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Conditioning regimen

Autologous peripheral blood stem cell transplantation with BCVAC conditioning in childhood acute myeloid leukemia

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Summary:

Autologous peripheral blood stem cell transplantation (APBSCT) after intensifying conditioning is one of the post-remission therapeutic options in childhood acute myeloid leukemia (AML) patients without a matched family donor, but the optimal conditioning regimen has not been defined. This study was performed to evaluate the efficacy of a novel conditioning regimen without busulfan or total body irradiation. In total, 28 children with AML underwent APBSCT with BCVAC (BCNU, etoposide, cytosine arabinoside and cyclophosphamide) conditioning regimen during first remission. The event-free survival rate was 71.43% for all patients and the only cause of treatment failure was relapse. Eight male patients recurred at 1–11 months (median 5 months) after APBSCT. One patient remains alive with salvage therapy after relapse. With the exception of fever, mucositis and diarrhea, no serious complications occurred during APBSCT, including veno-occlusive disease (VOD), and there was no transplantation-related mortality. One patient developed secondary MDS after APBSCT but recovered hematologically on medication. APBSCT with BCVAC conditioning was found to be a safe and effective alternative option for patients with childhood AML in first remission, without a matched family donor.

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The goal of therapy for patients with acute myeloid leukemia (AML) is to eradicate their leukemia while allowing them to lead normal lives. After the introduction of anthracyclines and cytosine arabinoside (Ara-C) as an induction therapy in the 1960s, approximately 50–75% of AML patients achieved remission,[1] but without some types of post-remission therapies, almost all relapsed. As a post-remission therapy, allogeneic stem cell transplantation with a matched family donor has shown survival advantage in several large randomized studies of childhood AML.[2–4] However, many patients have no appropriate donor and need another treatment, such as chemotherapy, autologous stem cell transplantation, or stem cell transplantation (SCT) with a matched unrelated donor (MUD).

Although there is some debate about the efficacy of autologous SCT as post-remission therapy, we choose autologous peripheral blood stem cell transplantation (APBSCT) in patients without a matched family donor during first remission. Although autologous SCT has advantages, that is, the absence of the necessity to find an HLA identical donor, the absence of development of graft–versus-host disease (GVHD), leukemia cell contamination in the stem cell source is the main problem in the autologous setting. To reduce leukemia cell contamination, 5-hydroperoxycyclophosphamide or mafosfamide was used as *ex vivo* purging, and consolidation chemotherapy was used as *in vivo* purging. Peripheral blood stem cells or bone marrow (BM) stem cells have been used as sources for autologous SCT. The use of peripheral blood stem cells has shown advantages over the use of BM, as their faster hematopoietic reconstitution results in lower infectious and hemorrhagic morbidity and mortality. Recently, APBSCT has begun to replace autologous bone marrow transplantation (ABMT).[5,6]

For optimal conditioning, busulfan- or total body irradiation (TBI)-based conditioning regimens were commonly used in the APBSCT setting, but these regimens have some associated toxicity; that is, 3–12% transplantation-related mortality (TRM) and long-term toxicity.[7,8] Although many combinations of chemotherapeutic drug and/or irradiation have been studied as conditioning regimens, the optimum regimen remains undefined.

To improve treatment outcome, we designed a novel conditioning regimen without busulfan or TBI for childhood AML patients in first remission. After consolidation chemotherapy, patients received APBSCT with BCVAC (BCNU, etoposide, Ara-C and cyclophosphamide) conditioning as a post-remission therapy if a matched family donor was not available.

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Patients and methods

Patient selection

From January 1997 to May 2002, 28 pediatric AML patients (aged <15 years) without a matched family donor in the first remission were treated with APBSCT and a BCVAC conditioning regimen in Seoul National University Children's Hospital. The diagnosis of AML was made according to the French–American–British (FAB) criteria. Patients with FAB M3 type were excluded from APBSCT treatment.

Induction and consolidation

All patients received N4-behenoyl-1-β-D-arabinofuranosyl-cytosine (BH-AC), idarubicin, 6-thioguanine (6-TG) and intrathecal cytosine arabinoside (ITAra-C) as induction therapy. Primary refractory patients received a second cycle of induction therapy excluding the 6-TG. Once in remission, patients received four courses of consolidation therapy for *in vivo* purging (Table 1).

Mobilization therapy

After confirming continuing remission, patients received chemotherapy (Ara-C 3000 mg/m^2 twice daily i.v. for 2 consecutive days, and etoposide 70 mg/m^2 once daily i.v. for 4 consecutive days). G-CSF 10 ug/kg was injected subcutaneously from 7 days after the start of the chemotherapy until the last day of collection of mobilized peripheral blood stem cells.

When the total WBC count increased to more than 1.0×10^9/l after the nadir, and monocytes were detected in the peripheral blood, leukapheresis was started to collect mobilized peripheral blood stem cells using the CS-3000 plus (Baxter Healthcare, Deerfield, IL, USA) or COBE spectra (COBE Laboratories, Lakewood, CO, USA). Collection was continued for several days to obtain more than 6×10^8/kg of mononuclear cell (MNC). All collected products were cryopreserved with DMSO.

Conditioning regimen

All patients received a BCVAC conditioning regimen (BCNU 250 mg/m^2 once i.v. on day 1, and 200 mg/m^2 once i.v. on day 6, etoposide 200 mg/m^2 twice daily i.v. on days 2, 3, 4 and 5 (total eight doses), Ara-C 2000 mg/m^2 twice daily i.v. on days 2, 3, 4 and 5 (total eight doses) and cyclophosphamide 50 mg/kg once daily i.v. on days 7 and 8 (total dose 100 mg/kg)). Patients received adequate hydration, alkalinization therapy and allopurinol during conditioning, and mesna to prevent hemorrhagic cystitis. At the time of APBSCT (9 days after the start of conditioning), each bag was rapidly thawed in a 38°C water bath and infused through a central venous catheter over 5–10 min.

Supportive care

Patients received ciprofloxacin, fluconazole, isoniazid and acyclovir as infection prophylaxis. Intravenous immune globulin (0.5 g/kg/dose) was infused weekly until day 100, and then monthly until day 180. Daily sulfamethoxazole/trimethoprim was discontinued 3 days before APBSCT and restarted after WBC recovery. When fever exceeded 38°C, broad-spectrum antibiotics were administered. Amphotericin B was given when fever persisted for more than 5 days despite appropriate antibacterial treatment. Transfusions were given to maintain the hemoglobin level above 8 g/dl and the platelet count above 20×10^9/l. All blood products were irradiated to 20 Gy to avoid the risk of acute GVHD. G-CSF 300 ug/m^2 was administered from one day after APBSCT and discontinued when the absolute neutrophil count (ANC) increased to more than 1.0×10^9/l for 3 consecutive days. Patients received low-molecular weight heparin (nadroparine; fraxiparine, Sanofi-synthelabo, Paris, France) or lipo-PGE1 (eglandin; alprostadil, Welfide, Osaka, Japan) as prophylaxis for veno-occlusive disease (VOD).

Interleukin-2 (IL-2)

From July 1999, post-APBSCT immune therapy was added after APBSCT. Recombinant IL-2 (aldesleukin; proleukin, Chiron, Emeryville, CA, USA) 3×10^6 IU/m^2 once daily for 5 consecutive days was delivered subcutaneously every other week for 11 weeks (total 30 doses), starting when the ANC and platelet count increased to more than 1.0×10^9/l and 20×10^9/l, respectively, 28 days after APBSCT.

Table 1 Schema of induction and consolidation

Induction
BH-AC 300 mg/m^2 once daily i.v. on days 1–7
BH-AC ([a]) mg/m^2 once daily i.v. on days 8–10
Idarubicin 10 mg/m^2 once daily i.v. on days 1–3
6-TG 50 mg/m^2 twice daily p.o. on days 1–7

Consolidation
First: BH-AC 200 mg/m^2 once daily i.v. on days 1–5 and idarubicin 10 mg/m^2 once daily i.v. on days 1 and 2
Second: BH-AC 200 mg/m^2 once daily i.v. on days 1–5 and mitoxantrone 10 mg/m^2 once daily i.v. on days 1 and 2
Third: Etoposide 100 mg/m^2 once daily i.v. on days 1–5 and amsacrine 100 mg/m^2 once daily i.v. on days 1 and 2
Fourth: Etoposide 100 mg/m^2 once daily i.v. on days 1–5 and mitoxantrone 10 mg/m^2 once daily i.v. on days 1 and 2

[a]BH-AC dose adjusted according to BM status on day 8 [M1 (blasts <5%) 300 mg/m^2, M2 (blasts 5–25%) 400 mg/m^2, M3 (blasts >25%) 500 mg/m^2]. All patients received intrathecal Ara-C on day 1 of induction and all consolidation cycles.

Statistical analysis

Clinical and laboratory data were analyzed using standard statistical methods by SPSS version 10.0. Correlation between cell dose and hematopoietic reconstitution was analyzed using Pearson's correlation coefficients. The χ^2 test or the *t*-test was applied for comparison of means or categorical variables among subgroups. Event-free survival (EFS) was defined as the time from diagnosis to the first event (relapse or treatment-related death). For survival data, Kaplan–Meier life tables were constructed and the curves were compared by means of the log-rank test.

Results

Patient characteristics

The median times of APBSCT from diagnosis and from remission were 226 days (175–349 days) and 190.5 days (144–318 days), respectively. The presenting characteristics of the 28 patients are documented in Table 2.

Hematopoietic reconstitution and complication

The median infused cell doses of MNC- and CD34-positive cells were 19.6×10^8/kg ($6.2–47.2 \times 10^8$/kg) and 12.0×10^6/kg ($0.9–46.9 \times 10^6$/kg), respectively. The median numbers of days required for an ANC of more than 0.5×10^9/l and 1.0×10^9/l were 11.5 days (8–23 days) and 13 days (8–41 days), respectively. Spontaneous platelet recovery to more than 50×10^9/l required a median of 29 days (15–71 days), except in two patients (one relapsed before recovery and another developed secondary MDS). A median of 43 days (17–184 days) was required for platelet recovery to more than 100×10^9/l, except in four patients (three relapsed and one developed secondary MDS). Infused cell MNC doses ($\times 10^8$/kg) had no relationship to hematopoietic reconstitution. CD34-positive cell doses ($\times 10^6$/kg) were found to correlate inversely with the days required for an ANC of more than 0.5×10^9/l ($r = -6.81$, $P = 0.000$), ANC of more than 1.0×10^9/l ($r = -6.05$, $P = 0.001$) and platelet of more than 50×10^9/l ($r = -6.96$, $P = 0.000$).

Complications of APBSCT were fever (20 patients), mucositis (12 patients) and diarrhea (18 patients). No VOD or TRM occurred. IL-2 was delivered in 14 patients as post-APBSCT immune therapy. With the exception of a high fever, no other complications or TRM occurred during the IL-2 treatment. A 13-year-old male patient developed pancytopenia 5 months after APBSCT. Secondary MDS was suspected because of decreased granulopoiesis and the number of megakaryocytes with dyserythropoiesis on BM examination, but his blood profile and BM normalized after prednisolone, oxymetholone and alfacalcidol therapy for 4 months.

Table 2 Patient characteristics

Age at diagnosis (years)	
0–4	14
5+	14
Sex	
Male	17
Female	11
FAB classification	
M2	4
M4	10
M4Eo	4
M5	6
M6	1
M7	3
Poor prognostic factor	
$\geqslant$2 courses of induction	1
High initial WBC count*	2
Good prognostic factor	
t(8;21)	6
inv(16)	4
Day 8 induction BM status	
M1	23
M2	5

*WBC count of more than 100×10^9/l at diagnosis.

Treatment outcome

The EFS ($\pm$s.e.) of all 28 patients who received an APBSCT with BCVAC conditioning was $71.43 \pm 8.54\%$ (Figure 1). The median follow-up duration from diagnosis was 36.5 months (9–90 months), and follow-up duration from APBSCT was a median of 30.5 months (1–80 months).

Eight patients (28.5%) developed recurrence at 1–11 months (median 5 months) after APBSCT (Figure 2). All relapsed patients were male. One of them remains alive after a MUD BMT.

Risk factors of relapse after APBSCT

The EFS of patients who received APBSCT was longer for female patients (100%) and patients aged <5 years. No

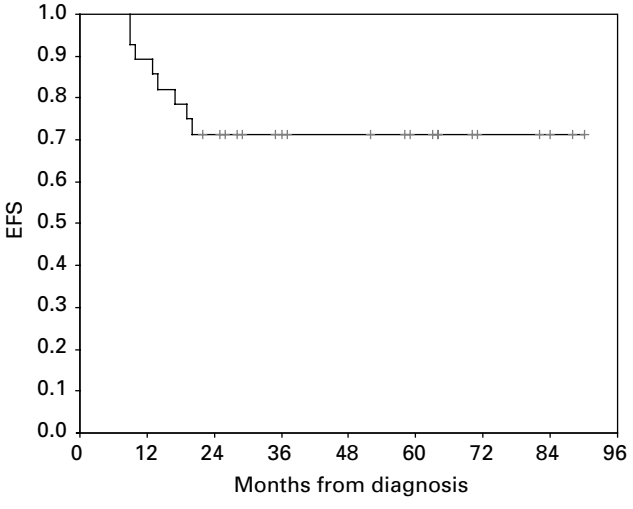

Figure 1 EFS of the patients who received APBSCT with the BCVAC preparative regimen. The EFS was $71.43 \pm 8.54\%$ and the only cause of treatment failure was relapse.

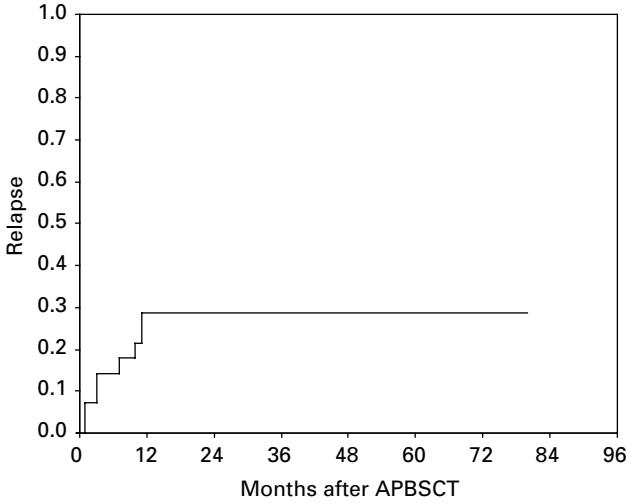

Figure 2 Relapse rate after APBSCT. Of the 28 patients who underwent APBSCT, eight (28.5%) relapsed within 1 year after APBSCT. One of them remains alive after BMT with a MUD.

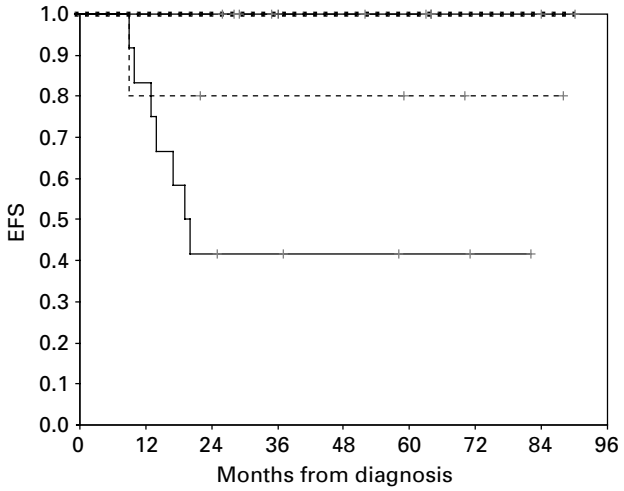

Figure 3 EFS of patients who underwent APBSCT grouped by sex and age. The EFS of female patients was 100%, and in males under 5 years it was $80.00 \pm 17.89\%$. The EFS of males older than 5 years was poorest; $41.67 \pm 14.23\%$ ($P = 0.027$).

Table 3 EFS *vs* prognostic factors

Prognostic factor	*EFS (%)*	*P-value (log rank test)*
Sex		
Male	52.94 ± 12.11	0.010
Female	100	
Age		
5+ years	50.00 ± 13.36	0.017
0–4 years	92.86 ± 6.88	
Cytogenetics		
t(8;21)	50.00 ± 20.41	0.296
inv(16)	100	
Others	72.22 ± 10.56	
IL-2 treatment		
No	64.29 ± 12.81	0.394
Yes	78.57 ± 10.97	

other significant risk factors predictive for relapse were identified, that is, FAB subtype, multiple induction, high initial WBC count ($>100 \times 10^9$/l), t(8;21), inv(16), day 8 induction BM status or infused cell dose. Patients who received IL-2 as post-APBSCT immune therapy showed no significant survival advantage (Table 3). As sex and age were significant prognostic factors, patients were divided into four groups according to age and sex. Among these four groups, the EFS of males >5 years was the worst (Figure 3).

Discussion

Autologous SCT is one of the options for post-remission treatment in childhood AML. Although there has been controversy about its efficacy,[2,4,7,9,10] patients without a matched family donor received APBSCT as a post-remission therapy in this study, because our experiences of chemotherapy alone were unsatisfactory. Autologous SCT has advantages over MUD transplantation of no GVHD and the availability of a stem cell source. As the risk of morbidity and mortality from GVHD in MUD transplantation is so high, this was not viewed as an option for treatment of pediatric patients with AML in first remission, as was shown in the large study conducted by the Children's Cancer Group.[2]

PBSCT was selected rather than BMT for autologous transplantation due to its low associated morbidity and mortality. APBSCT has several advantages over ABMT; rapid engraftment, low transplantation-related mortality and the avoidance of BM harvesting in an operating room under general anesthesia.[5]

Despite the original assumption that peripheral blood would be less contaminated than BM, the stem cell mobilization technique also mobilizes leukemia cells and some studies have found higher relapse rates after APBSCT.[7,11] Purging methods such as the *ex vivo* purging of BM with monoclonal antibodies or with a cytotoxic agent, such as 5-hydroperoxycyclophosphamide or mafosfamide, have been used to reduce leukemia cell contamination and have been reported to reduce relapse rate.[8]

The *ex vivo* purging of mobilized peripheral blood was at the preclinical stage at the beginning of this study,[12] and patients received four cycles of consolidation chemotherapy with different drug combinations as *in vivo* purging before mobilization, instead of *ex vivo* purging. Despite intensive *in vivo* purging, peripheral blood stem cell harvesting was successful, and adequate cell numbers were obtained from all patients.

The BCVAC regimen was used for conditioning in all patients receiving APBSCT. This was a modification of the MCVAC regimen (MCNU, etoposide, Ara-C and cyclophosphamide) first used in the University of Tokushima, Japan for lymphoid malignancies.[13,14] Busulfan- or TBI-based conditioning regimens have been used in the autologous SCT in AML setting in many studies,[7] but

these regimens were originally developed for allogeneic stem cell transplantation and have high immunosuppressive activity, which is not necessary in the autologous setting. A low toxicity BCNU-based regimen like BAVC (BCNU 800 mg/m^2, amsacrine 450 mg/m^2, etoposide 450 mg/m^2, and Ara-C 900 mg/m^2) was viewed as another option for childhood AML, but has proven to result in inferior survival.[8,15,16] BCVAC (BCNU 550 mg/m^2, etoposide 1600 mg/m^2, Ara-C 16 000 mg/m^2 and cyclophosphamide 100 mg/kg) used in this study is another BCNU-based regimen, which includes a high dose of Ara-C, and which is well known to have an anti-leukemic effect against AML cells.

The EFS (71.43 $\pm$ 8.54%) of the 28 patients, who received a BCVAC conditioning regimen in the present study, was comparable to the recent results for autologous SCT in pediatric AML patients.[7] The main cause of treatment failure was relapse. Lack of a graft-versus-leukemia effect is one of the disadvantages of autologous transplantation. The administration of IL-2 after autologous SCT is associated with clinical symptoms that resemble GVHD, and IL-2 therefore has been used as a form of immunotherapy after autologous SCT, to provoke a graft–versus-leukemia effect in childhood AML patients.[17,18] IL-2 was tried in our study, but without a significant improvement in outcome.

The toxicities of the BCVAC conditioning regimen were tolerable, and no TRM occurred. This low toxicity suggests that BCVAC could be used as a reduced-intensity regimen in a different setting. As patients who received BCVAC had no TRM, intensification of the conditioning regimen may be possible, although dose escalation may only increase the incidence and severity of complications. Radioisotope-labeled anti-CD33 or anti-CD45 monoclonal antibodies have recently been examined as a component of conditioning regimens in AML,[19] and the addition of these agents may have a positive role in the context of BCVAC conditioning.

Concerning treatment complications, secondary MDS was diagnosed in one patient 5 months after APBSCT. The incidence of secondary MDS/AML after autologous transplantation ranges from 1.1 to 24%.[20] Recently, chemomobilization with etoposide was found to increase the risk of treatment-related MDS/AML in autologous transplantation.[21] Etoposide and Ara-C delivered before the mobilization of peripheral blood stem cell in our study may have increased the risk of secondary MDS. Long-term follow-up of patients is needed to define the risk of secondary MDS, secondary malignancies and other delayed toxicities.

As all treatment failures in the present study were due to relapse, we believe it is necessary to select patients who may derive a survival benefit from APBSCT as a post-remission treatment, and to assess the risk factors predicting relapse. An initial high WBC count ($>100\times10^9$/l), monosomy 7, more than one course for complete remission, secondary AML or prior MDS and extramedullary leukemia (non-CNS) are well-known adverse factors; moreover, FAB M1 type with Auer rods, t(8;21), inv16/M4Eo type and t(15;17) are well-known favorable factors in childhood AML.[22] FAB M0 or M6 type, no *in vivo* purging and BVAC preparative regimens were recently found to be adverse prognostic factors in a large retrospective pediatric study.[8] Although 10 patients with favorable cytogenetic abnormalities who might be cured by chemotherapy were enrolled in the present study, no significant survival difference was observed. It is not possible to assess the prognostic value of FAB M6 because only one patient who relapsed after APBSCT was enrolled. Age and sex were identified as prognostic factors in this study. An older age was previously shown to be a poor prognostic factor in a pediatric AML study.[23] The reason why sex is a prognostic factor could not be determined.

BVAC and BCVAC are BCNU-based conditioning regimens without busulfan or TBI. The EFS of patients who received autologous SCT with BVAC (35%) without *ex vivo* purging in a large European prospective pediatric study proved to be worse than that of the patients who received conditioning regimens based on busulfan (59%) or TBI (63%), although there was no TRM with BVAC.[8] In the present study, patients received the BCVAC conditioning regimen without *ex vivo* purging, safely and with a somewhat better EFS (71.43%) than BVAC. This promising result may come from the mobilization of autologous stem cell after *in vivo* purging with four cycles of chemotherapy and the use of a higher dose of Ara-C and etoposide in the conditioning regimen.

To improve the treatment outcome in childhood AML, we have planned several strategies. Firstly, some newly diagnosed patients were recently selected to receive SCT using other stem cell sources such as BM from a MUD or cord blood. Together with improvements in SCT and the increased number of donors registered in the Korean Marrow Donor Program, the number of pediatric patients receiving SCT from unrelated donors is increasing. Now an unrelated donor search is performed for patients without a family donor, especially for those with high-risk factors. Secondly, detection of minimal residual leukemia has improved. Leukemia cell contaminations in stem cell sources contribute to relapse. The detection of minimal residual disease in mobilized products using FISH or other molecular methods has also been introduced. Finally, post-APBSCT immune therapy has been introduced. In addition to the IL-2 therapy mentioned above, we are planning to perform dendritic cell and NK cell therapy.

In conclusion, APBSCT with BCVAC conditioning after consolidation chemotherapy as an *in vivo* purging proved to be a safe and effective treatment for childhood AML patients without a matched family donor. The main problem encountered during this study was BM relapse. SCT from an unrelated donor, minimal residual leukemia detection and post-APBSCT immune therapy offer promising means of improving treatment outcome.

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