological malignancies has improved dramatically in the recent years [1]. Not only the development of effective anti-tumor therapies, management of infectious complications, either bacterial, fungal, and/or viral, is also critical to maintain and to further improve their prognoses.

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Respiratory syncytial virus (RSV) is a common cause of lower respiratory tract infection (LRTI) mostly in young children, especially in infants [2, 3]. In healthy individuals, RSV generally causes mild and self-limited upper respiratory tract infections (URTI) only. However, in young children, especially those with malignancies undergoing cytotoxic chemotherapy, RSV could cause severe LRTI and is one of the most life-threatening pathogens among all the pathogenic viruses.

Most infants experience initial infection with RSV before the age of 2 years, however, permanent immunity cannot be acquired [4]. The frequency of recurrent infection is high regardless of age, and patients with immunodeficiency are at highest risk of fatal complications. Several reports have suggested that the prognosis of RSV disease in hematological malignancies is poor [5–9]. It has been reported that the mortality rate was high among infants with acute myeloid leukemia (AML) enrolled in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 study, and one of the causes was respiratory complication caused by RSV infection [10]. Because RSV-specific therapy is not yet established, progression of RSV-induced URTI to LRTI is associated with high mortality [6, 9]. A recent study of high-risk pediatric patients, including those with malignancies, noted 12.5 % mortality rate following RSV infections [6, 11].

We, therefore, retrospectively investigated data related to the clinical course, prognosis, and follow-up of RSV disease in Japanese infants with hematological malignancies.

Patients and methods

The subject of this retrospective study was patients with newly diagnosed acute leukemia or lymphoma, and those who suffered from RSV infection at the age of 24 months or younger during curative therapy for their malignant conditions. The survey period of the RSV-infection occurrence was 3 years from April 2006 to March 2009. RSV infection had to be confirmed by rapid RSV testing by immunochromatographic analysis of rhinopharyngeal swabs along with UTRI and/or LTRI symptoms. The presence of applicable patients was confirmed in the primary survey, and information on demographics, laboratory and clinical data, treatment, and outcome were collected for all the subject cases in the secondary survey. These data were collected by questionnaire sent to all 180 centers across the nation that joined the JPLSG studies for the primary survey and selected centers with applicable cases for the secondary survey. The present research was approved by the Institutional Review Board of Fukuoka University Hospital and by the steering committee of the JPLSG.

Results

From the primary survey, we were able to obtain the information from 148 of the 180 JPLSG institutions (82 %). Sixteen cases from the 12 institutions were identified, but 4 cases were excluded from the present analysis because of the following reasons: three were aged older than 24 months at RSV infection and RSV infection was not confirmed by the appropriate RSV testing in one case. As a result, the remaining 12 cases were further analyzed. Patient characteristics are shown in Table 1.

All patients were positive for RSV antigen testing of rhinopharyngeal swabs. Median age at RSV infection was 10 months (range 2–21 months). There were seven boys and five girls. The primary diseases of the 12 patients were all acute leukemia: acute lymphoblastic leukemia (ALL, $n = 4$) and AML ($n = 8$). Patients #11 and #12 had trisomy 21 without heart disease or other complications. No patients had received palivizumab prophylaxis. The timing of RSV disease onset was September to November in eight cases and December to February in four cases.

Two patients (Patients #1 and #6) developed RSV disease before the initiation of induction chemotherapy, which was postponed until the respiratory symptoms had resolved (4 and 29 days, respectively). As a result, these two survived without severe complications. In the remaining ten patients, RSV infection developed during induction therapy in six cases and during consolidation therapy in four cases.

Most common symptoms were cough ($n = 12$) and fever ($n = 10$). In patients with URTI alone (Patients #1, #3 and #7), symptoms resolved within 4–11 days. Other nine patients developed LRTI with symptoms such as wheezing, tachypnea, and retraction (Patients #2, #4–6, and #8–12), and their median disease duration was 28 days (range 11–52 days). Four patients (Patients #4, #8–10) developed severe bronchiolitis, pneumonia, or acute respiratory distress syndrome (ARDS) after 10–39 days, which were all fatal in spite of intensive care including mechanical ventilation. Other complications such as acute myocarditis or encephalopathy were not encountered.

Laboratory data at onset of RSV disease are described as follows. Mean serum C-reactive protein (CRP) level was 0.81 mg/dL (range 0.03–2.64 mg/dL), and immunoglobulin (Ig) G values were $\geq$500 mg/dL (range 518–1055 mg/dL) in all patients. Peripheral white blood cell count was $\leq$1000/μL (range 100–700/μL) in six patients and the lymphocyte count was $\leq$500/μL (range 0–476/μL) in seven patients. Treatment after RSV infection involved intravenous infusion of antibiotics in 11 patients (excluding Patient #1), for a median period of 21 days (rang 11–66 days). Oxygen administration was used in eight of nine patients with LRTI: four of these patients survived

Table 1 Characteristics, treatment, and outcome of the 12 infants who suffered from RSV disease

	Sex	Primary disease	Age at onset of RSV disease (month)	Chemotherapy regimen (phase)	Symptoms	Diagnosis	WBC at onset (10^9/L)	ANC/ lym at onset of RSV disease (10^9/L)	IgG at onset of RSV disease (mg/dl)	Steroid	IVIG	Anti-viral therapy	Oxygen adminis-tration	Out-come
1	F	ALL	13	MLL03 (pre-induction)	Cough, fever	URTI	117.8	5.9/14.1	518	No	No	No	No	Recov-ered
2	F	ALL	2	MLL03 (induction)	Cough	Bronchitis	0.1	-	553	No	No	No	Yes	Recov-ered
3	M	ALL	8	MLL03 (induction)	Cough, fever	ARDS	1.2	0.3/0.9	845	Yes	Yes	Palivi-zumab/ rivabi-rin	Yes	Died (RSV infec-tion)
4	M	ALL	21	JACLS HR02 (consolidation)	Cough, nasal discharge	URTI	0.2	NR	689	No	Yes	No	No	Recov-ered
5	F	AML	11	AML-05 (induction)	Cough, fever	Bronchitis	1.6	7.7/7.8	785	Yes (pulse)	Yes	Palivi-zumab	Yes	Recov-ered
6	M	AML	4	AML-05 (pre-induction)	Cough, fever	Pneumonia	23.3	0.1/1.9	1055	Yes	Yes	Palivi-zumab	Yes	Recov-ered
7	F	AML	20	AML-05 (consolidation)	Cough, fever	URTI	0.1	NR	618	No	Yes	No	No	Recov-ered
8	M	AML	7	AML-05 (induction)	Cough, fever	ARDS	1	0.06/0.09	668	Yes	Yes	No	Yes, MV	Died (RSV infec-tion)
9	M	AML	7	AML-05 (pre-induction)	Cough, fever	Pneumonia	7.8	2.4/4.3	844	Yes (pulse)	Yes	Palivi-zumab	Yes, MV	Died (RSV infec-tion)
10	M	AML	14	CCLSG AML9805RE (induction)	Cough, fever	Bronchitis	1.5	0/0.07	567	Yes (pulse)	Yes	No	Yes, MV	Died (RSV infec-tion)
11	M	AML	17	AML99D (consolidation)	Cough, fever	Bronchitis	0.1	NR	592	No	Yes	No	No	Recov-ered
12	F	AML	9	AML99D (consolidation)	Cough, fever	Bronchitis	0.7	2.0/4.8	538	Yes	Yes	No	Yes	Recov-ered

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *ANC* absolute neutrophil count, *ARDS* acute respiratory distress syndrome, *F* female, *IVIG* intravenous immunoglobulin, *lym* lymphocyte, *M* male, *MV* mechanical ventilation, *NR* not recorded, *URTI* upper respiratory tract infections, *MLL03* The JPLSG trial for MLL-gene-rearrangement positive infantile ALL of the JPLSG [19], *JACLS HR02* The Japan Association of Childhood Leukemia Study (JACLS) trial for high risk pediatric B-precursor ALL, *AML-05* The JPLSG study for pediatric AML[10], *CCLSG AML9805RE* The Children's Cancer and Leuekmia Study Group (CCLSG) trial for refractory pediatric AML, A*ML99D* The Japanese AML cooperative study group trial for patients with Down syndrome (DS) and AML [20]

and their symptoms improved after 14–52 days of oxygen administration, while the other four patients did not survive despite receiving intensive care including mechanical ventilation, high-dose steroid treatment, etc.

Discussion

RSV is one of the most important respiratory viruses causing severe infections in patients with hematological malignancies. Previous reports showed that the occurrence of RSV infection during AML chemotherapy was about 10 %, and the RSV-associated mortality rate was 0.2 % [6, 10]. In our survey, RSV occurred in 12 patients, and 4 of the 12 patients died from RSV infection. This suggested that the prognosis was also poor in our study population.

RSV infection occurred most frequency during the induction phase ($n = 8$), which was the case in all the four fatal cases. Two patients were diagnosed with RSV infection prior to treatment and their induction chemotherapy was postponed until the symptoms had resolved: serious complications did not occur in these patients (Patients #1 and #5). It is therefore important to consider postponing chemotherapy in patients who are diagnosed with RSV infection prior to starting chemotherapy.

High mortality rate has been reported in cases with hematological malignancies complicated by RSV infection [5–7, 12]. In particular, the mortality rate was 50 % or higher for patients in whom disease status progressed from URTI to LRTI [6, 11]. Various reports have shown that progression to LRTI is an important prognostic factor [6, 11, 12]. In our study, symptoms improved within approximately 10 days in the three patients who only had upper respiratory symptoms. Nine patients had LRTI. Four of them developed severe pneumonia or ARDS and did not survive despite receiving intensive care including mechanical ventilation. Four of the five surviving patients required several weeks of oxygen administration. Other reported risk factors are aged under 3 years, old age, and lymphocytopenia [6, 7, 13, 14]. In our study, lymphocytopenia was detected in seven patients (58.3 %).

Effective treatment for RSV infection has not been established yet [6, 7, 13, 14]. Although efficacy has not been confirmed, steroids are often used to manage RSV infection. In our study, steroids were used in seven patients with progressive LRTI, although symptoms were not improved in any of these patients. Moreover, steroid pulse therapy was administered in four of these patients, and three of them died. Although many institutions used intravenous immunoglobulin therapy aiming an antiviral effect, no obvious effect could be seen in our series. Use of palivizumab, anti-RSV humanized monoclonal antibody, is established for preventing severe RSV infections for young children with congenital heart disease, with history of premature infancy, and/or with history of bronchopulmonary dysplasia. In the current study, no patients underwent prophylaxis with palivizumab, but four patients received palivizumab for treatment after developing severe respiratory failure, and symptoms improved in two of them. This finding suggests the effect of palivizumab as a potential treatment choice and warrants for evaluation in future studies. As of anti-viral agents, aerosolized ribavirin therapy has been used for high-risk patients, but this formula is generally not available in Japan [15–18]. Moreover, ribavirin has shown poor efficacy when used as monotherapy. Some reports have suggested that ribavirin is effective in preventing the progression of LRTI when combined with high-titer immunoglobulin, palivizumab, or other treatment options during the URTI period. Further investigation is necessary since the number of patients reported in each study is small [15–18]. In our study, aerosolized ribavirin and intramuscular palivizumab were used after progression to LRTI in one patient (Patient #11), but unfortunately did not work. To summarize, there are no established effective treatment for RSV infections to date, and more effective anti-viral therapy for RSV is urgently required for these high-risk cases.

Because RSV only causes mild symptoms when infected in healthy individuals including families or health care providers, it is quite difficult to completely prevent RSV transmission to the patient. Therefore, it is important to immunize the patients themselves to protect them from RSV. Since August 2013, prophylactic administration of palivizumab has been expanded for use in patients younger than 2 years with immunodeficiency in Japan, including patients with malignancies [7]. It is expected that this expansion of palivizumab indication would effectively prevent severe RSV infection in Japanese patients with hematological malignancies, although its efficacy must be further evaluated. However, as many of the cases suffer from RSV infections before or during the initial induction course as shown in the present study, prophylactic palivizumab might not be in time for effective prevention if the patients are already exposed to RSV shortly before or at diagnosis. In that sense, universal vaccination of RSV, which is currently underway, would be more effective.

In conclusion, the prognosis of RSV disease in pediatric hematological malignancies was poor. Because the effective treatment strategy is not yet established, the effective prophylaxis and treatment strategies for severe RSV disease must be investigated.

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Compliance with ethical standards

Conflicts of interest The authors have no financial relationships or other conflicts of interest to disclose relevant to this article to disclose.

References

1. Pui CH, Evans WE. A 50-year journey to cure childhood acute lymphoblastic leukemia. Semin Hematl. 2013;50(3):185–96.
2. Hall CB, Powell KR, MacDonald NE, Gala CL, Menegus ME, Cohen HJ, et al. Respiratory syncytial viral infection in children with compromised immune function. N Engl J Med. 1986;315:77–81.
3. Nair H, Nokes DJ, Gessner BD, Dherani M, Madhi SA, Cambell H, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systemic review and meta-analysis. Lancet. 2010;375:1545–55.
4. Lambert L, Sagfors AM, Openshaw PJ, Culley FJ. Immunity to RSV in early-life. Front Immunol. 2014;5:466.
5. Wendt CH, Hertz MI. Respiratory syncytial virus and parainfluenza virus infections in the immunocompromised host. Semin Respir Infect. 1995;10:224–31.
6. Shachor-Meyouhas Y, Zaidman I, Kra-Oz Z, Arad-Cohen N, Kassis I. Detection, control and management of a respiratory syncytial virus outbreak in a pediatric hematology-oncology department. J Pediatr Hematol Oncol. 2013;35:124–8.
7. Mori M, Morio T, Ito S, Morimoto A, Hara T, Saji T, et al. Risk and prevention of severe RS virus infection among children with immunodeficiency and Down's syndrome. J Infect Chemother. 2014;20:455–9.
8. Sung L, Alonzo TA, Gerbing A, Aplenc R, Woods WG, Gamis AS, et al. Respiratory syncytial virus infections in children with acute myeloid leukemia: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2008;51:784–6.
9. Camplin RE, Whimbey E. Community respiratory virus infections in bone marrow transplant recipients: the M.D. Anderson Cancer Center experience. Biol Blood Marrow Transplant. 2001;7(Suppl):8S–10S.
10. Tomizawa D, Tawa A, Watanabe S, Saito AM, Taga T, Adachi S, et al. Appropriate dose reduction in induction therapy is essential for the treatment of infants with acute myeloid leukemia: a report from the Japanese Pediatric Leukemia/Lymphoma Study Group. Int J Hematol. 2013;98:578–88.
11. Ljungman P. Respiratory syncytial virus infection in stem cell transplant patients: the European experience. Biol Blood Marrow Transplant. 2001;7(Suppl):5S–7S.
12. Anak S, Aay D, Unvar A, Garipardic M, Agaoglu L, Devecioglu O, et al. Respiratory syncytial virus infection outbreak among pediatric patients with oncologic diseases and/or BMT. Pediatr Pulmonol. 2010;45:307–11.
13. Lehners N, Schinitzler P, Geis S, Puthenparambil J, Benz MA, Egerer G, et al. Risk factors and containment of respiratory syncytial virus outbreak in a hematology and transplant unit. Bone Marrow Transplant. 2013;48:1548–53.
14. Sandherr M, Einsele H, Hebart H, Kahl C, Kiehl M, Comely OA, et al. Antiviral prophylaxis in patients with haematological malignancies and solid tumours: guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society for Hematology and Oncology (DGHO). Ann Oncol. 2006;17:1051–9.
15. Shah DP, Ghantoji SS, Shah JN, El Toum KK, Jiang Y, Chemaly RF, et al. Impact of aerosolized ribavirin on mortality in 280 allogeneic hematopoietic stem cell transplant recipients with respiratory syncytial virus infections. J Antimicrob Chemother. 2013;68:1872–8.
16. Shah JN, Chemaly RF. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. Blood. 2011;117:2755–63.
17. Adams RH. Preemptive treatment of pediatric bone marrow transplant patients with asymptomatic respiratory syncytial virus infection with aerosolized ribavirin. Biol Blood Marrow Transplant. 2001;7(Suppl):16s–8s.
18. Chávez-Bueno S, Mejias A, Merryman RA, Ahmad N, Jafri HS, Ramilo O. Intravenous palivizumab and ribavirin combination for respiratory syncytial virus disease in high-risk pediatric patients. Pediatr Infect Dis J. 2007;26:1089–93.
19. Koh K, Tomizawa D, Moriya-Saito A, Watanabe T, Miyamura T, Ishii E, et al. Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene- rearrangement- positive acute lymphoblastic leukemia. Leukemia. 2015;29:290–6.
20. Kudo K, Kojima S, Tabuchi K, Yabe H, Tawa A, Tsukimoto I, et al. Prospective study of pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with down syndrome and acute myeloid leuekmia: the Japanese Childhood AML Cooperative Study Group. J Clin Oncol. 2007;25:5442–7.

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Preserved High Probability of Overall Survival with Significant Reduction of Chemotherapy for Myeloid Leukemia in Down Syndrome: A Nationwide Prospective Study in Japan

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Background. On the basis of results of previous Japanese trials for myeloid leukemia in Down syndrome (ML-DS), the efficacy of risk-oriented therapy was evaluated in the Japanese Pediatric Leukemia/Lymphoma Study Group AML-D05 study. ***Procedure.*** All patients received induction chemotherapy that consisted of pirarubicin, intermediate-dose cytarabine, and etoposide. Patients who achieved complete remission (CR) after initial induction therapy were stratified to the standard risk (SR) group and received four courses of reduced-dose intensification therapy. Patients who did not achieve CR were stratified to the high risk (HR) group and received intensified therapy that consisted of continuous or high-dose cytarabine. ***Results.*** A total of 72 patients were eligible and evaluated. One patient died of sepsis during initial induction therapy. Sixty-nine patients were stratified to SR and two patients to HR. No therapy-related deaths were observed during intensification therapy. The 3-year event-free and overall survival rates were 83.3% ± 4.4% and 87.5% ± 3.9 %, respectively. Age at diagnosis less than 2 years was a significant favorable prognostic factor for risk of relapse ($P = 0.009$). ***Conclusions.*** The attempt of risk-oriented prospective study for ML-DS was unsuccessful, but despite the dose reduction of chemotherapeutic agents, the overall outcome was good, and further dose reduction might be possible for specific subgroups. Pediatr Blood Cancer 2016;63:248-254. © 2015 Wiley Periodicals, Inc.

Key words: acute myeloid leukemia; clinical trial; Down syndrome

INTRODUCTION

Myeloid leukemia in Down syndrome (ML-DS) has unique characteristics: predominance of acute megakaryoblastic leukemia; age predilection during the first 4 years of life; and higher sensitivity to chemotherapeutic agents, which translates into a good treatment response, as well as increased treatment-related toxicities compared with nonDS children with acute myeloid leukemia (AML). As a result, children with ML-DS are treated separately from nonDS AML children with less intensive treatment in recent clinical studies in developed countries.[1–5]

In Japan, a multi-institutional prospective study, designated the AML99 Down study, was conducted and enrolled 72 patients from 2000 to 2004 by the Japanese AML Cooperative Study Group (consisting of Tokyo Children's Cancer Study Group [TCCSG], Japan Association of Childhood Leukemia Study [JACLS], and Kyushu Yamaguchi Children's Cancer Study Group [KYCCSG]). It evaluated a slightly modified regimen from the previous trial using pirarubicin instead of daunorubicin and a reduced total number of treatment courses from six to five.[6] The 3-year overall survival (OS) and event-free survival (EFS) rates were 84% and 83%, respectively, and treatment-related mortality (TRM) was only 1.4%. In this study, failure to achieve M1 marrow after initial induction was a poor prognostic factor. During the same period, another group,

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

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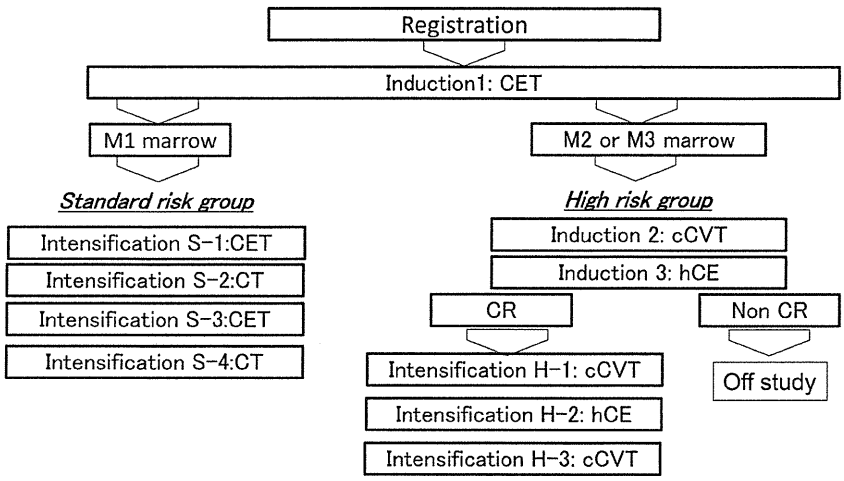

Fig. 1. Protocol scheme of the JPLSG AML-D05 study. CET: pirarubicin (25 mg/m^2 per day via 1-hr infusion on Days 1 and 2), cytarabine (100 mg/m^2 per day via 1-hr infusion on Days 1–7), etoposide (150 mg/m^2 per day via 2-hr infusion on Days 3–5). CT: CET without etoposide. cCVT: pirarubicin (40 mg/m^2 via 1-hr infusion on Day 1), vincristine (1 mg/m^2 intravenous on Day 2), cytarabine (100 mg/m^2 per day via 24-hr continuous intravenous infusion on Days 1–7). hCE: HDCA (1 g/m^2 via 2-hr infusion every 12 hr on Days 1–5), etoposide (100 mg/m^2 per day via 2-hr infusion on Days 2–4).

the Japanese Childhood Cancer and Leukemia Study Group (CCLSG), performed a prospective study using continuous or high-dose cytarabine combination chemotherapy for patients with ML-DS.[7] Of the 24 patients enrolled from 1998 to 2006, 21 achieved complete remission (CR), and three patients died during remission induction therapy because of serious infection. All but one patient maintained CR without serious complications. As shown in these previous studies, the majority of patients with ML-DS could be cured with relatively mild chemotherapy compared with children with AML in nonDS, but it is rarely salvageable once they relapse.[6] In order to further improve the outcome of children with ML-DS, it is necessary to identify a subgroup with a poor prognosis and prevent relapse for these cases. On the basis of these results, a nationwide prospective study for ML-DS by the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG), in which the four existing pediatric leukemia study groups (TCCSG, JACLS, KY-CCSG, and CCLSG) were merged, was planned and conducted.

PATIENTS AND METHODS

Patients

Between January 2008 and December 2010, patients with ML-DS entered the JPLSG AML-D05 study after informed consent was obtained. The eligibility criteria of this study were as follows: (i) patients of DS with myelodysplastic syndrome (MDS) or AML;[8] (ii) age older than 4 months and younger than 18 years old; (iii) patients with sufficient organ function; (iv) patients with cardiac disease were also eligible if without serious complications; (v) no history of previous chemotherapy (except use of cytarabine for transient abnormal myelopoiesis [TAM]) or radiation therapy. Patients with TAM and patients with central nervous system (CNS) leukemia were not eligible. This trial was registered with the UMIN Clinical Trials Registry (UMIN-CTR, URL: http://www.umin.ac.jp/ctr/index.htm), number UMIN000000989.

Treatment

The details of treatment in the JPLSG AML-D05 study are shown in Figure 1. The patients received one course of induction therapy with cytarabine, etoposide and pirarubicin (THP-adriamycin), designated as CET, which was the same as AML99 Down study induction, and they were then stratified into two risk groups (standard risk [SR] or high risk [HR]) according to the morphological response. Patients with a good response (SR: M1 marrow achieved after initial CET) received less intensive chemotherapy, in which the total dose of etoposide was reduced compared with the AML99 Down protocol (omitted at the 2nd and 4th courses). For those with a poor response (HR: M1 marrow not achieved after initial CET), a salvage regimen with more intensive use of cytarabine was given either by 24-hr continuous infusion or high-dose, as in the CCLSG AML 9805 Down study. No intrathecal chemotherapy was given.

ML-DS Older than 4 Years Old

ML-DS typically develops in patients younger than 4 years old and patients older than 4 years have different biology, such as no magakaryoblasts phenotype, no *GATA1* mutation of blasts, and poor prognosis compared with "typical ML-DS."[9] Actually, in the clinical therapeutic studies of International BFM Study Group (I-BFM) and Children's Oncology Group (COG) for ML-DS, patients older than 4 years were not included. However, an optimal therapeutic approach for these "atypical" ML-DS has not been established. Because there are a few case reports of the patients older than 4 years old in Japan who were successfully treated with less intensive regimen,[10,11] patients older than 4 years old were eligible for this AML-D05 study.

TABLE I. Characteristics of the Patients in the JPLSG AML-D05 Study

Characteristic	No.	%	Characteristic	No.	%
Age, months			*WBC (×10⁹/l)*		
Median	19		Median	4.9	
4–11	8	11	Range	1.2–38.8	
12–23	40	56	*Hb (g/dl)*		
24–35	14	19	Median	9.8	
36–48	5	7	Range	3.7–14.8	
49-	5	7	*Plt (×10⁹/l)*		
Sex			Median	36	
Male	40	56	Range	0.3–312	
Female	32	44	*Cytogenetics*		
21 trisomy mosaic			Normal karyotype[a]	19	26
Yes	3	4	Monosomy 7	10[b]	14
No	64	89	Sole trisomy 8[c]	3	4
Unknown	5	7	Complex	20[b]	28
History of TAM			Others	22	31
Yes	35	49	Unsuccessful	3	4
No	26	36	*GATA1 mutation*		
Unknown	11	15	Yes	28	38
Cardiac complication			No	6	8
Yes	48	67	Unknown	38	54
No	21	29	*FLT3-ITD*		
Unknown	3	4	Wild type	70	97
FAB classification			Mutated	1	1
M7	16	22	Unknown	1	1
RAEB-T	13	18			
RAEB	35	49			
RA	4	6			
Others	4	6			

[a]Normal karyotype means 47,XX,+21c or 47,XY,+21c. [b]Karyotype in five cases showed complex including monosomy 7. [c]Trisomy 8 only in addition to constitutional trisomy 21.

GATA1 Status

GATA1 status of blasts was examined retrospectively for a limited number of patients in this study. Target deep sequencing analysis was performed for detecting mutations in the second and third exons of the *GATA1* gene. Amplification of the locus was accomplished by long PCR reactions using KOD-FX-Neo DNA polymerase (TOYOBO, Osaka, Japan). Used Primers were 135 sense (5′-AGGTAGAAGCAGATGAGAGTGGA-3′) and AS3 (5′-GTGGGGTGGAGAGGAGAAGAGGGA-3′). The PCR products were processed for library preparation after determination of their quantity by the Qubit dsDNA HS Assay (Life Technologies, Invitrogen Division, Darmstadt, Germany). Libraries were prepared using the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA) according to the manufacturer's recommendation. Sequencing reactions were carried out using the MiSeq v2 (2 × 150 bp) chemistries (Illumina). The MiSeq re-sequencing protocol for amplicon was performed. The sequences were mapped on the human GRCh37/hg19 assembly and quality checked using the on-board software MiSeq Reporter, and analyzed by CLC Genomics Workbench software (CLC bio, Aarhus, Denmark).

Definitions and Statistics

The initial diagnosis of ML-DS was evaluated by central review (morphology, flow cytometry, and cytogenetics). In cases of dry tap marrow, the diagnosis was made by bone marrow biopsy. Evaluation of each treatment was performed after absolute neutrophil count (ANC) > 500/μl and platelets > 50,000/μl in peripheral blood were reached. Treatment response, also evaluated by central review, was defined as follows: M1 marrow, less than 5% blasts in the bone marrow; M2 marrow, more than 5% and less than 25% blasts; M3 marrow, more than 25% blasts; and CR, M1 marrow with regeneration of normal hematopoiesis and no leukemia-related symptoms or extramedullary leukemic invasion observed.

Descriptive statistical analyses to assess baseline characteristics and the clinical course of patients diagnosed with ML-DS were performed using Chi-square tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. OS was defined as the length of time from registration in the AML-D05 study to death from any cause. EFS was defined as the length of time from the diagnosis of AML to the last follow-up or first event (failure to achieve remission, relapse, secondary malignancy, or death from any cause). OS and EFS percentages and standard errors were calculated using the Kaplan–Meier method. A competing-risks regression based on Fine and Gray's proportional subhazards model was used to investigate risk factors that were associated with relapse after registration. Variables including sex (female vs. male), age at initial diagnosis (≤2 years vs. >2 years, ≤4 years vs. >4 years), WBC count at initial diagnosis (≥20,000/μl vs. <20,000/μl), FAB morphology at initial diagnosis (M7 vs. MDS vs. others), SR vs. HR, chromosomal abnormalities of blasts (normal karyotype;

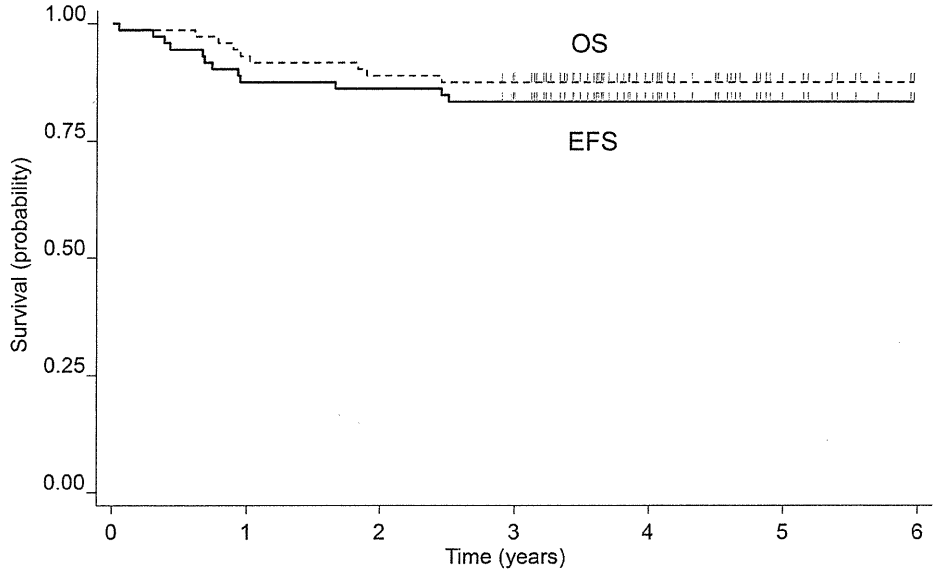

Fig. 2. Event-free and overall survival curves of the JPLSG AML-D05 study. The 3-year event-free and overall survival rates are 83.3% ± 4.4% and 87.5% ± 3.9 %, respectively.

47, XX, +21c or 47, XY, +21c vs. monosomy 7 vs. others), *FLT3*-ITD status of blasts (wild type vs. mutated), and *GATA1* status of blasts (normal vs. mutated), that were significantly associated with relapse on univariate analyses were considered for inclusion in the model. Significant variables associated with survival were then identified. Two-sided *P* values greater than 0.05 were interpreted with caution. All data analyses were performed using STATA version 13.0 (STATA Corp., College Station, TX). Follow-up data were actualized as of December 31, 2013.

RESULTS

Patient Characteristics

A total of 74 patients were registered in this study. Two patients were not eligible because of misdiagnosis and uncontrollable cardiac failure, respectively. For analysis of the results, 72 patients were eligible. The relevant initial clinical and hematological data of the 72 patients in this study are shown in Table I. Males predominated. Age ranged from 10 months to 206 months (median, 19 months), and all but five patients were younger than 4 years old. Three patients had mosaic 21 trisomy, 16 patients were diagnosed as M7, and 13 as refractory anemia with excess blasts in transformation (RAEB-T), 35 as RAEB, four as refractory anemia (RA), and four as others (M2, and about half the patients had a history of TAM). About two-thirds of the patients ($n = 48$) had cardiac disease at presentation. Morphologically, leukemic cells were M4, M5a, and M6 by the French–American–British (FAB) classification. The 11 of RAEB and one of RA patients had megakaryoblasts by bone marrow biopsy. Karyotype analysis showed sole monosomy 7 in one patient, monosomy 7 associated with a ring or marker chromosome excluding complex karyotype in four patients, monosomy 7 associated with a ring or marker chromosome including complex karyotype in three patients, t(8;21)(q22;q22) with FAB M2 morphology in one, complex karyotype (defined by the presence of a clone with at least three unrelated cytogenetic abnormalities) in 20 patients, and 19 patients with otherwise normal karyotype and sole constitutional trisomy 21. All but one patient were examined for *FLT3*-ITD status, and only one patient had mutated *FLT3*-ITD blasts.

Treatment Outcome

Overall, 69 (95.8%) of 72 patients achieved CR after initial CET and were stratified to the SR group. Two patients who did not achieve CR after induction therapy CET were stratified to the HR group, and both patients achieved CR by salvage induction therapy. One patient died of sepsis after initial CET. One patient was removed from the trial due to the physician's decision, with pathological cells in the bone marrow (less than 5%), during intensification therapy. No therapy-related deaths were observed during intensification therapy.

Ten patients relapsed in the bone marrow; nine were in the SR group, and two relapsed during intensification therapy. One patient was in the HR group and relapsed during intensification. No extramedullary relapse, including CNS, was observed.

The 3-year EFS and OS rates were 83.3% ± 4.4% and 87.5% ± 3.9%, respectively (Fig. 2). No patients with secondary cancer or severe cardiotoxicity were observed.

Toxic Events

A grade 4 adverse event (elevation of liver enzyme; AST/ALT) was observed in one patient (one event) during induction therapy with CET and in one patient (one event) during the intensification phases. No toxic deaths were observed during remission. Therapy-related mortality of this study was 1.4%.

TABLE II. Patients Older than 4 Years at Initial Diagnosis in the JPLSG AML-D05 Study

Registration code	Age (Mo)at diagnosis	Gender	History of TAM	WBC	Morphology	Chromosome	*GATA1* mutation	*FLT3* -ITD	Risk	Prognosis
#06	206	Male	No	11,300	FAB M2	47,XY,t(8;21) (q22;q22),+21c	No	Wild type	HR	DFS (68+ months)
#09	193	Male	Unknown	38,800	FAB M5a	Complex	No	Mutated	SR	BM relapse (19 months)
#19	204	Male	No	1,300	FAB M4	47,XY,+21c	No	Wild type	SR	DFS (59+ months)
#46	58	Female	Yes	1,200	RAEB	Complex	Not examined	Wild type	SR	DFS (47+ months)
#64	57	Male	No	6,710	FAB M6	47,XY,+21c	Not examined	Wild type	SR	BM relapse (30 months)

DFS, disease-free survival.

ML-DS Older than 4 Years Old

Table II shows the characteristics of all five patients older than 4 years at initial diagnosis. All four patients had nonFAB M7 phenotype, and three of them are alive without relapse. One patient who relapsed had *FLT3-ITD* mutated blasts.

Prognostic Factors

Several predictive factors for relapse were evaluated by univariate and multivariate analyses (Table III). Age at diagnosis less than 2 years old was a significant favorable prognostic factor on both univariate and multivariate analyses. Only one patient had *FLT3*-ITD, and this was a significant poor prognostic factor. Other factors, including sex, high WBC counts (>20,000/μl), FAB morphologies (non M7), and chromosomal abnormalities (normal karyotype or monosomy 7), did not adversely affect the risk of relapse.

DISCUSSION

We report a Japanese nationwide prospective study of ML-DS, which followed the previous Japanese strategy with a very low-intensity chemotherapy regimen. This study tested risk-oriented therapy and aimed to salvage the HR patients with a poor prognosis (M2 or M3 marrow after initial induction therapy with CET) based on a previous AML99 Down study, by adding more intensive use of cytarabine given either by 24-hr continuous infusion or high dose. About 10% of the patients had been anticipated to be stratified into the HR group, but unexpectedly, only two patients were actually assigned to this group, so that evaluation of the efficacy of this strategy was difficult. On the other hand, for the good prognosis group (SR; M1 marrow after initial induction therapy with CET), the cumulative dose of etoposide was reduced from 1,800 mg/m^2 to 900 mg/m^2 in the intensification phases compared to the previous AML99 Down study. The 3-year EFS of this group was 85.5% ± 4.2%, which was comparable with the previous AML 99 Down study,

TABLE III. Cumulative Relapse Rate of the JPLSG AML D-05 Study (Competitive Risk Model)

		Univariate analysis[a]			Multivariate analysis[a,b]					
					Model 1			Model 2		
	n	HR	(95% CI)	*P*	HR	(95% CI)	*P*	HR	(95% CI)	p
Sex (ref: female) male	68	2.08	(0.55–7.92)	0.284	1.79	(0.31–10.22)	0.514	1.59	(0.37–6.81)	0.533
Age (ref: <2 y) ≥2 y	68	7.92	(1.68–37.22)	0.009	7.33	(1.76–30.50)	0.006		–	–
Age (ref: <3 y) ≥3 y	68	5.96	(1.78–19.88)	0.004		–	–	18.20	(1.29–256.85)	0.032
Standard risk (ref: high risk) no	68	5.42	(0.57–51.69)	0.142	1.56	(0.11–22.62)	0.744	0.35	(0.00–39.27)	0.663
M7 phenotype (ref: M7+RAEB-T & RAEB or RA) Others	68	2.10	(0.23–19.17)	0.511	1.49	(0.09–25.89)	0.786	3.91	(0.45–33.92)	0.216
Chromosome abnormality										
Normal karyotype (ref: No) Yes	68	0.62	(0.13–2.89)	0.543	0.64	(0.09–4.45)	0.651	0.18	(0.02–1.45)	0.107
−7 (ref: No) Yes	68	2.54	(0.70–9.20)	0.156	3.72	(0.47–29.67)	0.215	2.98	(0.46–19.14)	0.250
Complex (ref: No) Yes	68	0.56	(0.12–2.53)	0.450	0.16	(0.01–2.25)	0.176	0.06	(0.00–1.33)	0.075
FLT3-ITD (ref: Negative) Positive	68	7.86	(3.93–15.74)	0.000	3.69	(0.64–21.12)	0.143	2.34	(0.30–18.38)	0.417

HR, hazard ratio; CI, confidence interval; ref, reference category. Model 1: adjustment for age (<2 y vs. ≥2 y), BMA2 CR (yes vs. no), M7 phenotype (M7+RAEB-T & RAEB or RA vs. others), chromosome abnormality: normal karyotype (no vs. yes), chromosome abnormality: −7 (no vs. yes), chromosome abnormality: complex (no vs. yes) and *FLT3*-ITD (negative vs. positive). Model 2: adjustment for age (<3 y vs. ≥3 y), BMA2 CR (yes vs. no), M7 phenotype (M7+RAEB-T & RAEB or RA vs. others), chromosome abnormality: normal karyotype (no vs. yes), chromosome abnormality: −7 (no vs. yes), chromosome abnormality: complex (no vs. yes) and *FLT3*-ITD (negative vs. positive).
[a]Competing-risks regression based on Fine and Gray's proportional subhazards model. [b]Forced entry method.

TABLE IV. Recent clinical trials for ML-DS

Study	Years	*N*	Daunorubicin (pirarubicin) (mg/m^2)	Ara-C (mg/m^2)	Etoposide (mg/m^2)	TRM (%)	OS (%)	EFS (%)	Ref.
BFM98 for DS	98–03	67	220–240	23–29,000	950	5	91	89 (3 y)	1
NOPHO AML93	88–02	41	300	48,600	1,600	5	NA	85 (8 y)	2
MRC AML10/12	88–02	46	670	10,600	0	15	74	74 (5 y)	3
CCG 2861/2891	89–99	160	320	15,800	1,600	4	79	77 (6 y)	4
COG A2971	99–03	132	320	27,200	0	3	84	79 (5 y)	5
LD-cytarabine	90–03	34	0	7,400	0	0	77	67(5 y)	13
AML99 DS	00–04	72	(250)	3,500	2,250	1	84	83 (4 y)	6
CCLSG 9805DS	98–06	24	(190)	12,600	200	12.5	88	83 (5 y)	7
JPLSG AML D05	08–10	72	(250)(SR) (170)(HR)	3,500(SR) 12,800(HR)	1,350(SR) 1,050(HR)	1	88	83(3 y)	

TRM, treatment-related mortality; OS, overall survival; EFS, event-free survival; NA, not evaluated; SR, standard risk; HR, high risk.

in which the 3-year EFS of patients who achieved M1 marrow after one cycle of induction CET was 87.3% ± 8.6%.

Age at diagnosis less than 2 years old was a significant favorable prognostic factor for risk of relapse. These results were comparable with the CCG 2891 study.[4]. Age more than 4 years old, which is thought to have different biology with poor prognosis, was not a poor prognostic factor. This AML-D05 study included patients older than 4 years, thought to be "atypical ML-DS;" statistical analyses only for the patients younger than 4 years old were also done, but age at diagnosis less than 2 years old was again a significant favorable prognostic factor for risk of relapse (Supplemental Table I). Monosomy 7, which was a significant poor prognostic factor in the AML 99 Down study, normal karyotype, and high white blood cell counts ($\geq 20 \times 10^9$/l), which were significant poor prognostic factors in large international retrospective study by I-BFM,[12] were not in this AML-D05 study. *GATA1* status, which was examined only a limited number of patients, was not a prognostic factor.

Recent clinical trials for ML-DS are summarized in Table IV. Less intensive chemotherapy compared with Western countries had been done in Japan for ML-DS, but treatment outcomes were comparable, and despite the dose reduction of chemotherapeutic agents compared with our previous studies, the overall outcome was good. Combined with the results of the Toronto group study with an ultra-low-dose cytarabine-based regimen, which contained no anthracyclines and no etoposide,[13] further dose reduction might be possible for specific subgroups. On the other hand, most relapses occurred in the SR group defined by morphological treatment response, and relapsed cases are rarely salvageable, even in those receiving stem cell transplantation.[14] Thus, in terms of treatment outcome, ML-DS is a heterogeneous disease and risk-oriented therapy as in the present study is a reasonable strategy for ML-DS. Unexpectedly, risk stratification by morphological treatment response in the present study did not work well, with few HR patients. To find a more accurate method for identification of the poor prognostic subgroup, we are currently analyzing the role of minimal residual disease with various methods (flow cytometry, PCR, WT1 expression, and *GATA1* mutation) in the ongoing JPLSG AML-D11 study (UMIN000007237), in which the treatment protocol is the same as the AML-D05 study. Moreover, new therapeutic approaches using new drugs such as MK-1775 (Wee1 inhibitor),[15] MNL9237 (Aurora kinase inhibitor) and histone deacetylase inhibitors will be needed for relapsed/refractory cases.[16,17]

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REFERENCES

1. Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia 2005;19:1355–1360.
2. Abildgaard L, Ellebaek E, Gustafsson G, Abrahamsson J, Hovi L, Jonmundsson G, Zeller B, Hasle H. Optimal treatment intensity in children with Down syndrome and myeloid leukaemia: Data from 56 children treated on NOPHO-AML protocols and review of the literature. Ann Haematol 2006;85:275–280.
3. Rao A, Hills RK, Stiller C, Gibson BE, de Graaf SS, Hann IM, O'Marcaigh A, Wheatley K, Webb DK. Treatment for myeloid leukaemia of Down syndrome: Population-based experience in the UK and results from the Medical Research Council AML 10 and AML 12 trials. Br J Haematol 2006;132:576–583.
4. Gamis AS, Woods WG, Alonzo TA, Buxton A, Lange B, Barnard DR, Gold S, Smith FO. Increased age at diagnosis has a significantly negative effect on outcome in children with Down syndrome and acute myeloid leukemia: A report from the Children's Cancer Group Study 2891. J Clin Oncol 2003;21:3415–3422.
5. Sorrell AD, Alonzo TA, Hilden JM, Gerbing RB, Loew TW, Hathaway L, Barnard D, Taub JW, Ravindranath Y, Smith EO, Arceci RJ, Woods WG, Gamis AS. Favorable survival maintained in children who have myeloid leukemia associated with Down syndrome using reduced-dose chemotherapy on Children's Oncology Group trial A2971: A report from the Children's Oncology Group. Cancer 2012;118:4806–4814.
6. Kudo K, Kojima S, Tabuchi K, Yabe H, Tawa A, Imaizumi M, Hanada R, Hamamoto K, Kobayashi R, Morimoto A, Nakayama H, Tsuchida M, Horibe K, Kigasawa H, Tsukimoto I. Prospective study of a pirarubicin, intermediate-dose cytarabine, and etoposide regimen in children with Down syndrome and acute myeloid leukemia: The Japanese Childhood AML Cooperate Study Group. J Clin Oncol 2007;25:5442–5447.
7. Taga T, Shimomura Y, Horikoshi Y, Ogawa A, Itoh M, Okada M, Ueyama J, Higa T, Watanabe A, Kanegane H, Iwai A, Saiwakawa Y, Kogawa K, Yamanaka J, Tsurusawa M. Continuous and high-dose cytarabine combined chemotherapy in children with Down syndrome and acute myeloid leukemia: Report from the Japanese Children's Cancer and Leukemia Study Group (JCCLSG) AML 9805 Down Study. Pediatr Blood Cancer 2011;57:36–40.
8. Baumann I, Niemeyer CM, Brunning RD, Porwit A. Myeloid proliferations related to Down syndrome. In Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thile J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edition. Lyon: IARC; 2008: p. 142–144.
9. Hasle H, Abrahamsson J, Arola M, Karow A, O'Marcaigh A, Reinhardt D, Webb DK, van Wering E, Zeller B, Zwaan CM, Vyas P. Myeloid leukemia in children 4 years or older with Down syndrome often lacks GATA1 mutation and cytogenetics and risk of relapse are more akin to sporadic AML. Leukemia 2008;22:1428–1430.
10. Sato A, Imaizumi M, Koizumi Y, Obara Y, Nakai H, Noro T, Saito T, Saisho T, Yoshinari M, Cui Y, Suzuki H, Funato T, Iinuma K. Acute myelogenous leukaemia with t(8;21)translocation of normal cell origin in mosaic Down's syndrome with isochromsome 21q. Br J Haematol 1997;96:614–616.
11. Kawamura M, Kaku H, Taketani T, Taki T, Shimada A, Hayashi Y. Mutations of GATA1, FLT3, MLL-partial tandem duplication, NRAS, and RUNX1 genes are not found in a 7-year-old Down syndrome patient with acute myeloid leukemia (FAB-M2) having a good prognosis. Cancer Genet Cytoget 2008;180:74–78.
12. Blink M, Zimmermann M, von Neuhoff C, Reinhardt D, de Haas V, Hasle H, O'Brien MM, Stark B, Tandonnet J, Pession A, Tousovska K, Cheuk DK, Kudo K, Taga T, Rubnitz

JE, Haltrich I, Balwierz W, Pieters R, Forestier E, Johansson B, van den Heuvel-Eibrink MM, Zwaan CM. Normal karyotype is a poor prognostic factor in myeloid leukemia of Down syndrome: A retrospective, international study. Haematologica 2014:299–307.

13. Al-Ahmari A, Shah N, Sung L, Zipursky A, Hitzler J. Long-term results of an ultra low-dose cytarabine-based regimen for the treatment of acute megakaryoblastic leukaemia in children with Down syndrome. Br J Haematol 2006;133:646–648.
14. Taga T, Saito AM, Kudo K, Tomizawa D, Terui K, Moritake H, Kinoshita A, Iwamoto S, Nakayama H, Takahashi H, Tawa A, Shimada A, Taki T, Kigasawa H, Koh K, Adachi S. Clinical characteristics and outcome of refractory/relapsed myeloid leukemia in children with Down syndrome. Blood 2012;120:1810–1815.
15. Caldwell JT, Edwards H, Buck SA, Ge Y, Taub JW. Targeting the wee1 kinase for treatment of pediatric Down syndrome acute myeloid leukemia. Pediatr Blood Cancer 2014;61:1767–1773.
16. Wen Q, Goldenson B, Silver SJ, Schenone M, Dancik V, Huang Z, Wang LZ, Lewis TA, An WF, Li X, Bray MA, Thiollier C, Diebold L, Gilles L, Vokes MS, Moore CB, Bliss-Moreau M, Verplank L, Tolliday NJ, Mishra R, Vemula S, Shi J, Wei L, Kapur R, Lopez CK, Gerby B, Ballerini P, Pflumio F, Gilliland DG, Goldberg L, Birger Y, Izraeli S, Gamis AS, Smith FO, Woods WG, Taub J, Scherer CA, Bradner JE, Goh BC, Mercher T, Carpenter AE, Gould RJ, Clemons PA, Carr SA, Root DE, Schreiber SL, Stern AM, Crispino JD. Identification of regulators of polyploidization presents therapeutic targets for treatment of AMKL. Cell 2012;150:575–589.
17. Stankov MV, El Khatib M, Kumar Thakur B, Heitmann K, Panayotova-Dimitrova D, Schoening J, Bourquin JP, Schweitzer N, Leverkus M, Welte K, Reinhardt D, Li Z, Orkin SH, Behrens GM, Klusmann JH. Histone deacetylase inhibitors induce apoptosis in myeloid leukemia by suppressing autophagy. Leukemia 2014;28:577–588.

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Mortality and morbidity in primarily resected hepatoblastomas in Japan: Experience of the JPLT (Japanese Study Group for Pediatric Liver Tumor) trials

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ABSTRACT

Background: In the Japanese Study Group for Pediatric Liver Tumor (JPLT) protocols (JPLT-1 and 2) for evaluating the cure rate of risk-stratified hepatoblastoma, primary resection was permitted in PRETEXT I and II cases, followed by postoperative chemotherapy.
Methods: In approximately 500 enrolled cases, resection was performed as the initial treatment in 60 cases, including all 18 PRETEXT I, 30 PRETEXT II, and 12 ruptured cases. The clinical features, surgical procedures, complications, and survival rates were compared in these three groups.
Results: All 18 PRETEXT I cases underwent complete resection by lobectomy or segmentectomy (n = 14) or nonanatomical partial hepatectomy (NPH) (n = 4). The 30 PRETEXT II cases underwent primary resection by right or left lobectomy (n = 16), NPH (n = 10), or other procedures (n = 4). Of these 30 cases, operational death occurred in 1 newborn, and recurrence occurred in 7 cases (14.6%), including 6 NPH cases and 4 older cases (aged >3 years). Of the 12 ruptured cases, 7 (58.3%) showed recurrence. Event-free survival rates at 5 years in the 3 groups were 88%, 70%, and 32%, respectively.
Conclusions: Primary resection for PRETEXT I or II HB cases should be performed by anatomical resection according to strict surgical guidelines. More intensified chemotherapy is required for primary resected cases whose tumors have ruptured.

Hepatoblastoma (HB) is the most common malignant hepatic tumor in infants and young children, representing approximately 1% of all pediatric malignancies [1,2]. Survival has improved over the past decades owing to clinical trials using a combination of chemotherapy and surgical resection [3]. Children with HB respond well to chemotherapy and have a high cure rate with a 3-year overall survival (OS) of 70%. Completeness of tumor removal is one of the most important prognostic factors. The standard treatment strategy for HB therefore consists of preoperative (neoadjuvant) chemotherapy, surgical resection, and postoperative chemotherapy [3,4]. At present, the Liver Tumor Study Group of the International Society of Pediatric Oncology (SIOPEL) group trials use surgical resection after preoperative chemotherapy in all cases of HB [5]. On the other hand, the JPLT and COG multicenter studies permit primary resection for early stages of the tumor including PRETEXT (Pre Treatment Extent of Disease staging System) I and II tumors. Moreover, HB sometimes occurs as a result of tumor rupture, and in such cases primary resection is performed to control intraperitoneal bleeding.

In this study, we investigated children who underwent resections as the initial treatment (so called primary resection) according to the JPLT-1 and JPLT-2 protocols. In these protocols, the chemotherapy regimen was based on CITA (cisplatin and pirarubicin). Because each cohort with primary resection was too small to be evaluated we evaluated the combined cases of primary resection.

1. Patients and methods

JPLT-1 was open to enrollment in 1991 and closed in 1999 [6], while JPLT-2 was open in 1999 and closed in 2012 [4]. Eligible patients included children younger than 15 years at diagnosis with previously untreated malignant liver tumors. The study retrospectively reviewed 48 children with HB (20 females, 28 males) who underwent primary resection according to the JPLT-1 (n = 154) and JPLT-2 protocol (n = 335) cohorts. In addition, the study reviewed 12 cases with ruptured tumor who underwent primary resection in JPLT 2 study. In the JPLT-2 protocol, tumor extension was graded using the PRETEXT system [7], whereas the JPLT-1 protocol was graded using the Japanese surgical

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classification [6]. Therefore, JPLT-1 cases were classified retrospectively using the PRETEXT system. Ethics approval was obtained from the ethics committee of each institution of JPLT. Written, informed consent was obtained from the parents of the HB patients before enrollment.

After a detailed history and a thorough physical examination, blood was collected for liver function tests and tumor markers including alpha-fetoprotein (AFP). Other routine investigations included a chest X-ray, upper gastrointestinal endoscopy, abdominal ultrasound, contrast-enhanced computed tomography (CT), and/or magnetic resonance imaging (MRI) to investigate patients with clinical or radiological suspicion of intrahepatic or extrahepatic metastases.

The outlines of the JPLT-1 and JPLT-2 studies have been described previously [6,8]. In the JPLT-1, patients with early stage tumors (stage I or II) received the 91A protocol which was divided into two subgroups. In this protocol, the 91A1 patients (n = 12) received one course of intraarterial chemotherapy before surgical resection, while the 91A2 patients (n = 18) underwent initial primary surgery. The 91A1 patients, whose ages at diagnosis ranged from 6 to 96 months (median 15 months) included 10 males and 2 females. In the 91A2 patients, whose ages at surgery ranged from 4 to 160 months (median 18 months), included 12 males and 6 females. In JPLT-2, all PRETEXT I cases (n = 14) first received a primary resection, while some PRETEXT II cases (n = 16) underwent primary resection under facility judgment. The ages at surgery ranged from 0 to 141 months (median 33.5 months) including 18 males and 12 females. The remaining PRETEXT II cases (n = 76) received two courses of up-front chemotherapy consisted of cisplatin 40 mg/m^2 on one day followed by pirarubicin 30 mg/m^2 on day 2 (low CITA regimen) and then underwent surgical resection. Twelve cases with ruptured HB also underwent primary resection. These tumors consisted of 6 PRETEXT I, 5 PRETEXT II and 1 PRETEXT III, with 2 patients having hepatic vain involvement and 2 pulmonary metastases.

In most cases, a central pathological review including microscopic resectability was performed after resection. JPLT-1 91A and 91B for early stage protocol consisted of 5 and 6 courses of postoperative low CITA regimen, respectively. All JPLT-2 PRETEXT 1 or 2 patients also received four courses of a postoperative low CITA regimen. For postoperative follow-up, serum AFP levels and abdominal ultrasound were carried out monthly in all patients. Patients received an abdominal contrast-enhanced CT scan or MRI once every 2–3 months in the first 2 years after surgery, and once every 6 months thereafter. Further investigations were carried out when clinically indicated or when tumor recurrence was suspected.

Complete resection was defined as resection of all tumor sites based on surgical findings and postsurgical images. In the JPLT-1 and JPLT-2 protocols, surgical guideline did not set rules on the safety margin of primary resection. In this study, incomplete resection was defined in the case whose margin was positive by histological examination. Overall survival (OS) was defined as the period from the date of surgery until death or last contact. Patients who did not experience an event were censored on the date of their last contact. Event-free survival (EFS) was defined as the period from the date of surgery until occurrence of an event (recurrence, progressive disease, death, or diagnosis of a second malignant neoplasm) or last contact, whichever occurred first.

The chi-square or Mann–Whitney *U* test was used to examine the significance of the comparisons of clinicopathological factors and AFP levels between the different groups. A log rank test was used to evaluate survival rates. Statistical significance was defined as $P < 0.05$.

2. Results

In the JPLT-1 cohort, 18 primary resection cases were evaluated retrospectively using the PRETEXT classification as 4 PRETEXT I and 14 PRETEXT II cases. Among them, 17 cases (94%) undergoing complete resection. One incomplete resected case was aged 56 months with a PRETEXT II tumor (segments 5–8) which was resected by nonanatomical partial hepatectomy (NPH). The serum AFP level of this case was 257 IU. This patient died as a consequence of the tumor progression. Of the complete resection cases, 2 cases with partial hepatectomy for segment 6 and 7 tumors relapsed (Table 1).

In the JPLT-2 cohort, 30 cases received primary resection at first. In the 14 PRETEXT I cases, one case who had received a NPH had a recurrence of the tumor, although all cases survived disease-free. In the 16 PRETEXT II cases who had received a primary resection, 10 had a lobectomy, 4 a NPH, and 2 a segmentectomy (Table 1). Of these 16 cases, 15 had a microscopically complete resection, although 1 case (aged 1 month) underwent an NPH for segments 5–8 located in the tumor and had an incomplete resection. One case (aged 16 months) who underwent a right lobectomy for segments 5–8 located in a large tumor with hepatic vein involvement (V1) had an operative death owing to surgical complications. The remaining two NPH cases relapsed.

We compared the survival rates in the JPLT-1 cases between the 91A1 (TAC group) and 91A2 patients (primary resection group) (Fig. 1). The 12 91A1 cases were evaluated retrospectively into 3 PRETEXT I and 9 PRETEXT II cases, suggesting that there are no basic differences between these two groups. The survival rates of these two groups were similar. The primary resection cases did not have a worse outcome compared with the up-front TAC cases. In the JPLT-2 cases, all PRETEXT I cases survived despite one case relapsing. The event-free survival (EFS) and overall survival (OS) rates were 88.9% and 100%, respectively for PRETEXT I cases, and 73.5% and 93.8%, respectively for PRETEXT II primary resected cases (Fig. 2). In the JPLT-2 cohort, the EFS and OS rates of the PRETEXT II patients with neither vascular involvement nor metastasis who underwent preoperative chemotherapy (n = 76) were 78.9% and 89.6%, respectively. The survival rates of the primary resection and up-front chemotherapy cases were similar in the PRETEXT II cases (Fig. 2). The complications by chemotherapy were evaluated in JPLT-2 PRETEXT I or II survived patients who accomplished older than 5 years, 3 obvious healing loss, 5 growth retardation and 1 secondary malignancy were detected in the up-front chemotherapy patients, while only one healing loss was detected in the primary resected cases.

We compared several clinical factors between the relapsed and other cases in the primary resected cases (Fig. 3). Relapse occurred in 4 cases who underwent surgery when aged older than 3 years. The mean AFP levels in the nonrelapse and relapse cases were 184,293 and 68,706 ng/ml, respectively. There is no significant difference between these groups, but, 2 of 4 cases with a low AFP level

Table 1
Surgical procedures for initial primary resection.

Surgical procedure	JPLT-1		JPLT-2	
	I, n = 4	II, n = 14	I, n = 14	II, n = 16
Nonanatomical partial hepatectomy (NPH)	2	6 (3[a])	5	4 (1[a])
Segmentectomy	2	2	6	2
Lobectomy	0	6	3	10 (1[b]) 1[c]

Values in parentheses are relapse cases.
[a] Local relapse.
[b] Relapse as pulmonary metastasis.
[c] Operative death by surgical complication (aged 16 months, right lobectomy for segments 5–8 tumor).

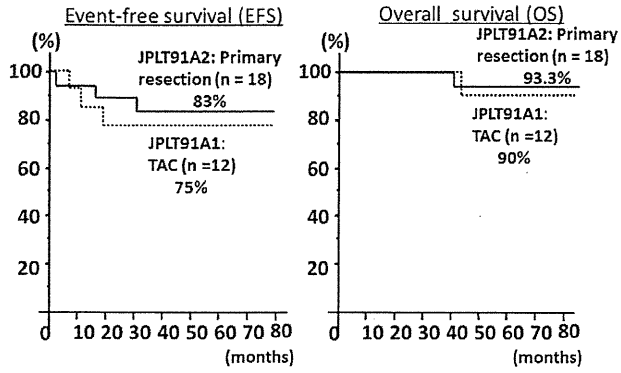

Fig. 1. Survival rates of the 91A protocol for stage 1 and 2 (retro-PRETEXT I and II) patients in the JPLT-1 HB study. 91A1 patients (dotted line, n = 12) received one course of intraarterial chemotherapy (TAC) before surgical resection, and the 91A2 patients (solid line, n = 18) underwent initial primary surgery.

<1000 ng/ml relapsed. The AFP levels after surgery (approximately one month after surgery) of relapsed cases were also similar to those of nonrelapsed but those after protocol treatment remained significantly higher in relapsed cases (mean 22 vs 3.6 ng/ml, $P < 0.01$). In the 30 PRETEXT II tumors, 16 had a lobectomy and 10 an NPH. Relapse occurred in 1 and 4 of these cases, respectively. Recurrence was significantly higher in the NPH cases ($P < 0.05$). One JPLT-1 case whose PRETEXT II tumor was resected by NPH was histologically evaluated as incomplete resection owing to microscopically residue of tumor cell. The AFP levels of this case were 257 ng/ml at diagnosis and remained high after treatment. Consequently, this case died of local relapse. Moreover, 2 of the 3 V1 or P1 positive cases locally relapsed, indicating that a sufficient surgical margin may be necessary for primary resections. Only one relapse in the patients who underwent lobectomy was pulmonary metastasis, suggesting that micrometastasis might have existed in this case.

In the JPLT-2 cases, 12 cases who were diagnosed by a tumor rupture had a primary resection in order to control intraperitoneal bleeding. These cases underwent emergency surgery and involved three advanced cases with PRETEXT III, hepatic vein involvement and/or pulmonary metastasis, who are usually contraindication for initially resection of primary tumor. The EFS rate of the ruptured cases who underwent primary resection was worse than those cases with a primary resection without rupture ($P < 0.01$) (Fig. 2).

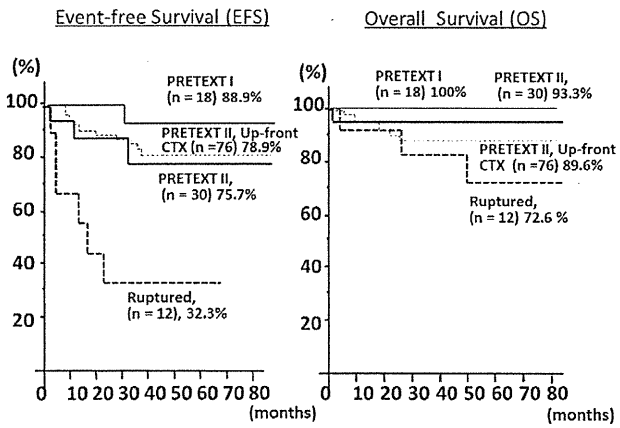

Fig. 2. Survival rates of the primary resection cases in the JPLT-2 HB study. The survival rates of the PRETEXT I (thin solid line, n = 14) and PRETEXT II patients (thick solid line, n = 16) were equivalent to the PRETEXT II patients with no vascular involvement who received up-front chemotherapy (thick dotted line, n = 76). The prognosis of the ruptured cases (thick dotted line, n = 12) was significantly worse ($P < 0.05$).

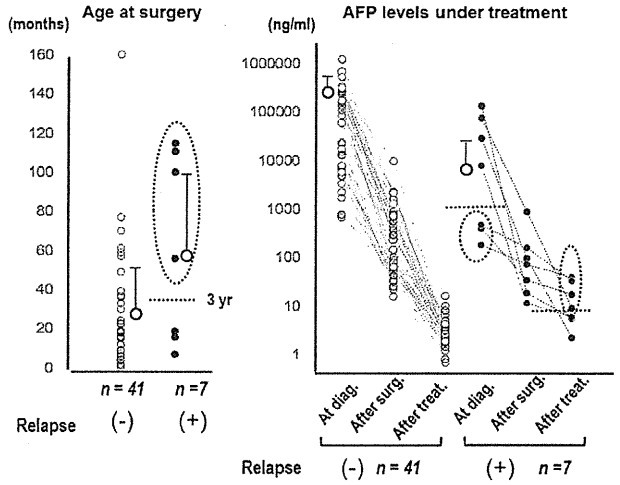

Fig. 3. Ages at surgery and serum AFP levels during treatment in primary resected cases. Relapse occurred in 4 cases who underwent surgery aged older than 3 years, and 2 of these cases with a low serum AFP level at diagnosis (<1000 ng/ml) relapsed. The AFP levels after surgery were similar between relapsed cases and no relapsed cases, but 4 of 7 relapsed cases remained with high AFP levels after treatment (>10 ng/ml). diag: diagnosis, surg: primary resection, and treat.: protocol treatment.

3. Discussion

Over the past two decades, multicenter cooperative study protocols for the treatment of children with HB have been developed in Japan, the United States, and Europe (SIOPEL study). The data of these studies have resulted in improved outcomes for HB using a combination of surgical resection and chemotherapy. Complete surgical resection is the most important factor in the prognosis of HB. Therefore, the timing of surgical resection should be considered in each case, with some patients with located HB initially undergoing a primary resection. In this study we determined the indications and guidelines for primary resection of HB.

Analysis of the JPLT-1 and JPLT-2 studies allowed the initial primary resection for patients with early stage tumors to be examined. The survival curves of these patients were not worse than other early stage patients who underwent up-front chemotherapies. These results indicated that primary resection was acceptable in PRETEXT I and II resectable cases. However, there were some patients who relapsed or had unfavorable outcomes. Older patients and those with low serum AFP levels (<1000 ng/ml) appeared to be at increased risk for relapse. Previous studies on HB prognosis have also reported that the age at diagnosis and low AFP levels are significant risk factors [9]. These results indicate that these tumors may have different biological characteristics.

Relapse occurred significantly higher in cases who had undergone nonanatomical partial hepatectomy (NPH) or incomplete resection. These results indicate that primary resection at the time of diagnosis should be performed using right or left anatomical lobectomy with sufficient surgical margins even if NPH is easy to be performed for complete resection. The surgical guidelines of the COG group showed that primary resection at diagnosis is only acceptable in patients with tumors where there is >1 cm radiographic margin at the middle hepatic vein, retrohepatic IVC, and the portal bifurcation, whereas trisegmentectomies and trisectorectomies are not permissible for primary resection at diagnosis [10]. Our results support these surgical guidelines. However, microintrahepatic metastasis and micopulmonary metastasis before surgery might be correlated with some relapses. The cases who underwent primary resection should receive approximately postoperative chemotherapy as well as close follow-up program.

The outcome of ruptured cases with primary resection had a poor prognosis. Although such cases sometimes have vascular involvement or distant metastasis, intraabdominal tumor dissemination may correlate with tumor progression. In oncologic emergency cases with

intraperitoneal bleeding caused by tumor rupture, primary tumor resection is effective for controlling tumor bleeding. However, as a consequence these patients have unfavorable outcomes, indicating that they should be treated as a high risk group after resection.

In conclusion, this paper describes a retrospective analysis of HB cases who had a primary resection at diagnosis, grouped as two JPLT clinical study cohorts (JPLT-1 and 2). The outcome of these patients indicated the effectiveness of primary resection at diagnosis in cases with early stage HB and identified the following risk factors for relapse: 1) age at diagnosis >3 years old, 2) low serum AFP level at diagnosis (<1000 ng/ml), 3) nonanatomical partial hepatectomy (NPH), 4) incomplete resection, and 5) ruptured cases. An improved understanding of these risk factors for primary resection at diagnosis affects prognosis in patients with early stage HB. However, greater understanding of the biological differences of resectable HB is necessary. Future clinical trials should be performed on primary resection in resectable cases under distinct surgical guidelines.

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References

[1] Litten JB, Tomlinson GE. Liver tumors in children. Oncologist 2008;13:812–20.
[2] Finegold MJ, Egler RA, Goss JA, et al. Liver tumors: pediatric population. Liver Transpl 2008;14:1545–56. http://dx.doi.org/10.1002/lt.21654.
[3] Hiyama E. Current therapeutic strategies for childhood hepatic malignant tumors. Int J Clin Oncol 2013;18:943–5. http://dx.doi.org/10.1007/s10147-013-0607-9.
[4] Hiyama E, Ueda Y, Onitake Y, et al. A cisplatin plus pirarubicin-based JPLT2 chemotherapy for hepatoblastoma: experience and future of the Japanese Study Group for Pediatric Liver Tumor (JPLT). Pediatr Surg Int 2013;29:1071–5. http://dx.doi.org/10.1007/s00383-013-3399-0.
[5] Perilongo G, Malogolowkin M, Feusner J. Hepatoblastoma clinical research: lessons learned and future challenges. Pediatr Blood Cancer 2012;59:818–21.
[6] Sasaki F, Matsunaga T, Iwafuchi M, et al. Outcome of hepatoblastoma treated with the JPLT-1 (Japanese Study Group for Pediatric Liver Tumor) Protocol-1: a report from the Japanese Study Group for Pediatric Liver Tumor. J Pediatr Surg 2002;37:851–6.
[7] Perilongo G, Shafford E, Plaschkes J. SIOPEL trials using preoperative chemotherapy in hepatoblastoma. Lancet Oncol 2000;1:94–100.
[8] Hishiki T, Matsunaga T, Sasaki F, et al. Outcome of hepatoblastomas treated using the Japanese Study Group for Pediatric Liver Tumor (JPLT) protocol-2: report from the JPLT. Pediatr Surg Int 2011;27:1–8. http://dx.doi.org/10.1007/s00383-010-2708-0.
[9] Czauderna P, Otte JB, Roebuck DJ. Comments on surgical treatment of locally advanced hepatoblastoma. Cancer 2012;118:4092–3. http://dx.doi.org/10.1002/cncr.26714 [author reply 4–5].
[10] Meyers RL, Tiao G, de Ville de Goyet J, et al. Hepatoblastoma state of the art: pretreatment extent of disease, surgical resection guidelines and the role of liver transplantation. Curr Opin Pediatr 2014;26:29–36. http://dx.doi.org/10.1097/MOP.0000000000000042.

Pediatr Blood Cancer 2015;62:1844–1850

Comparison of Chemotherapeutic Agents as a Myeloablative Conditioning With Total Body Irradiation for Pediatric Acute Lymphoblastic Leukemia: A Study From the Pediatric ALL Working Group of the Japan Society for Hematopoietic Cell Transplantation

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Background. As a partner of total body irradiation (TBI) in hematopoietic stem cell transplantation (HSCT) for pediatric acute lymphoblastic leukemia (ALL), various cytotoxic agents are used, but the optimal combination is still unclear. ***Procedure.*** We retrospectively analyzed 767 children who received TBI-based myeloablative allogeneic HSCT in complete remission (CR), using nationwide registry data of the Japan Society for Hematopoietic Cell Transplantation. Combinations of chemotherapy were categorized as follows: cyclophosphamide (CY) (n = 74), melphalan (L-PAM) (n = 139), CY + etoposide (VP16) (n = 408), CY + cytarabine (AraC) (n = 73), and others (n = 73). ***Results.*** Event-free survival (EFS) at 5 years after HSCT was 62.2% for CY, 71.4% for L-PAM, 67.6% for CY + VP16, 52.6% for CY + AraC, and 59.1% for others ($P = 0.009$). Further detailed comparison of LPAM and CY + VP16 demonstrated superior EFS for LPAM (83.2 ± 6.7%), with a marked difference compared with CY + VP16 (66.7 ± 4.9%) when limited to HSCT from a matched related donor (MRD), and this result was reproduced regardless of disease status (CR1 or CR2). However, EFS for CY + VP16 (68.3 ± 2.8%) was comparable to that for LPAM (64.5 ± 5.7%, $P = 0.37$) in HSCT from alternative donors, because higher non-relapse mortality attenuated the advantage of LPAM. ***Conclusions.*** For pediatric ALL in remission, LPAM could provide superior EFS for HSCT from MRD; however, compared to LPAM, CY + VP16 has similar EFS for HSCT from an alternative donor. Pediatr Blood Cancer 2015;62:1844–1850. © 2015 Wiley Periodicals, Inc.

Key words: acute lymphoblastic leukemia; chemotherapeutic agents; conditioning regimen; stem cell transplantation; total body irradiation

INTRODUCTION

The survival rate of pediatric acute lymphoblastic leukemia (ALL) has dramatically improved to 80%–90% in recent clinical trials,[1] but allogeneic hematopoietic stem cell transplantation (HSCT) is still indicated for a certain proportion of high-risk or relapsed ALL in children.[2–4] A total body irradiation (TBI)-based myeloablative regimen is generally considered as a standard regimen for ALL in children, except infants, because busulfan (BU) had an inferior survival outcome compared with TBI in HSCT for pediatric ALL.[5] To enhance the immunosuppressive and cytotoxic effects of TBI, a chemotherapeutic agent is usually also applied, and TBI and cyclophosphamide (CY) is the classic combination in allogeneic HSCT,[6] and is still considered as one of the standard regimens. However, the outcome of HSCT with TBI and CY for pediatric ALL had been unsatisfactory mainly due to relapse,[2–4,7] even when performed in complete remission (CR). Consequently, several investigators have attempted to add other agents to TBI-CY, or exchange CY for other cytotoxic agents.

Some reports showed that the addition of etoposide (VP16) or its replacement of CY could improve the survival rate by reducing the relapse rate,[8] as in adult ALL;[9] but there have been some studies with inconsistent results, mainly because of increased toxicity.[10,11] Other studies adopted cytarabine (AraC),[12] melphalan (L-PAM),[13] or thiotepa[14] in an attempt to improve the outcome of TBI-based HSCT for pediatric ALL. However, the numbers of patients included in these studies were limited, with varying disease status, conditioning regimens, and stem cell sources, and the optimal partner of TBI in myeloablative conditioning for pediatric ALL has yet to be established. Thus, the recommended or most frequently used conditioning regimens in recent clinical trials for

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pediatric ALL are not uniform, and include TBI-VP16,[3,15] TBI-CY,[16,17] and TBI-CY-AraC.[18]

In this study, we retrospectively analyzed HSCT for pediatric ALL with myeloablative TBI conditioning based on nationwide registry data of the Japan Society for Hematopoietic Cell Transplantation (JSHCT) in order to obtain fundamental information for establishing a standard conditioning regimen for children with ALL.

PATIENTS AND METHODS

This study was approved by the Institutional Ethics Committee of the University of Tokyo Hospital. A total of 767 patients were analyzed based on data reported to the JSHCT registry.[19] The patients were selected according to the following criteria: (i) patients diagnosed with ALL; (ii) aged 15 years or younger when receiving HSCT; (iii) allogeneic HSCT performed in first or second complete remission (CR1 or CR2); (iv) 12 Gy of TBI-based myeloablative conditioning;[20] (v) no prior HSCT; and (vi) HSCT performed between 2000 and 2012. Thirteen patients were excluded from this study due to insufficient data related to relapse date.

Mismatched donor was defined as one or more serological mismatch of six HLA antigens (A, B, and DR). The overall survival (OS) probability was calculated using Kaplan–Meier estimates. The duration of event-free survival (EFS) was defined as the time from HSCT to either treatment failure (relapse, death, or the diagnosis of secondary cancer) or the last day of observation when confirmed to be alive. Cumulative incidence curves were used in a competing-risk setting to calculate the probability of engraftment, graft-versus-host disease (GVHD), and non-relapse mortality (NRM). Univariate analyses of EFS were performed using the log-rank test, and Gray's test was used for group comparisons of cumulative incidences. Multivariate analysis was performed using the Cox proportional-hazard regression model, and the factors with $P < 0.2$ by the univariate analysis were entered into the multivariate analysis.

All statistical analyses were performed using EZR software.[21] A two-sided P-value of less than 0.05 was considered to be significant.

RESULTS

Patients

The characteristics of the 767 patients and transplantation are listed in Table I. The median age at HSCT was 9 years. The median follow-up period of surviving patients after HSCT was 5.1 years. Of the 767 patients, 500 received HSCT at CR1 and 383 at CR2. Chemotherapeutic agents with TBI were classified as CY (n = 74), L-PAM (n = 139), CY + VP16 (n = 408), CY + AraC (n = 73), and others (n = 73). Among the "Other" group, the combinations

TABLE I. Patient and Transplantation Characteristics

		Chemotherapeutic agents					
Characteristics	All (%)	CY (%)	L-PAM (%)	CY+VP16 (%)	CY + AraC (%)	Other (%)	*P*
All patients	767 (100)	74 (100)	139 (100)	408 (100)	73 (100)	73 (100)	
Age at HSCT							0.06
<10	427 (55.7)	32 (43.2)	72 (51.8)	242 (59.3)	44 (60.3)	37 (50.7)	
≥10	340 (44.3)	42 (56.8)	67 (48.2)	166 (40.7)	29 (39.7)	36 (49.3)	
Immunophenotype							0.40
Non-T lineage	597 (77.8)	60 (81.1)	109 (78.4)	307 (75.2)	62 (84.9)	59 (80.8)	
T-lineage	158 (20.6)	14 (18.9)	28 (20.1)	93 (22.8)	10 (13.7)	13 (17.8)	
Unknown	12 (1.6)	0 (0.0)	2 (1.4)	8 (2.0)	1 (1.4)	1 (1.4)	
Cytogenetics							<0.01
Low risk	47 (6.1)	6 (8.1)	10 (7.2)	24 (5.9)	2 (2.7)	5 (6.8)	
Hyperdiploid	32	5	6	16	1	4	
t(12;21)	15	1	4	8	1	1	
Intermediate risk	531 (69.2)	62 (83.8)	113 (81.3)	254 (62.3)	61 (83.6)	41 (56.2)	
t(1;19)	16	1	2	9	3	1	
Normal	324	41	71	140	48	24	
Other abnormality	191	20	40	105	10	16	
High risk	189 (24.6)	6 (8.1)	16 (11.5)	130 (31.9)	10 (13.7)	27 (37.0)	
t(9;22)	129	4	9	90	7	19	
MLL rearrangement	49	0	4	37	3	5	
Hypodiploid	11	2	3	3	0	3	
Disease status at HSCT							<0.01
CR1	435 (56.7)	41 (55.4)	58 (41.7)	270 (66.2)	24 (32.9)	41 (56.2)	
CR2	332 (43.3)	33 (44.6)	81 (58.3)	138 (33.8)	49 (67.1)	32 (43.8)	
Stem cell source							<0.01
Matched related donor	208 (27.1)	22 (29.7)	50 (36.0)	101 (26.5)	20 (27.4)	15 (20.5)	
Mismatched related donor	87 (11.3)	8 (10.8)	14 (10.1)	39 (9.6)	11 (15.1)	15 (20.5)	
Unrelated donor	291 (37.9)	32 (43.2)	52 (37.4)	168 (41.2)	21 (28.8)	18 (24.7)	
Cord blood	181 (23.6)	12 (16.2)	23 (16.5)	100 (24.5)	21 (28.8)	25 (34.2)	

P-value calculated by Fisher's exact test for independence of each categorical data. CR, complete remission; HSCT, hematopoietic stem cell transplantation; CY, cyclophosphamide; L-PAM, melphalan; VP16, etoposide.

included were L-PAM + VP16 (n = 38), CY + FLU (n = 15), and FLU only (n = 13).

Most cases of HSCT with CY used 120 mg/kg (or 3600 mg/m^2) CY, as in 487 (94.2%) of 517 HSCT with CY or CY + VP16. With VP16, 60 mg/kg (or 1800 mg/m^2) and 30 mg/kg (900 mg/m^2) doses were used in 307 (71.7%) and 39 (9.1%), respectively, of 428 HSCT with CY + VP16. In the L-PAM group (n = 139), 180 mg/m^2 of L-PAM was used in 73 (52.5%) HSCT, whereas 200 mg/m^2 or more was used in 45 (32.4%) HSCT. Detailed information regarding the distributions of CY, L-PAM, and VP16 is shown in Supplementary Figure 1.

Outcomes of HSCT

The association between outcome and clinical characteristics is shown in Table II. The estimated EFS and OS probabilities ± standard error after HSCT were 65.5 ± 1.8% and 68.6 ± 1.8%, respectively, at 5 years. The cumulative incidence of relapse at 5 years was 22.2 ± 1.6%, whereas that of NRM at 5 years was 11.9 ± 1.2%. Not surprisingly, the EFS of HSCT in CR1 (82.3 ± 2.0%) was better than that in CR2 (67.1 ± 2.9%) ($P = 0.009$). Of note, HSCT from an HLA-matched related donor had the lowest NRM incidence (6.9 ± 1.9%) ($P = 0.003$). Age, immunophenotype, and cytogenetic risk were not associated with survival.

Outcomes according to chemotherapeutic agent combination are shown in Figure 1. CY + VP16 and L-PAM provided better EFS than CY, CY + AraC, and others ($P = 0.009$, Fig. 1A). Compared with CY, CY + VP16 suppressed the relapse rate (23.4 ± 2.2% vs. 30.5 ± 5.5%) with an insignificant increase in the risk of NRM (9.0 ± 3.2% vs. 7.3 ± 3.2%). On the other hand, L-PAM was associated with the lowest relapse risk (14.6 ± 3.1%), but the increased risk of NRM (14.0 ± 3.1%) weakened the survival advantage. The group of "others" failed to exhibit improved outcome compared to CY, owing to the high incidence of NRM (23.9 ± 5.1%). L-PAM dose in the L-PAM group (i.e., TBI + L-PAM) was not significantly correlated with EFS: 68.2 ± 13.44% for 150 mg/m^2 or less (n = 14), 74.6 ± 5.8% for 151–199 mg/m^2 (n = 71), and 65.7 ± 7.8% for 200 mg/m^2 or more (n = 40) ($P = 0.38$).

Engraftment probabilities at day 60 after HSCT were similar in each group: 97.3 ± 2.1% for CY, 96.4 ± 1.6% for L-PAM, 97.1 ± 0.9% for CY + VP16, 95.9 ± 2.6% for CY+AraC, and 93.1 ± 3.1% for others. Acute GVHD incidence at day 100 after HSCT was also not associated with chemotherapeutic agents, and the incidence of acute GVHD (grade II–IV) was 37.0 ± 5.7% for CY, 42.3 ± 4.2% for L-PAM, 42.4 ± 2.5% for CY + VP16, 38.4 ± 5.7%, and 45.2 ± 5.9% for others ($P = 0.20$).

Multivariate analysis showed that HSCT at CR1 independently had a better effect EFS. The hazard ratio for events was smallest in L-PAM, although it did not reach statistical significance (Table III).

Comparison of L-PAM and CY + VP16 in HSCT From a Matched Related Donor

As L-PAM and CY + VP16 were considered as potentially optimal preparative agents to be combined with TBI, we performed further analysis focusing on a comparison of the two groups. To

TABLE II. Outcome of HSCT

Characteristics	n	CI of relapse (at 5 years)	*P*	CI of NRM (at 5 years)	*P*	EFS (at 5 years)	*P*
All patients	767	22.2±1.6		11.9±1.2		65.5±1.8	
Age at HSCT			0.16		0.03		0.77
<10	427	23.9±2.1		9.9±1.5		66.2±2.3	
≥10	340	20.2±2.4		15.5±2.1		64.3±2.8	
Immunophenotype			0.16		0.42		0.56
non-T	597	23.5±1.8		11.6±1.3		64.9±2.0	
T	158	16.7±3.1		15.1±3.0		68.2±3.9	
Disease status at HSCT			<0.001		0.02		<0.001
CR1	435	16.6±1.8		10.0±1.5		73.4±2.2	
CR2	332	29.3±2.6		15.4±2.0		55.2±2.9	
Cytogenetic risk[1]			0.91		0.10		0.51
Low	47	26.9±6.8		10.9±4.7		62.1±7.3	
Intermediate	531	21.1±1.8		13.9±1.6		65.0±2.2	
High	189	24.1±3.4		8.1±2.0		67.8±3.6	
Stem cell source			0.33		0.003		0.13
Matched related donor	208	26.4±3.2		6.9±1.9		66.8±3.5	
Mismatched related donor	87	20.2±4.4		14.0±3.8		65.8±5.1	
Unrelated donor	291	17.8±2.3		14.5±2.1		67.8±2.9	
Cord blood	181	25.2±3.4		14.5±2.6		60.3±3.8	
Chemotherapeutic agents			0.003		0.15		0.009
CY	74	30.5±5.5		7.3±3.2		62.2±5.8	
L-PAM	139	14.6±3.1		14.0±3.1		71.4±4.1	
CY+VP16	408	23.4±2.2		9. ±3.2		67.6±2.4	
CY + AraC	73	26.4±5.2		21.0±4.9		52.6±5.9	
Other	73	17.0±4.9		23.9±5.1		59.1±6.1	

[1]Low risk includes hyperdiploid and t(12;21); high risk includes hypodiploid, MLL rearrangement, and t(9;22). Other cytogenetic statuses are categorized as intermediate risk. *P*-values were calculated for the null hypothesis of equal hazard ratio or survival curves using Gray's test for CI of relapse and NRM, and log-rank test for EFS. CI, cumulative incidence; NRM, non-relapse mortality; OS, overall survival; CR, complete remission.