High-dose Chemotherapy and Autologous Stem Cell Rescue in Patients with High-risk Stage 3 Neuroblastoma: 10-Year Experience at a Single Center

High-dose chemotherapy and autologous stem cell rescue (HDCT/ASCR) was applied to improve the prognosis of patients with high-risk stage 3 neuroblastoma. From January 1997 to December 2006, 28 patients were newly diagnosed as stage 3 neuroblastoma. Nine of 11 patients with *N-myc* amplification and 5 of 17 patients without *N-myc* amplification (poor response in 2 patients, persistent residual tumor in 2 and relapse in 1) underwent single or tandem HDCT/ASCR. Patients without high-risk features received conventional treatment modalities only. While 8 of 9 patients underwent single HDCT/ASCR and the remaining one patient underwent tandem HDCT/ASCR during the early study period, all 5 patients underwent tandem HDCT/ASCR during the late period. Toxicities associated with HDCT/ASCR were tolerable and there was no treatment-related mortality. While the tumor relapsed in two of eight patients in single HDCT/ASCR group, all six patients in tandem HDCT/ASCR group remained relapse free. The 5-yr event-free survival (EFS) from diagnosis, in patients with *N-myc* amplification, was 71.6±14.0%. In addition, 12 of 14 patients who underwent HDCT/ASCR remained event free resulting in an 85.1± 9.7% 5-yr EFS after the first HDCT/ASCR. The present study demonstrates that HDCT/ASCR may improve the survival of patients with high-risk stage 3 neuroblastoma.

Key Words : *Neuroblastoma; High-dose Chemotherapy; Autologous Stem Cell Rescue; Prognosis; N-myc*

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INTRODUCTION

The prognosis of stage 3 neuroblastoma is generally good (1, 2). However, the prognosis of stage 3 neuroblastoma with unfavorable biologic factors, such as, *N-myc* amplification, is poor with conventional chemoradiotherapy (1, 3). In addition, the prognosis of stage 3 neuroblastoma with a poor response to conventional treatment (4-6) or with relapse during conventional treatment is also poor even when the tumor did not have unfavorable biologic factors.

A strategy using high-dose chemotherapy and autologous stem cell rescue (HDCT/ASCR) has been explored to improve the prognosis of patients with high-risk neuroblastoma (7-11). This strategy is based on the hypothesis that a dose escalation might improve the survival of children with high-risk neuroblastoma. The results of randomized trials, comparing HDCT/ASCR with chemotherapy alone, showed a better event-free survival (EFS) in the HDCT/ASCR arm than in the continuous chemotherapy arm (8, 11). Recently, investigators have examined the efficacy of double or triple tandem HDCT/ASCR to further improve the outcome of high-

risk neuroblastoma patients. Sung et al. (12) and George et al. (13) carried out a single arm trial of tandem transplantation as consolidation therapy, and reported improved long-term survival (5-yr progression-free survival 62% and 47%, respectively) with acceptable toxicity. Kletzel et al. also conducted a single arm trial of triple tandem transplantation and reported improved survival (3-yr EFS 57%) (14). They demonstrated that further dose escalation using sequential HDCT/ASCR might result in further improvements in the survival of patients with high-risk neuroblastoma.

However, a majority of patients in their studies had stage 4 tumors and only a small proportion of patients had stage 3 high-risk tumors. Therefore, the efficacy of HDCT/ASCR for stage 3 high-risk patients was not shown in their reports (7-14). The number of studies evaluating the efficacy of HDCT/ASCR only in patients with high-risk stage 3 neuroblastoma is limited. In this context, the present study examined the efficacy of HDCT/ASCR in order to determine if a dose intensification strategy might improve the EFS in patients with high-risk stage 3 neuroblastoma.

MATERIALS AND METHODS

Patients

From January 1997 to December 2006, 28 consecutive patients were newly diagnosed as stage 3 neuroblastoma at the Samsung Medical Center. A diagnosis of neuroblastoma was made based on either histological examination of the tumor specimens or bone marrow infiltrated with neuroblastoma cells and elevated urine catecholamine levels. The *N-myc* copy number was determined using Southern blot analysis or quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). The patients were staged according to the criteria reported by Brodeur et al. (15) and the extent of the disease was evaluated using computerized tomography, a technetium-99 (^{99}Tc) bone scan, bilateral bone marrow aspirates and biopsy specimens, and an iodine-131 or 123-metaiodobenzylguanidine scan. Measurement of serum lactate dehydrogenase, ferritin and neuron-specific enolase was part of the routine evaluation at diagnosis.

Treatment of patients

Fig. 1 summarizes the treatment. Most patients received 5-6 cycles of chemotherapy before definitive surgery, except for those patients that had a surgical resection before the administration of chemotherapy. If it was difficult to debulk the tumor, an additional 1-2 cycles of chemotherapy were given prior to the definitive surgery.
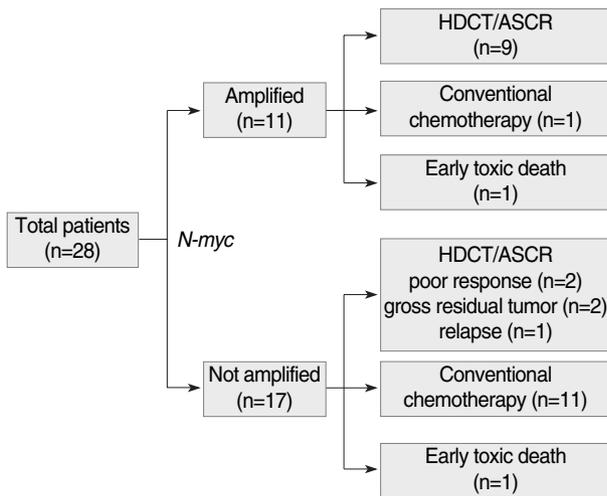

Fig. 1. Flow of the patients from diagnosis through HDCT/ASCR. All except two patients with *N-myc* amplification (early death in 1 and refusal to receive HDCT in 1) underwent HDCT/ASCR. In addition, 5 of 17 patients without *N-myc* amplification (poor response to chemotherapy in 2 patients, persistent gross residual tumor in 2 patients and relapse during chemotherapy in 1 patient) underwent HDCT/ASCR. Therefore, overall 14 patients underwent HDCT/ASCR. Eleven of 17 patients without *N-myc* amplification received conventional chemotherapy alone.

For patients with *N-myc* amplified tumors, patients with a very poor response to chemotherapy or patients with persistent gross residual tumor after surgery, the peripheral blood stem cells (PBSCs) were collected during the next chemotherapy cycle after surgery. After collecting the PBSCs, 0-2 additional cycles of chemotherapy were given prior to the HDCT/ASCR. For patients without N-myc amplification who responded well, continuous chemotherapy was provided after surgery (total 12 cycles).

Table 1 lists the chemotherapy regimens used in the present study. During the early period of the study (diagnosis by December 2003), the cisplatin, etoposide, doxorubicin and cyclophosphosphamide (CEDC) regimen was used as the primary treatment. In the late period (diagnosis from January 2004), alternating CEDC and ifosfamide, carboplatin and etoposide (ICE) regimens were used. While all the patients in the late study period received 9 cycles of chemotherapy prior to the HDCT/ASCR, 7 of 9 patients in the early study period received 6 or 7 cycles of chemotherapy. Each chemotherapy cycle was scheduled to be 28 days apart but some delays were permitted in order to allow the absolute neutrophil count (ANC) and platelet count to recover to 1,000/μL and 100,000/μL, respectively. The patients received 5-10 μg/kg of subcutaneous human granulocyte-colony stimulating fac-

Table 1. Conventional and high-dose chemotherapy regimens

Regimens	Dosage	Schedule
Conventional chemotherapy		
CEDC		
Cisplatin	60 mg/m²/day	Day 0
Etoposide	100 mg/m²/day	Days 2 & 5
Doxorubicin	30 mg/m²/day	Day 2
Cyclophophamide	30 mg/kg/day	Days 3 & 4
ICE		
Ifosfamide	1,200 mg/m²/day	Days 0-4
Carboplatin	400 mg/m²/day	Days 0 & 1
Etoposide	100 mg/m²/day	Days 0-4
First HDCT regimens		
CEM		
Carboplatin	300 mg/m²/day	Days -6, -5, -4, -3
Etoposide	200 mg/m²/day	Days -6, -5, -4, -3
Melphalan	140 mg/m²/day	Day -5
	70 mg/m²/day	Day -4
CEC		
Carboplatin	650 mg/m²/day	Days -7, -6, -5
Etoposide	650 mg/m²/day	Days -7, -6, -5
Cyclophosphamide	1,800 mg/m²/day	Days -4, -3, -2
Second HDCT regimens		
CTM		
Carboplatin	250 mg/m²/day	Days -6, -5, -4
Thiotepa	200 mg/m²/day	Days -6, -5, -4
Melphalan	160 mg/m²/day	Day -3
TM		
Thiotepa	300 mg/m²/day	Days -7, -6, -5
Melphalan	60 mg/m²/day	Days -4 & -3

HDCT, high-dose chemotherapy.

tor (G-CSF) daily if the ANC fell below 500/μL after chemotherapy.

PBSC collection

The patients received 5-10 μg/kg of subcutaneous G-CSF daily if the ANC fell below 500/μL after chemotherapy, and G-CSF was continued until the completion of leukapheresis. Leukapheresis was started when the white blood cell count exceeded 1,000/μL with a monocytosis after the nadir. The aim was to collect a minimum of 2×10^6 CD34$^+$ cells/kg (hopefully >5×10^6 cells/kg) in order to rescue the probable tandem HDCT with the PBSCs collected during a single leukapheresis round.

HDCT and ASCR

Table 1 lists the HDCT regimens used in the present study. While only one patient who was not in complete response (CR), even after the first HDCT/ASCR, underwent a second HDCT/ASCR in the early period of this study, all the patients in the late period underwent tandem HDCT/ASCR. The HDCT regimens employed for the first HDCT were carboplatin, etoposide and melphalan (CEM) in the early period and CEC in the late period. The regimens employed for the second HDCT were carboplatin, thiotepa, and melphalan (CTM) in the early period and TM in the late period. Total body irradiation (TBI) was not included in the HDCT regimen. The second HDCT/ASCR was given only if the platelet count exceeded 50,000/μL after the first HDCT/ASCR, without a transfusion, and if there was no evidence of significant organ dysfunction. Approximately half of the collected PBSCs were infused for marrow rescue at each HDCT session. Samsung Medical Center Institutional Review Board approved the protocols used for the HDCT/ASCR, and written informed consent was obtained from the parents of each patient.

Local radiotherapy

In the early period of this study, local radiotherapy (15 Gy/ 1.5 Gy in the case of small residual tumor and 21.6 or 30.6 Gy/1.8 Gy in the case of bulky residual tumor) was given to the primary site only if gross residual tumor remained after surgery. However, in the late period, local radiotherapy (15 Gy/1.5 Gy in the case of complete resection of the primary tumor and 21.6 Gy/1.8 Gy in the case of incomplete resection) was administered to all patients (4 weeks after the completion of conventional chemotherapy or 6 weeks after the second HDCT/ASCR).

Post-HDCT treatment

Thirteen-*cis*-retinoic acid (CRA) was used for 10 cycles (125 mg/m^2/day for 14 days per every 4 weeks) to differentiate the possible minimal residual tumor cells. Differentiation therapy with CRA was initiated 60-90 days after the HDCT/ ASCR or 30 days after the completion of conventional chemotherapy. Immunotherapy using interleukin-2 (IL-2) was administered (2×10^6 U/m^2/day, subcutaneous injection, days 0-4, every 4 weeks) with or without preceding induction therapy (3×10^6 U/m^2/day, continuous infusion, days 0-4 and 7- 11) (16). IL-2 immunotherapy was initiated when the platelet count exceeded 50,000/μL, without a transfusion, after the HDCT/ASCR. The first dose of IL-2 was provided on the first day of the CRA treatment cycle and was continued until 1 yr after the HDCT/ASCR. Immunotherapy was not used in patients who received conventional chemotherapy alone.

Response criteria

The international response criteria were used to evaluate the treatment response (15). Briefly, a CR was defined as no identifiable tumor with normal catecholamine levels. A very good partial response (VGPR) was defined as a decrease in the primary tumor by 90-99% with normal catecholamine levels with or without any residual ^{99}Tc bone changes. A partial response (PR) was defined as a reduction of the primary tumor and metastatic tumor by more than 50%. The toxicity was recorded according to the common toxicity criteria of the National Cancer Institute of the U.S.A.

Statistics

The survival rate along with the standard error (SE) was estimated using the Kaplan-Meier method. An event was defined as the occurrence of a relapse, progression or treatment-related death. The differences in the survival rates between the two groups (single HDCT versus tandem HDCT, *N-myc* amplified versus not amplified) were compared using the log-rank test. The differences in the ANC and platelet recovery between the first and second HDCT/ASCR were analyzed using a paired t-test. *P* values <0.05 were considered significant.

RESULTS

Patient characteristics

Table 2 lists the clinical and biological characteristics of all 28 patients. The median age of the 28 patients (15 males and 13 females) at diagnosis was 23.5 months (range 1-95), and 19 patients (67.9%) were over 12 months of age at diagnosis. Eleven out of 28 tumors (39.3%) were *N-myc* amplified ($\geq$ 3 copies) and 10 (35.7%) had unfavorable Shimada pathology. The primary site of the tumors was the abdomen in 23 patients, mediastinum in 4 patients and neck in 1 patient.

Treatment prior to HDCT/ASCR

Fig. 1 shows the flow of patients from diagnosis through HDCT/ASCR. All except two patients with *N-myc* amplification (early death in 1 and refusal to receive HDCT in 1) underwent HDCT/ASCR. In addition, five of 17 patients without *N-myc* amplification (poor response to chemotherapy in 2 patients, persistent gross residual tumor in 2 patients and relapse during chemotherapy in 1 patient) underwent HDCT/ASCR. Therefore, overall, 14 patients underwent

HDCT/ASCR. Eleven of 17 patients without *N-myc* amplification received conventional chemotherapy alone.

Table 3 lists the clinical and biological characteristics of 14 patients who underwent HDCT/ASCR. Overall, a median of 8 cycles (range 6-14) of chemotherapy was administered before the first HDCT/ASCR. Five out of 14 patients received local radiotherapy after surgery. The tumor status prior to the first HDCT was CR in 7, VGPR in 5 and PR in 2.

Feasibility of PBSC collection

The PBSCs were collected during the sixth to eighth chemotherapy cycle. A median of 6.5×10^6 CD34$^+$ cells/kg (range 2.1-17.9) were collected during a median of four leukapheresis events (range 2-11). Generally, the leukapheresis procedure was well tolerated and no patient developed significant toxicity except for thrombocytopenia requiring a transfusion after leukapheresis in all patients. During the late period of this study, quantitative RT-PCR for tyrosine hydroxylase mRNA was performed to detect possible tumor cell contamination in the PBSCs collected from five patients, and no mRNA transcript was detected (12).

HDCT/ASCR

Fourteen out of 28 patients underwent at least one HDCT/ASCR. The median age at the first HDCT/ASCR was 30.5 months (range 16-70). The tumor status after the first HDCT/ASCR was CR in 12 and VGPR in 2. One out of nine patients in the early period and all five in the late period underwent a second HDCT/ASCR. The median time from the first infusion of PBSCs to the initiation of the second HDCT was 82.5 days (range 66-88).

Table 2. Clinical and biological characteristics at diagnosis

Parameters	Number (%) (n=28)
Sex	
Female	13 (46.4)
Male	15 (53.6)
Age	
<12 months	9 (32.1)
≥12 months	19 (67.9)
N-myc gene	
Amplified (≥3 copies)	11 (39.3)
Not amplified (<3 copies)	17 (60.7)
Pathology (Shimada classification)	
Favorable	17 (60.7)
Unfavorable	10 (35.7)
Undetermined	1 (3.6)
Primary site	
Abdomen	23 (82.1)
Others	5 (17.9)
Lactate dehydrogenase (IU/L)	1,002 (367-6,744)*
Ferritin (ng/mL)	76 (10-1,129)
Neuron-specific enolase (ng/mL)	45 (8-529)
Vanyl-mandellic acid (mg/day)	5.3 (0.2-54.1)

*Median (range).

Table 3. Characteristics of patients who underwent HDCT/ASCR

UPN	Age at Dx (mon)	*N-myc*	Pathology (Shimada)	Primary site	Pre-HDCT chemotherapy	Reason for HDCT	Tumor status at HDCT1	HDCT1 regimen	HDCT2 regimen	RT timing	FU from HDCT1 (mon)	Outcome
5	27	A	UF	Abdomen	CEDC×6	High-risk	CR	CEM	–	ND	110 +	Ds free
7	7	NA	F	Abdomen	CDEC×9	Persistent	PR	CEM	CTM	After surgery and HDCT2	94 +	Ds free
8	31	A	UF	Abdomen	CEDC×6	High-risk	CR	CEM	–	ND	15	Relapse
10	15	A	F	Abdomen	CEDC×6	High-risk	VGPR	CEM	–	After surgery	83 +	Ds free
11	33	A	UF	Abdomen	CEDC×6	High-risk	CR	CEM	–	ND	79 +	Ds free
12	63	A	UF	Abdomen	CEDC×6	High-risk	VGPR	CEM	–	After surgery	7	Relapse
14	10	NA	F	Abdomen	CEDC×13, ICE×1	Relapse	CR	CEM	–	After relapse	58 +	Ds free
17	17	A	UF	Abdomen	CEDC×6	High-risk	VGPR	CEM	–	After surgery	57 +	Ds free
18	25	A	F	Chest	CEDC×3, ICE×4	High-risk	VGPR	CEM	–	After surgery	53 +	Ds free
21	7	A	F	Abdomen	CEDC×5, ICE×4	High-risk	VGPR	CEC	TM	After HDCT2	19 +	Ds free
22	13	NA	F	Chest	CEDC×5, ICE×4	Poor response	CR	CEC	TM	After HDCT2	18 +	Ds free
23	32	NA	F	Abdomen	CEDC×5, ICE×4	Poor response	CR	CEC	TM	After HDCT2	16 +	Ds free
24	38	NA	UF	Abdomen	CEDC×5, ICE×4	Persistent	PR	CEC	TM	After HDCT2	15 +	Ds free
25	19	A	Unknown	Abdomen	CEDC×5, ICE×4	High-risk	CR	CEC	TM	After HDCT2	12 +	Ds free

UPN, unique patient number; Dx, diagnosis; HDCT, high-dose chemotherapy; HDCT1, first HDCT; HDCT2, second HDCT; RT, radiotherapy; FU, follow-up; A, amplified; NA, not amplified; F, favorable; UF, unfavorable; ND, not done; ds, disease; mo, months.

A median of 4.0×10^6 CD34$^+$ cells/kg (range 0.6-13.0) were infused for the first HDCT/ASCR, and the median time required to reach an ANC of 500/μL and a platelet count of 20,000/μL, without transfusion for the previous 7 days, was 10 days (range 8-14) and 24 days (range 16-38), respectively. A median of 4.1×10^6 CD34$^+$ cells/kg (range 1.5-7.3) were infused for the second HDCT/ASCR and the median time required to reach an ANC of 500/μL and a platelet count of 20,000/μL was 10 days (range 8-14) and 19.5 days (range 14-220), respectively. When the analysis was confined to only those patients who had received tandem transplantation, there were no significant differences in the ANC and platelet recovery between the first and second HDCT/ASCR.
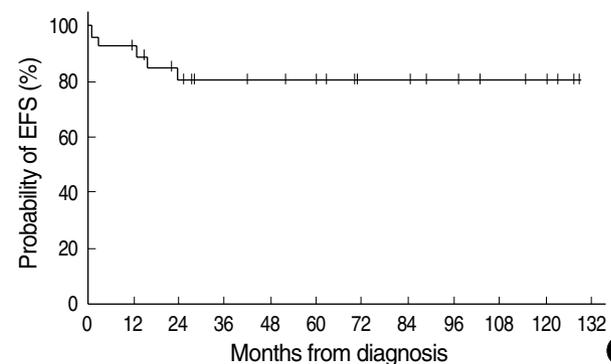
Table 4 lists the grade 3 and 4 toxicities that developed during the first and second HDCT/ASCR. The median duration of high fever (body temperature $\geq 38.0^{\circ}$C) during the first and second HDCT/ASCR was 5 and 6.5 days, respectively. There were three microbiologically documented infections (2 in the first and 1 in the second HDCT/ASCR). The

grade 3 and 4 toxicities that frequently developed in more than 1/3 of patients were vomiting, elevation of liver enzymes and hypokalemia. There was no toxic death during the first and second HDCT/ASCR. No secondary malignancy developed in this study.

Feasibility of post-HDCT/ASCR therapy

Skin eruption, particularly on the face, was a common side effect of the CRA treatment. High fever, thrombocytopenia and local inflammation were frequent side effects of the IL-2 therapy. However, all patients tolerated the differentiation therapy and immunotherapy well and there was no grade 3 or 4 toxicity that required the discontinuation of treatment during the post-HDCT/ASCR therapy.

Survival of patients

The tumor relapsed in three out of all 28 patients and there were two toxic deaths during the conventional chemotherapy. Therefore, 23 out of 28 patients remained event free with a median follow-up of 63 months (range 12-129) from diagnosis. The 5-yr EFS rate ($\pm$SE) after diagnosis for all 28 patients was $80.8 \pm 7.8\%$ (Fig. 2A). The 5-yr EFS rate, for all 11 patients with *N-myc* amplification and the 17 patients without, was $71.6 \pm 14.0\%$ and $87.8 \pm 8.1\%$, respectively (Fig. 2B).

The tumor relapsed in 2 out of 14 patients who underwent HDCT/ASCR and the remaining 12 patients remained event free with a median follow-up of 55 months (range 12-110) from the first HDCT/ASCR. The 5-yr EFS rate after the first HDCT/ASCR for all 14 patients who underwent single or tandem HDCT/ASCR, was $85.1 \pm 9.7\%$ (Fig. 3A). While the tumor relapsed in two of eight patients who underwent single HDCT/ASCR, all six patients who underwent the tandem HDCT/ASCR remained event free (Fig. 3B).
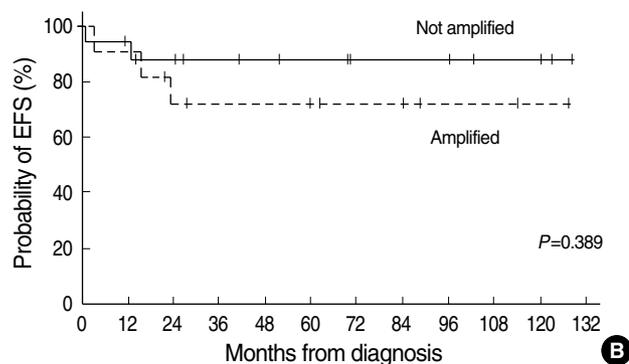
Table 4. Grade 3, 4 toxicities during HDCT/ASCR

Toxicities	First HDCT (n=14)	Second HDCT (n=6)
Days of BT $\geq 38.0^{\circ}$C	5 (2-11)*	6.5 (4-8)*
Documented infection	2 (14.3%)	1 (16.7%)
Stomatitis	2 (14.3%)	3 (50.0%)
Vomiting	4 (28.6%)	2 (33.3%)
Frequency of diarrhea ≥ 10/day	7 (50.0%)	6 (100%)
Elevation of liver enzyme	14 (100%)	4 (66.7%)
Azotemia	0 (0%)	0 (0%)
Metabolic acidosis	0 (0%)	0 (0%)
Hyponatremia	3 (21.4%)	0 (0%)
Hypokalemia	12 (85.7%)	3 (50.0%)
Hepatic veno-occlusive disease	1 (7.1%)	0 (0%)
Treatment-related mortality	0 (0%)	0 (0%)

*Median (range).
BT, body temperature.

Fig. 2. Results of Kaplan-Meier analysis for the EFS in all 28 patients. The tumor relapsed in 3 out of 28 patients and there were 2 toxic deaths during conventional chemotherapy. Therefore, 23 out of 28 patients remained event free with a median follow-up of 63 months (range 12-129) from diagnosis. **(A)** The 5-yr EFS rate ($\pm$SE) after diagnosis for all 28 patients was $80.8 \pm 7.8\%$. **(B)** The 5-yr EFS rate for all 11 patients with *N-myc* amplification and 17 patients without was $71.6 \pm 14.0\%$ and $87.8 \pm 8.1\%$, respectively.
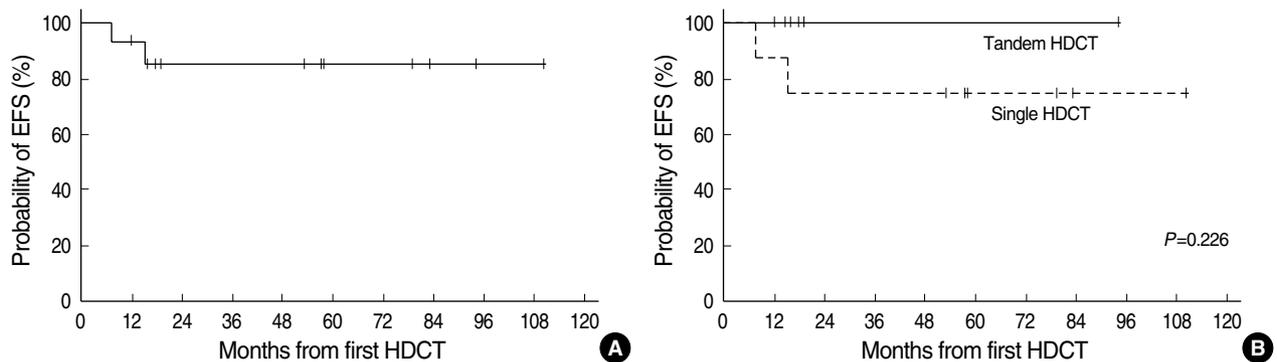
Fig. 3. Results of Kaplan-Meier analysis for the EFS of 14 patients who underwent HDCT/ASCR. The tumor relapsed in 2 out of 14 patients and the remaining 12 patients remained event free with a median follow-up of 55 months (range 12-110) from the first HDCT/ASCR. (**A**) The 5-yr EFS rate after the first HDCT/ASCR for all 14 patients was 85.1±9.7%. (**B**) While tumor relapsed in 2 of 8 patients in single HDCT/ASCR group, all 6 patients in tandem HDCT/ASCR group remained event free.

DISCUSSION

Although the prognosis of stage 3 neuroblastoma is generally good (1, 2), the prognosis of stage 3 neuroblastoma with unfavorable biologic factors, especially *N-myc* amplification, is poor with conventional chemoradiotherapy (1, 3). In addition, the prognosis of stage 3 neuroblastoma with a poor response to conventional treatment (4-6) or with a relapse during conventional treatment is also poor even when the tumor did not have unfavorable biologic factors. In this context, HDCT/ASCR was given to patients with high-risk stage 3 neuroblastoma in the present study. As a result, the 5-yr EFS from diagnosis, in patients with *N-myc* amplified tumor, was 71.6±14.0%. In addition, 12 out of 14 patients who underwent HDCT/ASCR remained event free resulting in an 85.1±9.7% 5-yr EFS after the first HDCT/ASCR. Survival rate in the present study is higher than those reported in the previous trials in which conventional chemotherapy was given for the treatment of high-risk stage 3 neuroblastoma (1, 3). These findings suggest that HDCT/ASCR might improve the survival of patients with high-risk stage 3 neuroblastoma.

Generally, the prognosis of stage 3 neuroblastoma without *N-myc* amplification is good with conventional chemotherapy alone (1, 2). However, even in these patients, the prognosis is poor, if the tumor response to conventional chemotherapy is poor (4) or gross residual tumor remained after surgery (5, 6). In this context, HDCT/ASCR was given to two patients with a poor response to conventional chemotherapy and two patients with a gross residual mass after surgery as well as patients with *N-myc* amplified tumor. These four patients remained event free; the 5-yr EFS for patients without *N-myc* amplification was 87.8±8.1% in the present study. In the present study, HDCT/ASCR contributed to the excellent survival rate in patients without *N-myc* amplification in whom the tumor response to conventional chemotherapy was poor.

In our recent report, on patients over 1 yr of age with stage 4 neuroblastoma, the EFS in patients who received TBI was better than for patients who did not receive TBI (12). This suggested that a regimen with TBI was better than a regimen without, for the survival of patients, over 1 yr of age, with stage 4 neuroblastoma. However, TBI was not included in the HDCT regimen for high-risk stage 3 patients in the present study. Although the tumor relapsed in 2 of 14 patients who underwent HDCT/ASCR, the remaining 12 high-risk patients remained event free without TBI. TBI may increase the frequency and severity of short-term side effects as well as long-term side effects such as a secondary malignancy several years after treatment (17, 18). The fact that there was no toxicity-related death during the HDCT/ASCR, in the present study, might be attributed to the absence of TBI in our HDCT regimen. Taken together, these findings suggest that our regimen without TBI might be safe and effective for patients with high-risk stage 3 neuroblastoma.

Although the strategy with the HDCT/ASCR improved the survival of patients with high-risk neuroblastoma, the survival rate after a single HDCT/ASCR has been unsatisfactory (7-11). Our recent report, which examined the efficacy of tandem HDCT/ASCR to further improve the treatment outcome of stage 4 neuroblastoma patients over 1 yr of age, showed a better long-term EFS compared to studies reporting on a single HDCT/ASCR strategy (12). The strategy using a tandem HDCT/ASCR was based on the hypothesis that further dose escalation might result in further improvements in the EFS of high-risk neuroblastoma patients. In the present study, the tandem HDCT/ASCR was given only if a CR was not achieved, even after the first HDCT/ASCR, in the early period of the study. However, all patients underwent tandem HDCT/ASCR during the late study period. As a result, while the tumor relapsed in two of eight patients in the single HDCT group, all 6 patients in the tandem HDCT group remained relapse free. These findings suggest that the tandem HDCT strategy may be better than the single HDCT strategy for improved survival even in patients with high-risk stage 3 neuroblastoma, although it was not

statistically significant in the present study. Further study will be needed to evaluate whether tandem HDCT/ASCR is better than single HDCT/ASCR in the treatment of high-risk stage 3 neuroblastoma. In six patients who underwent tandem HDCT/ASCR, the short-term toxicities were acceptable during the second HDCT/ASCR. There was no life-threatening toxicity during the second HDCT/ASCR. However, long-term follow-up is needed to evaluate the long-term toxicities of the tandem HDCT/ASCR.

According to previous reports, patients treated for longer periods, prior to HDCT/ASCR, had better long-term EFS than those patients treated for shorter periods (9, 12). While all the patients in the late study period received 9 cycles of chemotherapy prior to the HDCT/ASCR, most of the patients in the early study period received 6 or 7 cycles of chemotherapy. The two patients in whom the tumor relapsed after the HDCT/ASCR received 6 cycles of chemotherapy prior to HDCT/ASCR, although they were in the CR or VGCR prior to the HDCT/ASCR. Similarly, the tumor relapsed in two of six patients who received 6 cycles of chemotherapy prior to the HDCT, although the tumor status prior to the first HDCT was CR in three patients and VGPR in three, respectively. However, it is unclear whether the short duration of conventional chemotherapy was related to the high relapse rate after HDCT/ASCR in the present study, because all but one patient in the single HDCT/ASCR group received 6 or 7 cycles of chemotherapy prior to the first HDCT/ASCR while all patients in the tandem HDCT/ASCR group received 9 cycles of chemotherapy.

Immunotherapy with IL-2 along with CRA was provided in an attempt to control the minimal residual disease after the HDCT/ASCR. These two drugs were used simultaneously because their major toxicities do not overlap and their different mechanisms of action could synergistically eradicate any minimal residual disease. While the efficacy of CRA has been demonstrated (19-21), there have been few studies that have evaluated the efficacy of IL-2 therapy (16). Therefore, it is unclear if IL-2 therapy contributed to the improved survival in the present study. However, there was no life-threatening toxicity and an increase in the number of natural killer cells was observed after IL-2 therapy (22). More study will be needed to evaluate the efficacy of the IL-2 treatment after HDCT/ASCR.

The present study demonstrates that HDCT/ASCR, especially tandem HDCT/ASCR, may improve the prognosis of patients with high-risk stage 3 neuroblastoma. However, throughout this study, multiple modifications were made in the treatment plan, which resulted in significant variability over time between the patients. This variability may create doubt as to whether tandem HDCT/ASCR actually resulted in the improved outcomes. For example, a longer pre-HDCT treatment in the tandem HDCT group compared with the single HDCT group might have had an impact on the EFS in the present study. Further study will be needed to evaluate the efficacy of tandem HDCT/ASCR in the treatment of high-risk stage 3 neuroblastoma.

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