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Clinical Significance of Persistent Tumor in Bone Marrow during Treatment of High-risk Neuroblastoma

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The records of 63 high-risk neuroblastoma patients with bone marrow (BM) tumors at diagnosis were retrospectively reviewed. All patients received nine cycles of induction chemotherapy followed by tandem high-dose chemotherapy and autologous stem cell transplantation (HDCT/auto-SCT). Follow-up BM examination was performed every three cycles during induction chemotherapy and every three months for one year after the second HDCT/auto-SCT. BM tumor cells persisted in 48.4%, 37.7%, 23.3%, and 20.4% of patients after three, six, and nine cycles of induction chemotherapy and three months after the second HDCT/auto-SCT, respectively. There was no difference in progression-free survival (PFS) rate between patients with persistent BM tumor and those without during the induction treatment. However, after tandem HDCT/auto-SCT, the PFS rate was worse in patients with persistent BM tumor than in those without (probability of 5-yr PFS 14.7% ± 13.4% vs. 64.2% ± 8.3%, $P = 0.009$). Persistent BM tumor during induction treatment is not associated with a worse prognosis when intensive tandem HDCT/auto-SCT is given as consolidation treatment. However, persistent BM tumor after tandem HDCT/auto-SCT is associated with a worse prognosis. Therefore, further treatment might be needed in patients with persistent BM tumor after tandem HDCT/auto-SCT.

Keywords: Neuroblastoma; Bone Marrow Tumors; Prognosis; Treatment

INTRODUCTION

Neuroblastoma is the most common extracranial solid tumor of children. Many patients have metastatic disease at diagnosis. The bone marrow (BM) is a common metastatic site, and many high-risk patients have BM tumor at diagnosis. The current treatment for high-risk neuroblastoma consists of induction treatment, high-dose chemotherapy and autologous stem cell transplantation (HDCT/auto-SCT) as consolidation treatment, and 13-*cis*-retinoid acid treatment to reduce relapse from possible residual disease. Regular evaluation of tumor response during and after treatment is needed to assess treatment efficacy.

Several researchers have reported that persistent BM tumor after a certain period of induction treatment is associated with poor outcomes (1-3). However, these studies differed in the timing of BM examination and used strategies with various treatment intensities and durations. Therefore, it remains controversial whether persistent BM tumor is really a significant risk factor. In our previous retrospective reports, a longer induction treatment followed by tandem HDCT/auto-SCT to increase treatment intensity was associated with a better outcome in patients with high-risk neuroblastoma (4). We have prospectively used tandem HDCT/auto-SCT following nine cycles of induction chemotherapy since January 2004. Interestingly, many patients with persistent BM tumor during induction treatment were found to remain progression free. This finding suggests that the significance of persistent BM tumor might differ from what was previously reported when treatment intensity and duration are increased. For this reason, we retrospectively evaluated the significance of persistent BM tumor in patients with high-risk neuroblastoma treated at our center.

MATERIALS AND METHODS

Patients

Among 197 patients who were newly diagnosed with neuroblastoma at Samsung Medical Center from January 2004 to December 2012, 88 had high-risk tumors. In the present study, 63 high-risk neuroblastoma patients with BM tumor at diagnosis were retrospectively reviewed. Patients were staged according to the International Neuroblastoma Staging System (5). *MYCN* amplification was determined using competitive PCR, quantitative RT-PCR (qRT-PCR), or fluorescence in situ hybridization. Tumors were classified as histologically favorable or unfavorable according to the International Neuroblastoma Pathology Classification (6). Serum ferritin, neuron-specific enolase (NSE), lactic acid dehydrogenase (LDH), and 24-hr urine vanillylmandelic acid (VMA) were measured at diagnosis. Stage 4 tumors

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in patients older than 1 yr or any *MYCN*-amplified tumors were stratified as high-risk tumors.

Treatment of patients

For induction chemotherapy, CEDC (cisplatin + etoposide + doxorubicin + cyclophosphamide) and ICE (ifosfamide + carboplatin + etoposide) regimens were used in an alternating manner (Table 1). Overall, nine cycles of induction chemotherapy were administered prior to HDCT/auto-SCT. Excisional biopsy of the primary tumor was performed at diagnosis if the tumor was resectable. Otherwise, incisional or percutaneous needle biopsy was performed, and definitive surgery was deferred until after six cycles of chemotherapy. Peripheral blood stem cells (PBSCs) were collected during the recovery phase after the seventh chemotherapy cycle. After induction treatment, tandem HDCT/auto-SCT was administered. The CEC (carboplatin + etoposide + cyclophosphamide) regimen was used for the first HDCT. As the second HDCT regimen, a TM (thiotepa + melphalan)-total body irradiation (TBI) regimen was used for patients who were diagnosed up until December 2008. For patients diagnosed from January 2009 onward, TBI was substituted with high-dose ^{131}I-metaiodobenzylguanidine (MIBG) treatment to reduce late adverse effects (Table 1). Local radiotherapy was applied to the primary site in all patients about 6 weeks after the second HDCT/auto-SCT. Differentiation therapy with 13-*cis*-retinoic acid (125 mg/m^2/day for 14 days every 4 weeks) along with immunotherapy using interleukin-2 (2×10^6 U/m^2/day for 5 days every 4 weeks) was administered until one year after HDCT/auto-SCT to reduce relapse from possible residual tumor cells (7).

Table 1. Induction and high-dose chemotherapy regimens

Regimen/drugs	Dose	Schedule
Induction regimens		
CEDC		
Cisplatin	60 mg/m^2/dose	Day 0
Etoposide	100 mg/m^2/dose	Days 2, 5
Doxorubicin	30 mg/m^2/dose	Day 2
Cyclophosphamide	30 mg/kg/dose	Days 3, 4
ICE		
Ifosfamide	1,200 mg/m^2/dose	Days 0-4
Carboplatin	400 mg/m^2/dose	Days 0-1
Etoposide	100 mg/m^2/dose	Days 0-4
First HDCT regimen		
Carboplatin	650 mg/m^2/dose	Days -7, -6, -5
Etoposide	650 mg/m^2/dose	Days -7, -6, -5
Cyclophosphamide	1,800 mg/m^2/dose	Days -4, -3, -2
Second HDCT regimen		
2004-2008		
Thiotepa	200 mg/m^2/dose	Days -8, -7, -6
Melphalan	60 mg/m^2/dose	Days -5, -4
TBI	3.33 Gy/dose	Days -3, -,2, -1
2009-2012		
Thiotepa	200 mg/m^2/dose	Days -6, -5, -4
Melphalan	60 mg/m^2/dose	Days -3, -2
^{131}I-MIBG	12 or 18 mCi/kg	Day -21

HDCT, high-dose chemotherapy; TBI, total body irradiation; ^{131}I-MIBG, ^{131}I-metaiodobenzylguanidine.

BM examination

BM examination (bilateral aspiration and biopsies) was performed at diagnosis, after every three cycles of induction chemotherapy, and then every three months for the first year after tandem HDCT/auto-SCT. Hematoxylin and eosin (H&E) sections were prepared from paraffin embedded biopsy specimens fixed in 10% buffered formalin. Tumor cells in the BM biopsy were confirmed by immunohistochemical staining with antibodies against NSE (1:100, polyclonal, CAT 18-0042, Invitrogen, Carlsbad, CA, USA) and CD56 (1:200, CD564, NCL-56-5041, Novocastra, Newcastle upon Tyne, UK) (8, 9). The degree of BM tumor differentiation (undifferentiated neuroblasts, differentiating neuroblasts, and ganglion cells), stroma composition (neuropil and Schwannian stroma), and presence of rosettes, necrosis, fibrosis, hemorrhage, calcification, and foam cells was evaluated. The percent tumor area was calculated as the proportion of the area occupied by tumor cells in the total BM area.

Detection of tyrosine hydroxylase (TH) transcripts

The levels of TH transcripts in PBSCs were measured. PBSC samples were collected in EDTA tubes and mononuclear cells were separated. The mononuclear cells were lysed in Trizol reagent (Invitrogen, Carlsbad, CA, USA), and RNA was extracted according to the manufacturer's instructions. When RNA could not be extracted on collection day, the specimen was immediately stored below -70°C and RNA was extracted later (Ambion, Austin, TX, USA). Complementary DNA was generated using an RNA PCR kit (Applied Biosystems, Foster City, CA, USA). Real-time qRT-PCR was performed using the Real-Q TH Quantification Kit (BioSewoom, Seoul, Korea) on a LightCycler (Roche Diagnostics, Mannheim, Germany). qRT-PCR was performed under the following cycling conditions: denaturation at 95°C for 10 min, 45 cycles at 95°C for 10 sec, 60°C for 10 sec, and 72°C for 30 sec, followed by a cooling step at 40°C for 30 sec. Samples were regarded as TH transcript positive if a cycle threshold value was 42 or less. The levels of TH transcripts in biopsied BM samples were also measured according to the methods which we previously reported (10). In brief, fresh biopsied BM tissues were directly lysed in Trizol agent immediately after biopsy, and the following RNA isolation steps were identical to those used for PBSCs.

Statistical analysis

The Mann-Whitney U-test and Kruskal Wallis test were used to compare continuous variables between groups. The chi-square test was used to compare frequencies between groups. The progression-free survival (PFS) rate and 95% confidence interval were determined using the Kaplan-Meier method. Differences

in the PFS rates between groups were compared using the log-rank test. *P* value < 0.05 were considered significant.

Ethics statement

The institutional review board (IRB) of Samsung Medical Center approved this study and waived the requirement for informed consent (IRB No. 2014-12-123).

RESULTS

Patient characteristics

A total of 63 high-risk patients (39 boys and 24 girls) had BM tumors at diagnosis during the study period. Median age at diagnosis was 39.5 months (range 1-231). Twenty-two patients had *MYCN*-amplified tumors and 47 patients had histologically unfavorable tumors. Three patients experienced progression during induction treatment. The remaining 60 patients underwent the first HDCT/auto-SCT, and three patients died from toxicities during the first HDCT/auto-SCT. Therefore, 57 patients underwent the second HDCT/auto-SCT. Twenty patients experienced relapse/progression after the second HDCT/auto-SCT (nine patients within one year after the second HDCT/auto-SCT).

Result of BM examination

A total of 484 BM biopsies from 63 patients were reviewed. The proportion of patients with persistent BM tumor gradually decreased during induction treatment and follow-up after tandem HDCT/auto-SCT (Fig. 1A). BM tumor cells were persistent in 14 (23.3%) of 60 biopsies at the end of induction and 11 (20.4%) of 54 biopsies at three months after the second HDCT/auto-SCT. In patients with persistent BM tumor, the BM tumor area decreased significantly during the first three cycles of chemotherapy; however, it did not change during further follow-up (Fig. 1B). The evidence of tumor maturation (proportions of ganglion cells and Schwannian stroma) gradually increased during treatment and follow-up (Fig. 1C and D).

Detection of TH mRNA transcripts

TH transcripts in PBSCs were positive in 1 of 22 patients with persistent BM tumor and 1 of 37 patients without BM tumor after six cycles of induction chemotherapy (*P* = 1.000). While the former patient remains progression free, the latter patient experienced progression. When the presence of BM tumor was evaluated with qRT-PCR for TH, the results were correlated with those with histologic examination (sensitivity 71.6% and specificity 82.8%). TH transcripts were positive in 25 (17.2%) of 145

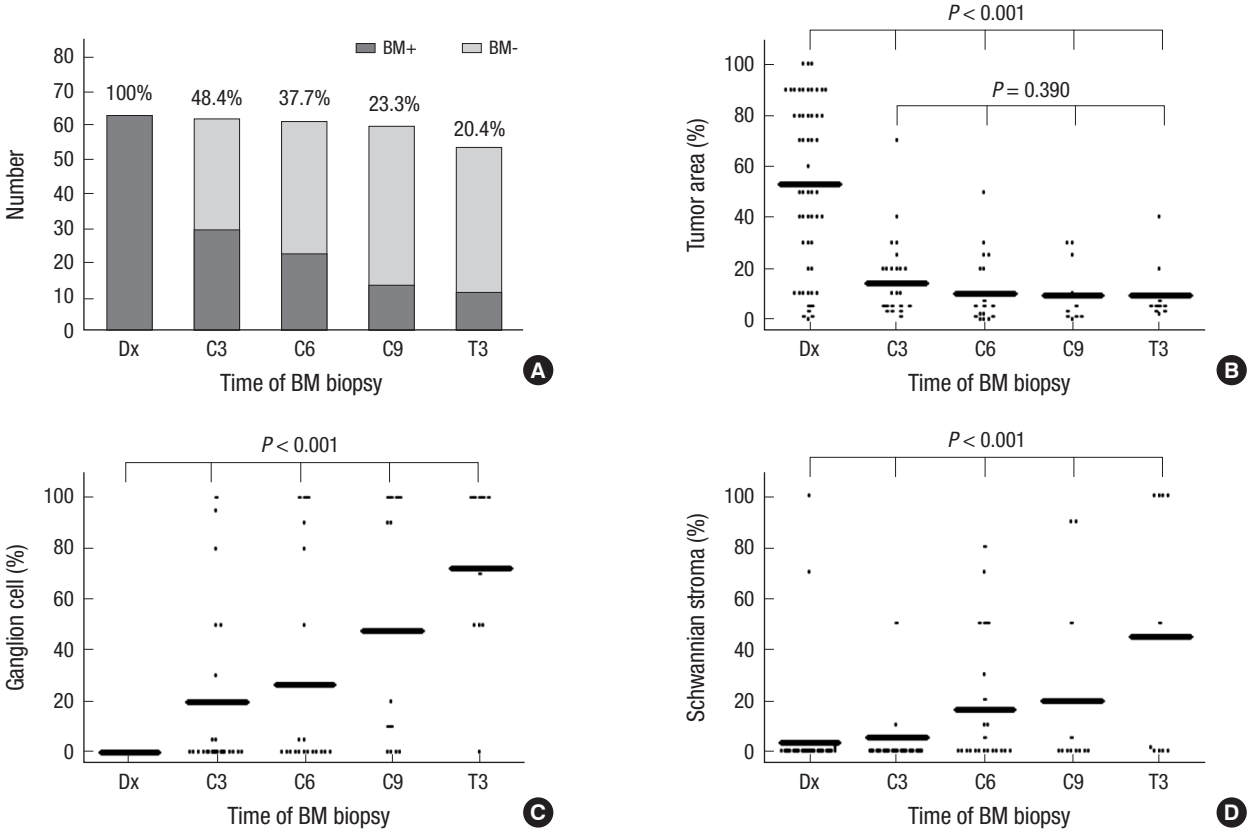

Fig. 1. Results of bone marrow examination. (**A**) Proportion of patients with persistent BM tumor gradually decreases during follow-up. (**B**) In patients with persistent BM tumor, tumor area in BM decreases significantly during the first three cycles of chemotherapy (Dx-C3); however, it does not change during further follow-up (C3-T3). Proportions of ganglion cell (**C**) and Schwannian stroma (**D**) gradually increase during treatment and follow-up.

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BM samples without tumor in histological examination.

PFS according to presence/absence of BM tumors

There was no difference in the PFS rates between patients with persistent BM tumor and those without at each time point during induction treatment (Fig. 2A-C). However, the PFS rate in patients with BM tumor was worse than those without at three months after tandem HDCT/auto-SCT (probability of 5-yr PFS 14.7 ± 13.4% vs. 64.2 ± 8.3%, $P = 0.009$, Fig. 2D). When TH transcript positive BM samples without histological evidence were regarded as having persistent BM tumors, the prognostic significance of persistent BM tumors was not changed during induction treatment. However, after tandem HDCT/auto-SCT, the prognostic significance of persistent BM tumors disappeared (probability of 5-yr PFS 60.7 ± 9.3% vs. 42.9 ± 13.8%, $P = 0.359$). While seven of 11 patients with histologically persistent BM tumors experienced relapse/progression, only one of seven TH transcript positive patients without histological evidence experienced relapse/progression ($P = 0.014$).

Factors determining early BM response

Various clinical and pathologic parameters associated with BM response were analyzed after the first three cycles of chemotherapy (Table 2). High NSE level (> 100 ng/mL) and low 24 hr urine VMA level (< 15 mg/day) at diagnosis were associated with lower frequency of persistent BM tumor after the first three cycles of chemotherapy. Younger age (< 18 months) and *MYCN* amplification were also associated with lower frequency of persistent BM tumor, but with borderline significance.

Pathologic parameters associated with poor outcome

BM specimens were analyzed in 29 of 30 patients with persistent BM tumor after the first three cycles of induction chemotherapy to evaluate a possible association between pathologic findings after three chemotherapy cycles and subsequent prognosis (Table 3). A higher proportion of differentiating neuroblasts in the BM tumor was associated with a higher probability of subsequent progression/relapse. However, there was no difference in the presence of rosettes, necrosis, fibrosis, hemorrhage, calcifications, and foam cells between patients who experienced

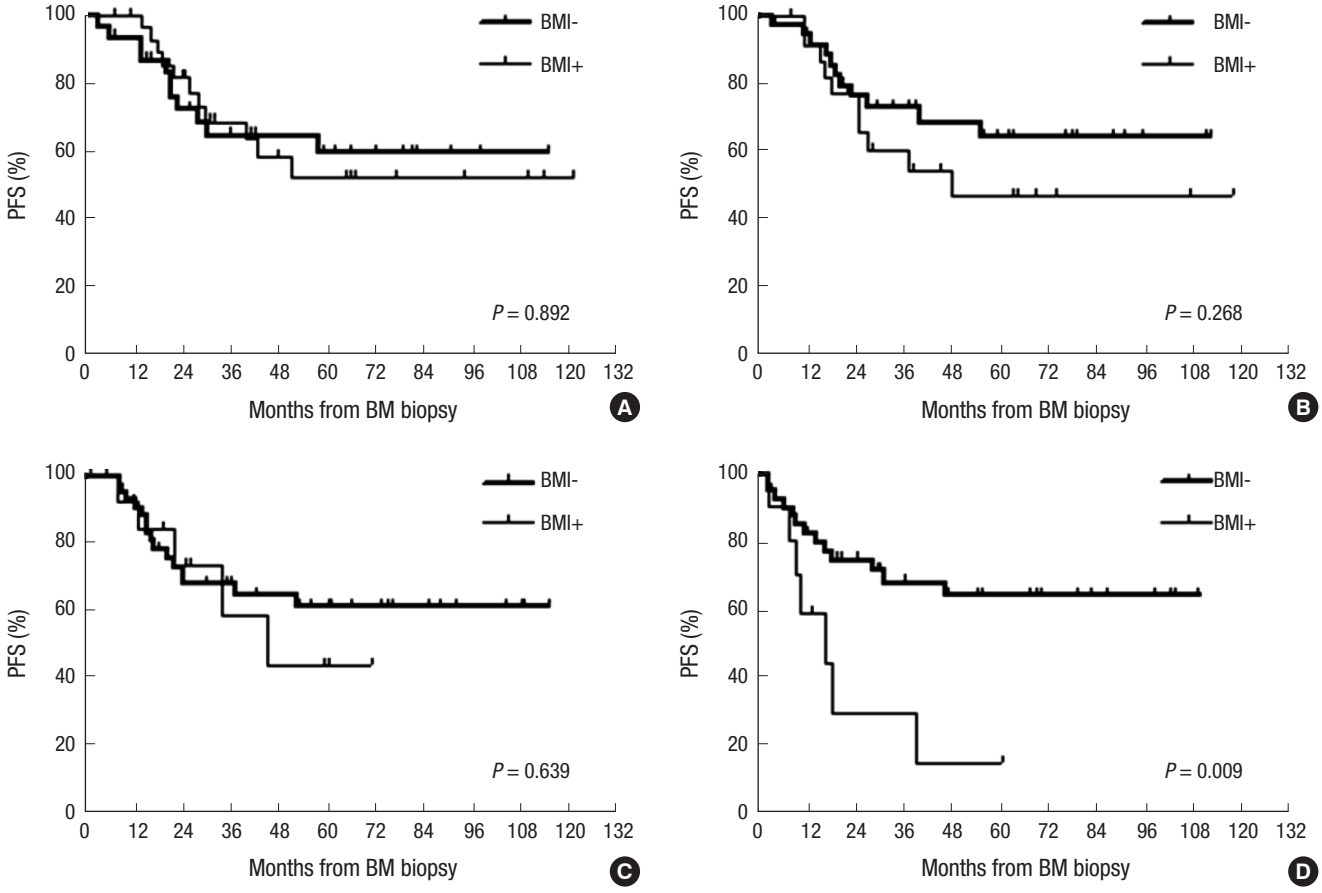

Fig. 2. PFS according to presence/absence of persistent BM tumor. There was no difference in progression free survival (PFS) rates between patients with persistent BM involvement (BMI) of tumor and those without at three (**A**), six (**B**), and nine (**C**) cycles of induction chemotherapy. (**D**) However, the PFS rate in patients with BMI of tumor was worse than those without at three months after tandem HDCT/auto-SCT.

subsequent relapse/progression and those who did not. There was also no difference in percent tumor area between the two patient groups.

DISCUSSION

According to previous studies, persistent BM tumor during induction treatment is a poor prognostic factor (1-3). However, these studies differed in the timing of BM examination and used various regimens with different treatment intensity and duration. Some studies used single HDCT/auto-SCT after a relatively short induction treatment (1, 2) while others used conventional chemotherapy (3). In our previous reports for high-risk neuroblastoma, we showed that longer induction treatment and intensive consolidation using tandem HDCT/auto-SCT is associated with better outcomes (4, 7). We hypothesized that the significance of persistent BM tumor might differ from what has been previously reported when intensive tandem HDCT/auto-SCT is administered as a consolidation treatment. The results indicate that persistent BM tumor during induction treatment was not associated with worse prognosis. This finding suggests that the prognostic significance of persistent BM tumor during induction treatment may be irrelevant if intensive consolidation treatment is given. However, histologically persistent BM tumor even after tandem HDCT/auto-SCT was associated with worse prognosis. This finding suggests that further treatment with a novel strategy is needed in patients with persistent BM tumor after tandem HDCT/auto-SCT.

Various clinical and pathologic parameters associated with early BM response after the first three cycles of chemotherapy were analyzed. Higher NSE level and lower urine VMA level at diagnosis were associated with lower frequency of persistent BM tumors after the first three cycles of chemotherapy. Younger age and *MYCN* amplification were also associated with lower frequency of persistent BM tumors, but with borderline significance. Higher urine VMA level (i.e., more mature tumor) at diagnosis and higher proportion of differentiating (not undifferentiated) neuroblasts in BM tumor after three cycles of chemotherapy were associated with lower frequency of persistent BM tumors after the first three cycles of chemotherapy and higher probability of subsequent progression, respectively. These findings suggest that favorable tumor biology might not indicate a favorable prognostic factor in high-risk patients who receive intensive treatment. We previously reported that the degree of tumor volume reduction during the early phase of induction che-

Table 2. Clinical factors determining BM response after three cycles of chemotherapy

Parameters	Persistent BM tumor (n = 30)	*P* value
Age at diagnosis		0.055
< 18 months (n = 7)	1 (14.3%)	
≥ 18 months (n = 55)	29 (52.7%)	
MYCN amplification		0.090
Absent (n = 41)	23 (56.1%)	
Present (n = 21)	7 (33.3%)	
Pathology (INPC)		0.596
Favorable (n = 11)	6 (54.5%)	
Unfavorable (n = 46)	21 (45.7%)	
Unknown (n = 5)	3 (60.0%)	
Differentiation		0.136
Undifferentiated (n = 14)	3 (21.4%)	
Poorly differentiated (n = 25)	12 (48.0%)	
Differentiating (n = 12)	8 (66.7%)	
Ganglioneuroblastoma (n = 6)	3 (50.0%)	
Unknown (n = 5)	4 (80.0%)	
LDH (U/L)		0.174
< 1,500 (n = 29)	16 (55.2%)	
≥ 1,500 (n = 27)	10 (37.0%)	
Unknown (n = 6)	4 (66.7%)	
Ferritin (ng/mL)		0.696
< 300 (n = 29)	13 (44.8%)	
≥ 300 (n = 28)	14 (50.0%)	
Unknown (n = 5)	3 (60.0%)	
NSE (ng/mL)		0.031
< 100 (n = 26)	16 (61.5%)	
≥ 100 (n = 33)	11 (33.3%)	
Unknown (n = 3)	3 (100%)	
24-hr urine VMA (mg/day)		0.030
< 15 (n = 26)	8 (30.8%)	
≥ 15 (n = 32)	19 (59.4%)	
Unknown (n = 4)	3 (75.0%)	

BM, bone marrow; INPC, International Neuroblastoma Pathology Classification; LDH, lactic acid dehydrogenase; NSE, neuron-specific enolase; VMA, vanillylmandelic acid.

Table 3. Pathologic characteristics according to outcome in patients with persisting tumor cells in BM after three cycles of chemotherapy

Characteristics	Progression free (n = 19)	Progression (n = 10)	*P* value
Presence of neuropil	14 (73.7%)	9 (90.0%)	0.303
Presence of rosettes	5 (26.3%)	4 (40.0%)	0.449
Presence of necrosis	0 (0%)	1 (10.0%)	0.161
Presence of fibrosis	5 (26.3%)	1 (10.0%)	0.303
Presence of hemorrhage	1 (5.3%)	0 (0%)	0.460
Presence of calcifications	0	0	1.000
Presence of foam cells	0	0	1.000
Tumor area (% BM area)*	5 (1-70)	15 (3-30)	0.247
Undifferentiated neuroblast (% total tumor cells)†	63 (0-100)	40 (0-97)	0.464
Differentiating neuroblast (% total tumor cells)†	15 (0-80)	45 (3-100)	0.035
Ganglion cell (% total tumor cells)†	0 (0-100)	0 (0-50)	0.588
Schwannian stroma (% tumor area)‡	0 (0-50)	0 (0-50)	0.796

*Tumor area ÷ BM area × 100; †Percent of neuroblasts or ganglion cells among total tumor cells; ‡Stroma area ÷ tumor area × 100. BM, bone marrow.

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motherapy was higher in undifferentiated and *MYCN*-amplified tumors (11). Similarly, the current findings suggest that tumors with unfavorable biology in high-risk neuroblastoma patients show better treatment response when treated with longer and more intensive protocols.

The detection of minimal residual disease (MRD) status in PBSCs might be crucial in high-risk neuroblastoma because PBSCs contaminated with tumor cells are thought to contribute to relapse (12, 13). Detection of TH transcripts by RT-PCR is one way to assess whether PBSCs are contaminated with tumor cells. The present study evaluated whether persistent BM tumor at time of PBSC collection is related to tumor cell contamination in PBSCs. There was no difference in TH positivity of PBSCs between patients with persistent BM tumor and those without. These findings suggest that it might not be necessary to defer PBSC collection even when BM tumors were persistent after 6 cycles of chemotherapy. However, it is not clear whether PBSC could be collected without tumor cell contamination in the earlier treatment period when BM tumors were persistent.

The significance of minimal residual BM tumors detected only with qRT-PCR after tandem HDCT/auto-SCT was different from that of histologically persistent BM tumors. There was no difference in the PFS between patients with MRD in the BM and those without after tandem HDCT/auto-SCT. These findings suggest that minimal residual BM tumors which can be detected only with qRT-PCR might be controlled by subsequent differentiation treatment and immunologic treatment.

In conclusion, persistent BM tumor during induction treatment is not associated with a worse prognosis when intensive tandem HDCT/auto-SCT is given as consolidation treatment. However, persistent BM lesions after tandem HDCT/auto-SCT are associated with a worse prognosis. Therefore, further treatment with a novel strategy might be required in patients with persistent BM tumor after tandem HDCT/auto-SCT.

DISCLOSURE

The authors have no competing financial conflicts of interest to declare.

AUTHOR CONTRIBUTION

Conception and coordination of the study: Sung KW. Design of ethical issues: Choi YB, Lee NH. Acquisition of clinicopathological data: Bae GE, Kim JS, Sung KW. Analysis and interpretation of data: Choi YB, Sung KW. Manuscript preparation: Choi YB, Sung KW. Critical review of manuscript: Lee SH, Yoo KH, Koo HH. Manuscript approval: all authors.

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REFERENCES

1. Seeger RC, Reynolds CP, Gallego R, Stram DO, Gerbing RB, Matthay KK. *Quantitative tumor cell content of bone marrow and blood as a predictor of outcome in stage IV neuroblastoma: a Children's Cancer Group Study. J Clin Oncol 2000; 18: 4067-76.*
2. Fukuda M, Miyajima Y, Miyashita Y, Horibe K. *Disease outcome may be predicted by molecular detection of minimal residual disease in bone marrow in advanced neuroblastoma: a pilot study. J Pediatr Hematol Oncol 2001; 23: 10-3.*
3. Cai JY, Pan C, Tang YJ, Chen J, Ye QD, Zhou M, Xue H, Tang JY. *Minimal residual disease is a prognostic marker for neuroblastoma with bone marrow infiltration. Am J Clin Oncol 2012; 35: 275-8.*
4. Sung KW, Lee SH, Yoo KH, Jung HL, Cho EJ, Koo HH, Lee SK, Kim J, Lim DH, Suh YL, et al. *Tandem high-dose chemotherapy and autologous stem cell rescue in patients over 1 year of age with stage 4 neuroblastoma. Bone Marrow Transplant 2007; 40: 37-45.*
5. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, De Bernardi B, Evans AE, Favrot M, Hedborg F, et al. *Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 1993; 11: 1466-77.*
6. Peuchmaur M, d'Amore ES, Joshi VV, Hata J, Roald B, Dehner LP, Gerbing RB, Stram DO, Lukens JN, Matthay KK, et al. *Revision of the International Neuroblastoma Pathology Classification: confirmation of favorable and unfavorable prognostic subsets in ganglioneuroblastoma, nodular. Cancer 2003; 98: 2274-81.*
7. Sung KW, Son MH, Lee SH, Yoo KH, Koo HH, Kim JY, Cho EJ, Lee SK, Choi YS, Lim DH, et al. *Tandem high-dose chemotherapy and autologous stem cell transplantation in patients with high-risk neuroblastoma: results of SMC NB-2004 study. Bone Marrow Transplant 2013; 48: 68-73.*
8. Ahn S, Lee JJ, Ha SY, Sung CO, Kim J, Han J. *Clinicopathological analysis of 21 thymic neuroendocrine tumors. Korean J Pathol 2012; 46: 221-5.*
9. Lee SS, Kang M, Ha SY, An J, Roh MS, Ha CW, Han J. *Morphologic analysis of pulmonary neuroendoscrine tumors. Korean J Pathol 2013; 47: 16-20.*
10. Lee ST, Suh YL, Ko YH, Ki CS, Sung KW, Kim HJ, Kim JW, Kim SH, Chueh H, Lee SH, et al. *Measurement of tyrosine hydroxylase transcripts in bone marrow using biopsied tissue instead of aspirates for neuroblastoma. Pediatr Blood Cancer 2010; 55: 273-8.*
11. Yoo SY, Kim JS, Sung KW, Jeon TY, Choi JY, Moon SH, Son MH, Lee SH, Yoo KH, Koo HH. *The degree of tumor volume reduction during the early phase of induction chemotherapy is an independent prognostic factor in patients with high-risk neuroblastoma. Cancer 2013; 119: 656-64.*
12. Burchill SA, Kinsey SE, Picton S, Roberts P, Pinkerton CR, Selby P, Lewis IJ. *Minimal residual disease at the time of peripheral blood stem cell harvest in patients with advanced neuroblastoma. Med Pediatr Oncol 2001; 36: 213-9.*
13. Moss TJ, Cairo M, Santana VM, Weinthal J, Hurvitz C, Bostrom B. *Clonogenicity of circulating neuroblastoma cells: implications regarding peripheral blood stem cell transplantation. Blood 1994; 83: 3085-9.*