J Korean Med Sci 2001; 16: 175-82
ISSN 1011-8934

Clinical Impacts of Tumor Cell Contamination of Hematopoietic Stem Cell Products in Metastatic Breast Cancer Patients undergoing Autologous Peripheral Blood Stem Cell Transplantation: Multicenter Trial

To determine whether the tumor cell contamination of peripheral blood stem cells influences clinical impacts on high-dose chemotherapy in patients with metastatic breast cancer, we analyzed carcinoembryonic antigen (CEA) mRNA in the apheresis products by nested RT-PCR (reverse transcriptase-polymerase chain reaction). A total of 38 metastatic breast cancer patients and ten normal healthy subjects as a negative control were included. Twenty out of 38 (51.3%) apheresis products from patients with metastatic breast cancer were positive for CEA mRNA. CEA mRNA was noted in 54.8% (17/31) of patients mobilized with chemotherapy plus G-CSF and 42.8% (3/7) of patients with G-CSF alone. There was no significant difference in age, estrogen receptor, menopausal status, mobilization method, disease free interval, or number of metastasis sites (1 vs ≥2) between positive and negative groups. The presence of CEA mRNA in apheresis products did not influence the time to progression and overall survival in both groups. However, both the univariate and the multivariate analysis disclosed that the number of metastasis was associated with survival significantly. We suggest that the tumor cell contamination does not predict poor treatment outcome in patients with metastatic breast cancer.

Key Words: *Breast Neoplasms; Peripheral Stem Cell Transplantation; RNA, Messenger; Hematopoietic Stem Cells*

Myung-Ju Ahn, Yun-Hee Noh*, Yong-Sung Lee†, Young-Yeul Lee, Il-Young Choi, In-Soon Kim, Eun-Kyung Joh†, Dong-Bock Shin†, Si-Young Kim§, Kyung-Sam Cho§, Hyo-Cheul Kim‖, Hyun-Soo Kim‖, Cheolwon Suh¶, Sang-Hee Kim¶, Jung-Ae Lee, Young-Suck Park****

Department of Internal Medicine and †Biochemistry, Hanyang University College of Medicine; Department of Biochemistry, *Kon-Kuk University College of Medicine; †Gachon University College of Medicine; §Kyung Hee University College of Medicine; ‖Ajou University College of Medicine; ¶University of Ulsan College of Medicine; **Hallym University College of Medicine, Korea

Received: 26 July 2000
Accepted: 12 December 2000

Address for correspondence
Myung-Ju Ahn, M.D.
Section of Hematology-Oncology, Department of Internal Medicine, Hanyang University Kuri Hospital, 249-1 Kyomung-dong, Kuri 471-020, Korea
Tel: +82.31-560-2236, Fax: +82.31-553-7369
E-mail: ahnmj@hanyang.ac.kr

INTRODUCTION

Autologous peripheral blood stem cell transplantation (APBSCT) is a commonly used treatment modality to manage a variety of advanced solid tumors, including breast cancer (1-3). Unfortunately, most of those patients who received APBSCT experienced relapse and succumbed because the therapy was unable to eradicate the existing disease. It has been known that bone marrow or peripheral blood stem cell harvests from patients with breast cancer and other malignancies are contaminated with tumor cells (4-8). Although several studies reported that infusion of tumor cells in stem cell products may be a contributing factor to poor clinical outcome and post-transplant relapse in leukemia, lymphoma, and neuroblastoma, the exact role of reinfusion of tumor cells in post-transplant relapse of metastatic breast cancer is not fully determined (9-12).

Carcinoembryonic antigen (CEA) is a notable tumor marker for gastric and colorectal cancer that is also expressed by the majority of breast cancers. There are reports of CEA as an RT-PCR (reverse transcriptase-polymerase chain reaction) marker to detect carcinoma cells in lymph nodes, bone marrow, and peripheral blood in breast cancer patients (13-15). Most promising was the report that the detection of positive axillary lymph nodes increased from 26% via histological analysis to 66% by RT-PCR analysis for CEA (13). Also it has been reported that RT-PCR analysis with CEA or mammaglobin marker confirmed immunohistochemistry-positive sentinel lymph node results of breast cancer and that, in some cases, RT-PCR analysis detected marker expression even in immunohistochemistry-negative sentinel lymph node (16).

We investigated whether the tumor cell contamination of peripheral blood stem cells, which was determined by nested RT-PCR of CEA mRNA, influences the clinical

outcome of high-dose chemotherapy in patients with metastatic breast cancer.

MATERIALS AND METHODS

Patient population

A total of 38 metastatic breast cancer patients underwent high dose chemotherapy (HDC) with APBSCT at eight institutes in Korea between December 1995 and November 1998. Peripheral blood stem cells were mobilized with chemotherapy plus G-CSF or G-CSF only. Aliquots of processed stem cells were cryopreserved and stored in liquid nitrogen until use. Ten normal healthy subjects were studied as a negative control.

Cell lines and peripheral blood samples

A colon cancer cell line, Colo 201 was used to test the potential sensitivity of RT-PCR for the detection of tumor cells in the blood. The mononuclear cells from normal healthy donors were prepared from 10 to 14 mL of peripheral blood by Ficoll-Hypaque gradient.

RNA preparation

RNA from peripheral mononuclear cells or peripheral blood stem cells was obtained following the thiocyanate, phenol-chloroform method described by Chomzinsky and Sacchi (17). Total RNA was spectrophotometrically quantified at 260/280 nm, and its quality was tested in 1% agarose gel to seek for intact 28S and 18S RNAs.

Nested RT-PCR

cDNA was synthesized from 5 μg of total RNA in a 25 μL reaction mixture containing 5 μL of 5×reverse transcriptase reaction buffer, 200 μM dNTP, 100 μM of random hexamer, 50 units of RNasin, 2 μL of 0.1 M dithiothreithol, and 200 units of Moloney leukemia virus reverse transcriptase. The mixture was incubated at 37℃ for 60 min, heated to 95℃ for 10 min, and then chilled on ice. CEA-specific oligonucleotide primers used for nested PCR were synthesized as previously described (15, 18). The first PCR product exhibited a 160 bp fragment and the second PCR product showed a 131 bp fragment. Nested PCR was performed according to the method described by Gerhard et al. (15). To ensure that the RNA was of sufficient purity for RT-PCR, PCR assay with primers specific for the gene β-actin cDNA was carried out in each case. The primer sequences for β-actin primers were as follows: 5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3' and 5'-CTAGAAGCATTGCGGGTGGACGATGGAGGG-3'. Each series of RT-PCR reactions included RNA-negative samples and normal healthy blood samples as a negative control and Colo 201 cell samples as a positive control.

Marker mRNA detection sensitivity

Total RNA was isolated from colon cancer cell line and quantitated to determine the sensitivity of the assay. Serially diluted Colo 201 cells from 10^4 to 1 were mixed in 10^6 mononuclear cells from normal healthy subject and RT-PCR was performed. The CEA mRNA could be detected at the level of 1 colon cancer cell in 10^6 mononuclear cells as previously described (18, 19).

High dose chemotherapy with APBSCT

Patients were treated with three or four cycles of induction chemotherapy with FAC (5-fluorouracil, adriamycin, cyclophosphamide), FEC (5-fluorouracil, epirubicin, cyclophosphamide), or Taxol containing regimen. For the mobilization of peripheral blood stem cells, G-CSF 5 μg/kg was administered by subcutaneous injection or G-CSF combined with cyclophosphamide 4 g/m^2 or induction chemotherapy. Peripheral blood stem cells were collected with Fenwal CS-3000 (Baxter Healthcare, Deerfield, IL, U.S.A.). Cryopreservation was performed using controlled rate freezer. Patients were treated with various types of high dose chemotherapy according to individual center protocol and the details are listed in Table 1. After 48 hr of chemotherapy, rapidly thawed stem cells were infused via central line. All patients provided informed consent.

Statistical analysis

Frequencies of CEA mRNA positivity were compared with various clinical characteristics using Pearson's chi-square test. Relapse free survival was calculated from day 0 (infusion of stem cells) until 1) the day of death or 2) the first signs or symptoms of disease proved to be relapse or progression of breast cancer, or 3) the last day of follow-up. Overall survival was calculated from day 0 until the day of death or the last follow-up. Patients who did not relapse or die were censored at the last day of follow-up. Survival curves were calculated using the Kaplan-Meier method and compared with the frequencies of tumor cell contamination using log-rank test. Multivariate analyses were performed using Cox's proportional hazards regression model to identify prognostic factors for survival. The regression analysis was performed using forward stepwise method. All statistical analyses were

Table 1. Patients characteristics in metastatic breast cancer

Patient	Age (yr)	Site	Mobilization	HDC	CEA mRNA	PFS	OS
1	54	B,L,Pl	Cy+G-CSF	CBP	Negative	0	2
2	38	B,L,Br	FEC+G-CSF	CBP	Negative	0	3
3	47	B,L,H	FEC+G-CSF	CBP	Positive	6	7
4	46	L,Pl,Br	FAC+G-CSF	CBP	Negative	0	1
5	34	H	FEC+G-CSF	CBP	Negative	11	13
6	38	LN	FEC+G-CSF	CBP	Negative	27+	27+
7	45	B,L	FEC+G-CSF	CBP	Positive	27+	27+
8	43	B	G-CSF	CBP	Positive	27+	27+
9	41	L,H	G-CSF	CBP	Positive	12	23+
10	46	Skin	G-CSF	MM	Negative	3	5
11	43	B,L	G-CSF	MM	Negative	0	2
12	50	B,L	G-CSF	MM	Positive	4	5
13	55	Pl	FEC+G-CSF	CBP	Negative	12	25+
14	44	B,Pl	G-CSF	CBP	Negative	3	4
15	52	B,L,H,Pl	FEC+G-CSF	CBP	Negative	23+	23+
16	32	B	FEC+G-CSF	CBP	Negative	6	15
17	40	B,L,H,Pl	Tax+C+G-CSF	CBP	Negative	17+	17+
18	44	B	Tax+C+G-SCF	CBP	Positive	12+	12+
19	33	B	G-CSF	ICE	Negative	8+	8+
20	46	B,H	Tax+G-CSF	CBP	Negative	12	21
21	58	B	Cy+G-CSF	CBP	Negative	1	1
22	57	M	Cy+G-CSF	ICE	Negative	50+	50+
23	41	L,Pl	Cy+G-CSF	ICE	Positive	4	15
24	40	B	Cy+G-CSF	ICE	Positive	0	7
25	42	B	FAC+G-CSF	MM	Positive	14	33+
26	52	L	Cy+G-CSF	ICE	Positive	5	17
27	36	BB,S	FAC+G-CSF	ICE	Positive	6	7
28	53	B	Cy+G-CSF	ICE	Positive	14	15+
29	39	L	Cy+G-CSF	CBPT	Positive	9+	9+
30	30	B,Pl,M	Cy+G-CSF	CBPT	Positive	0	2
31	52	LN	Cy+G-CSF	CECb	Positive	9	11
32	56	LN	Cy+G-CSF	CECb	Positive	28+	28+
33	44	LN	Cy+G-CSF	CECb	Positive	24+	24+
34	50	BB,S	Cy+G-CSF	CECb	Positive	4	13+
35	57	LN	Cy+G-CSF	CECb	Negative	9+	9+
36	29	Ovary	Tax+P+G-CSF	CTCb	Positive	7	10
37	30	L	Cy+G-CSF	CTCb	Positive	6	27+
38	43	L,B,LN	Cy+G-CSF	CTCb	Negative	10	14

Site, metastasis site; B, bone; BB, contralateral breast; L, lung; Pl, pleura; H, liver; LN, lymph node; M, bone marrow; Br, brain; S, soft tissue; PFS, progression free survival; OS, overall survival; FEC, 5-fluorouracil+epirubicin+cyclophosphamdie; Tax+P, taxol+cisplatin; Tax+C, taxol+carboplatin; Cy, cyclophosphamide; HDC, high dose chemotherapy; CBP, cyclophosphamide+BCNU+cisplatin; CBPT, cyclophosphamide+BCNU+cisplatin+taxol; MM, melphalan; ICE, ifosfamide+carboplatin+etoposide; CECb, cyclophosphamide+etoposide+carboplatin; CTCb, cyclophosphamide+thiothepa+carboplatin

two-sided at a significance level of $p=0.05$, and performed using a SPSS 8.0® statistical software (Systat, U.S.A.).

RESULTS

Patients characteristics

The characteristics of the 38 metastatic breast cancer patients are listed in Table 1. The median age was 44 yr (range: 29-58). The most common sites of metastasis were bone, lung and liver, and 19 out of 38 patients had more than one metastasis site.

RT-PCR results and clinical data

No CEA mRNA was detected in all normal subjects. Twenty out of 38 patients (51.3%) were found to be positive for CEA mRNA. Representative data are shown

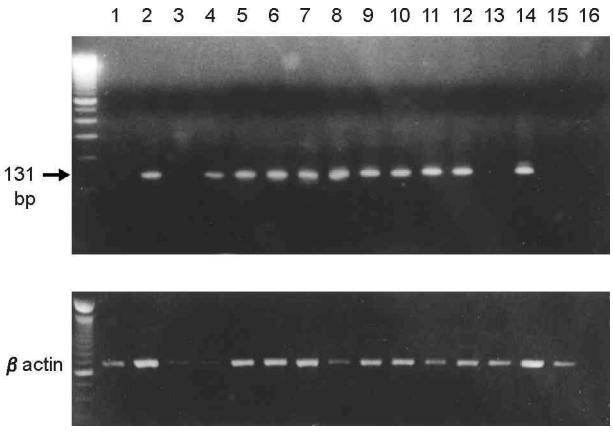

Fig. 1. Representative RT-PCR data for CEA mRNA in stem cells from metastatic breast cancer. Lane 1, 3, 13: CEA negative from metastatic breast cancer patients; Lane 2, 4-12; CEA positive from metastatic breast cancer patients; Lane 14: colo201 cells as a positive control; Lane 15-16: normal healthy blood and dH20 without RNA as a negative control, respectively. β-actin serves for intact RNA and RT as an internal control.

in Fig. 1. There was no significant difference in age, estrogen receptor status, menopausal status, mobilization methods, site of metastasis, disease free interval, or number of metastasis sites between CEA mRNA positive and negative groups (Table 2). CEA mRNA was found in the apheresis products in 54.8% (17/31) of patients mobilized with chemotherapy plus G-CSF and 42.8% (3/7) of patients with G-CSF alone (Table 2). With median 14 months of follow-up (range: 1-50+ months), the median relapse or progression free survival and overall survival were 10 and 14 months, respectively, in patients who did not receive tumor cells, compared with 9 and 23 months, respectively, in those who received tumor cells. Ten out of twenty patients who received contaminated stem cells are still alive (range: 9-33 months), whereas eight out of 18 who were infused with non-contaminated stem cells are alive (range: 8-50 months). There was no significant difference in progression free or overall survival (p=0.5497, p=0.3086) between CEA mRNA positive and negative group (Fig. 2). However, shorter progression free interval (p=0.0013) and poor overall survival (p=0.0033) were correlated with the number of metastasis sites (1 vs ≥2) (Fig. 3). The median progression free interval and overall survival were 4 and 7 months, respectively, in patients with more than one metastasis sites, whereas in patients with one metastasis site the median progression free interval was 14 months and the median overall survival was not reached yet. When multivariate analysis was performed using

Table 2. Patient characteristics in metastatic breast cancer according to CEA mRNA

	CEA mRNA				*p* value
	Negative (n=18)		Positive (n=20)		
	Number	(%)	Number	(%)	
Age (yr)					
≤50	11/26	(42.3)	15/26	(67.7)	0.489
>50	7/12	(58.3)	5/12	(41.7)	
Estrogen receptor					
Negative	3/9	(33.3)	6/9	(66.7)	0.580
Positive	3/5	(60.0)	2/5	(40.0)	
Mobilization					
G-CSF	4/7	(57.1)	3/7	(42.9)	0.687
G-CSF+Chemotherapy	14/31	(45.1)	17/31	(54.9)	
Menopause					
Premenopause	10/25	(40.0)	15/25	(60.0)	0.307
Postmenopause	8/13	(61.5)	5/13	(38.5)	
Disease free interval					
≤2 yr	8/20	(40.0)	12/20	(60.0)	0.516
>2 yr	10/18	(55.6)	8/18	(44.4)	
Site of metastasis					
Bone	11/20	(55.0)	9/20	(45.0)	0.466
Lung	7/15	(46.7)	8/15	(53.3)	
Liver	4/6	(66.7)	2/6	(33.3)	
Soft tissue	1/3	(33.3)	2/3	(66.7)	
Others	12/21	(57.1)	9/21	(42.9)	
No. of metastasis site					
1	8/19	(42.1)	11/19	(57.9)	0.746
≥2	10/19	(52.6)	9/19	(47.4)	

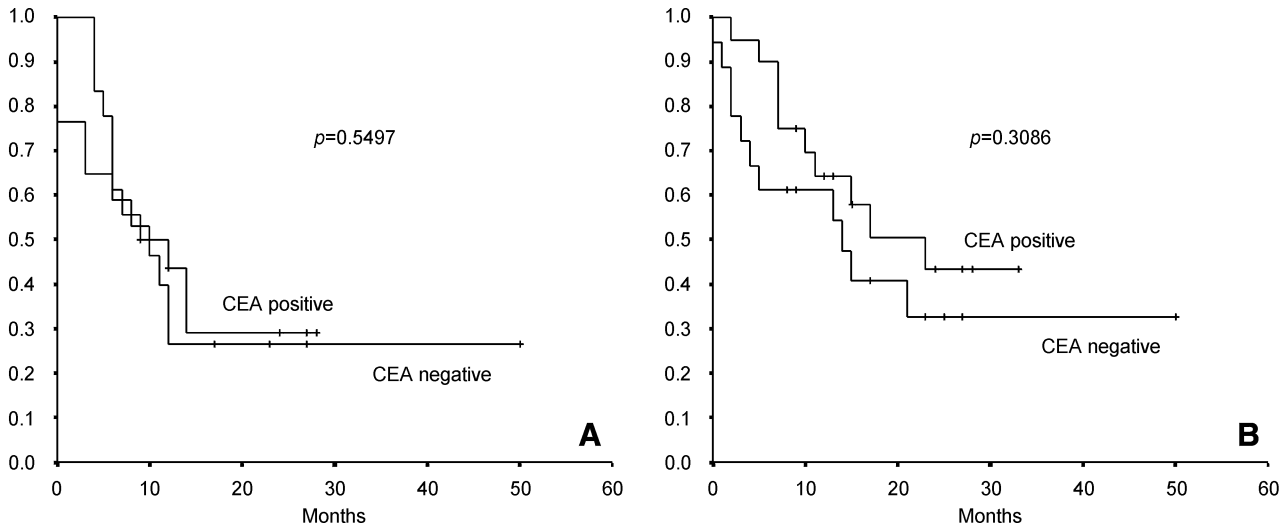

Fig. 2. **A:** Progression free survival for patients with CEA mRNA positive (n=20) or negative (n=18) in metastatic breast cancer. **B:** Overall survival for patients with CEA mRNA positive (n=20) or negative (n=18) in metastatic breast cancer.

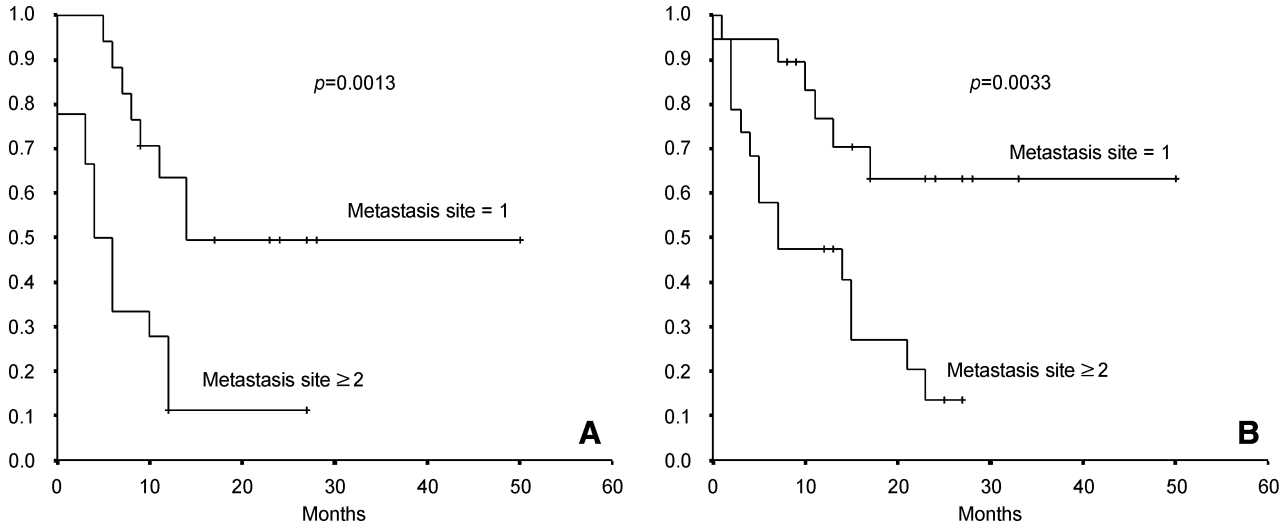

Fig. 3. **A:** Progression free survival for patients with number of metastasis=1 (n=19) or ≥2 (n=19) in metastatic breast cancer. **B:** Overall survival for patients with number of metastasis=1 (n=19) or ≥2 (n=19) in metastatic breast cancer.

Table 3. Multivariate analysis of prognostic factors predicting survival in 38 metastatic breast cancer patients

Factors	*b*	SE (*b*)	*p*-value	Odds ratio (95% CI*)
Age group	0.736	0.560	0.895	1.076 (0.358-3.231)
Metastasis number	1.317	0.5104	0.010	3.733 (1.361-10.233)
Mobilization	0.519	0.551	0.345	0.594 (0.202-1.702)
Disease free interval	0.401	0.476	0.399	0.669 (0.262-1.702)
CEA mRNA	0.429	0.476	0.367	0.651 (0.255-1.656)

* 95% confidence interval

Cox's proportional hazards regression model is as follows: $h_i(t)=h_o(t)\ \exp\ (\beta_1$ age$+\beta_2$ metastasis site$+\beta_3$ mobilization$+\beta_4$ disease free interval$+\beta_5$ CEA). Age: 0=≤50, 1=>50; Metastasis site: 0=1, 1=≥2; Mobilization: 0=G-CSF, 1=Chemo+G=CSF; Disease free interval: 0=≤2 yr, 1=>2 yr; CEA: 0=negative, 1=positive

Cox's proportional hazards regression model to identify the prognostic factors for survival, only the number of metastasis site was associated (odds ratio 3.733) (Table 3).

DISCUSSION

We demonstrated that more than 50% of metastatic breast cancer patients undergoing APBSCT are contaminated with malignant cells in apheresis products. Sensitive immunocytochemical or molecular diagnositc techniques have detected occult tumor cells in 10% to 80% of peripheral stem cell apheresis products from patients with metastatic breast cancer undergoing high-dose chemotherapy (6, 9, 20). The cytokeratins (CK) expression, such as CK8, CK18, CK19, and CK20, are commonly used markers for detection of most epithelial cell tumors, based on specific traits of the tissue in which the tumor originates (21-24). Also, the selective expression of certain antigens such as CEA or estrogen receptors are used (15, 18). However, cytokeratin markers (CK8, CK18, CK19) or estrogen receptors may not be considered adequate for detecting tumor cells since they are expressed to a greater or less extent by peripheral blood mononuclear cells (25, 26). Moreover, RT-PCR is widely used to the presence of tumor cells with high sensitivity detecting a tumor cell among 10^6 mononuclear cells (13, 20-23, 27, 28). We found that CEA mRNA was not detected in the peripheral blood mononuclear cells from all normal healthy controls, indicating that CEA mRNA may be a reliable marker for the detection of tumor cell contamination.

Goeminne et al. (29) reported that CEA transcription can be induced by G-CSF since CEA RT-PCR expression was observed in 95% of G-CSF mobilized stem cell samples derived from patients with breast cancer and also in five out of seven peripheral blood mononuclear cells (PBMC) from healthy individuals incubated in vitro with G-CSF, suggesting a loss of specificity of CEA RT-PCR for tumor cell detection in peripheral blood mononuclear cells. However, we observed that only 50% of metastatic breast cancer patients showed CEA mRNA positive even though all the patients were administered G-CSF for mobilization of stem cells during leukapheresis. Considering the sensitivity of RT-PCR analysis (14-16) and other previous reports (13-16), this discrepant result should be elucidated through further studies.

Recently, it has been reported that the apheresis products mobilized with chemotherapy and G-CSF were less contaminated than those mobilized with G-CSF alone (30). However, results of our trial are similar to those previously reported by others in a retrospective study (31, 32), suggesting that tumor cell contamination in apheresis products was not affected by the mobilization method. We also noted that there was no significant difference in tumor cell contamination among the metastasis sites.

Although several reports suggested that tumor cells present in stem cell transplants or bone marrow may contribute to early relapse or shorter disease free survival in high-risk breast cancer patients treated with high dose chemotherapy (33, 34), it is still controversial whether the reinfusion of tumor cells in apheresis products may play a role in early progression or poor clinical outcome of metastatic breast cancer patients (35-37). Brockstein et al. (35) reported that six of 26 advanced breast cancer patients had contaminating tumor cells detectable by immunocytochemistry in bone marrow harvests and these 6 patients had a trend toward decreased overall survival compared with those patients without tumor cells. However, recent study with relatively large number of patients by Cooper et al. (37) reported that microscopic tumor was frequently detected by immunocytochemistry in 23 out of 57 (40%) hematopoietic stem cell products of metastatic breast cancer, but did not predict for inferior treatment outcome in tumor cell positive group. Although in our small series of patients 20 of 38 (51.3%) were found to be positive for CEA mRNA, we also could not find any significant difference in progression free or overall survival between CEA mRNA positive group and negative group. Rather, only the number of metastasis sites (1 vs $\geq$2) was correlated with shorter progression free and poor overall survival by univariate analysis, indicating that tumor burden may be one of the most important factors to predict the prognosis in the management of metastatic breast cancer. Multivariate analysis also showed that only the number of metastasis site was associated with survival (odds ratio: 3.733).

In summary, our studies indicate that a significant number of apheresis products from metastatic breast cancer patients undergoing APBSCT are contaminated with tumor cells by using molecular diagnostic techniques. Although it seems from our study with small cohort of patients that the contaminating malignant cells in stem cell products may not predict early relapse or poor clinical outcome, further studies with large number of patients and the comparison with other prognostic factors and characteristics of the tumor may determine the significance of tumor cell contamination in apheresis products.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Korea

Research Foundation Grant (KRF-F-00177). We thank JA Kim for her technical assistance. We thank Dr Moran Ki and Dr Jinkyung Park for her help with the statistical analysis.

REFERENCES

1. Antman KH, Rowlings PA, Vaughen WP, Pelz CJ, Fay JW, Fields KK, Freytes CO, Gale RP, Hillner BE, Holland HK, Kennedy MJ, Klein JP, Lazarus HM, McCarthy PL Jr, Saez R, Spitzer G, Stadtmauer EA, Williams SF, Wolff S, Sobocinski KA, Armitage JO, Horowitz MM. *High dose chemotherapy with autologous hematopoietic stem cell support for breast cancer in North America. J Clin Oncol 1997; 15: 1870-97.*
2. Gradishar WJ, Tallman MS, Abrams JS. *High dose chemotherapy for breast cancer. Ann Intern Med 1996; 125: 599-604.*
3. Peters WP, Shpall EJ, Jones RB, Oslen GA, Bast RC, Gockerman JP, Moore JO. *High-dose combination alkylating agents with bone marrow support as initial treatment for metastatic breast cancer. J Clin Oncol 1988; 6: 1368-76.*
4. Berger U, Bettelheim R, Mansi JL, Easton D, Coombes RC, Neville AM. *The relationship between micrometastases in the bone marrow, histopathologic features of the primary tumor in breast cancer and prognosis. Am J Clin Pathol 1988; 90: 1-6.*
5. Diel IJ, Kaufmann M, Goerner R, Costa SD, Kaul S, Bastert G. *Detection of tumor cells in bone marrow of patients with primary breast cancer: a prognostic factor for distant metastases. J Clin Oncol 1992; 10: 1534-9.*
6. Ross AA, Cooper BW, Lazarus HM, Mackay W, Moss TJ, Ciobanu N, Tallman MS, Kennedy MJ, Davidson NE, Sweet D. *Detection and viability of tumor cells in peripheral blood stem cell collections from breast cancer patients using immunohistochemical and clonogenic assay techniques. Blood 1993; 82: 2605-10.*
7. Shpall EJ, Jones RB, Bearman SL, Franklin WA, Archer PG, Curiel T, Bitter M, Claman HN, Stemmer SM, Purdy M. *Transplantation of enriched CD34 positive autologous marrow into breast cancer patients following high dose chemotherapy: Influence of CD34 positive peripheral blood progenitors and growth factors on engraftment. J Clin Oncol 1994; 12: 28-36.*
8. Passos-Coelho J, Ross AA, Davis JM, Huelskamp AM, Clarke B, Noga SJ, Davidson NE, Kennedy MJ. *Bone marrow micrometastases in chemotherapy-responsive advanced breast cancer: effect of ex vivo purging with 4-hydroxycyclophosphamide. Cancer Res 1994; 54: 2366-71.*
9. Sharp JG, Joshi SS, Armitage JO, Bierman P, Coccia PF, Harrington DS, Kessinger A, Crouse DA, Mann SL, Weisenburger DD. *Significance of detection of occult non-Hodgkin's lymphoma in histologically uninvolved bone marrow by culture technique. Blood 1992; 79: 1074-80.*
10. Gribben JG, Freedman AS, Neuberg D, Roy DC, Blake KW, Woo SD, Grossbard ML, Rabinowe SN, Coral F, Freeman GJ. *Immunologic purging of marrow assessed by PCR before autologous bone marrow transplantation for B-cell lymphoma. N Engl J Med 1991; 325: 1525-33.*
11. Brenner MK, Rill DR, Moen RC, Krance RA, Mirro J Jr, Anderson WF, Ihle JN. *Gene marking to trace the origin of relapse after autologous bone marrow transplantation. Lancet 1993; 341: 85-6.*
12. Rill DR, Santana VM, Roberts WM, Nilson T, Bowman LC, Krance RA, Heslop HE, Moen RC, Ihle JN, Brenner MK. *Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells. Blood 1994; 84: 380-3.*
13. Mori M, Mimori K, Inoue H, Shibuta K, Baba K, Nakashima H, Hartaguchi M, Tsuji K, Ueo H, Barnard GF, Akiyoshi T. *Detection of cancer micrometastases in lymph nodes by reverse transcriptase-polymerase chain reaction. Cancer Res 1995; 55: 3417-20.*
14. Mori M, Mimori K, Ueo H, Tsuji K, Shiraishi T, Barnard G, Sugimachi K, Akiyoshi T. *Clinical significance of molecular detection of carcinoma cells in lymph nodes and peripheral blood by reverse transcriptase-polymerase chain reaction in patients with gastrointestinal or breast carcinomas. J Clin Oncol 1998; 16: 128-32.*
15. Gerhard M, Juhl H, Kalthoff H, Schreiber HW, Wagener C, Neumaier M. *Specific detection of carcinoembryonic antigen expressing tumor cells in bone marrow aspirates by polymerase chain reaction. J Clin Oncol 1994; 12: 725-9.*
16. Min CJ, Tafra L, Vervanac KM. *Identification of superior markers for polymerase chain reaction detection of breast cancer metastases in sentinel lymph nodes. Cancer Res 1998; 58: 4581-4.*
17. Chomzynsky P, Sacchi N. *Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156-9.*
18. Mori M, Mimori K, Ueo H, Karimine N, Barnard GF, Sugimachi K, Akiyoshi T. *Molecular detection of circulating solid carcinoma cells in the peripheral blood: the concept of early systemic disease. Int J Cancer 1996; 68: 739-43.*
19. Ahn MJ, Noh YH, Kim WC, Kim HS, Lee YY, Jung TJ, Choi IY, Kim IS, Lee YS. *Detection of tumor cell contamination in peripheral blood stem cells by RT-PCR in high risk or metastatic breast cancer patients and its clinical implications. J Korean Stem Cell Transplant 1999; 4: 1-11.*
20. Datta YH, Adams PT, Drobyski WR, Ethier SP, Terry VH, Roth MS. *Sensitive detection of occult breast cancer by reverse-transcriptase polymerase chain reaction. J Clin Oncol 1994; 12: 475-82.*
21. Traweek ST, Liu J, Battifora H. *Keratin gene expression in non-epithelial tissues. Detection with polymerase chain reaction. Am J Pathol 1993; 142: 1111-8.*
22. Burchill SA, Bradbury MF, Pittman KM, Southgate J, Smith B, Selby P. *Detection of epithelial cancer cells in peripheral blood by reverse transcriptase-polymerase chain reaction. Br*

J Cancer 1995; 71: 278-81.

23. Krismann M, Todt B, Schroder J, Gareis D, Muller KM, Seeber S, Schutte J. *Low specificity of cytokeratin 19 reverse transcriptase-polymerase chain reaction analyses for detection of hematogenous lung cancer dissemination. J Clin Oncol 1995; 13: 2769-75.*
24. Moll R, Lowe A, Laufer J, Franke WW. *Cytokeratin 20 in human carcinomas. A new histodiagnostic marker detected by monoclonal antibodies. Am J Pathol 1992; 140: 427-47.*
25. Johnson PWM, Burchill SA, Selby PJ. *The molecular detection of circulating tumor cells. Br J Cancer 1995; 72: 268-76.*
26. Lopez-Guerrero JA, Bolufer-Gilabert P, Sanz-Alonso M, Barragan-Gonzalez E, Palau-Perez J, De La Rubia-Comos J, Sempere-Talens A, Bonanad-Biox S. *Minimal illegitimate levels of cytokeratin K19 expression in mononucleated blood cells detected by a reverse transcription PCR method (RT-PCR). Clin Chim Acta 1997; 263: 105-16.*
27. Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmuller G. *Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 1992; 340: 685-9.*
28. Brossart P, Schmier JW, Kruger S, Willhauck M, Scheibenbogen C, Mohler T, Keilholz U. *A polymerase chain reaction based semiquantitative assessment of malignant melanoma cells in peripheral blood. Cancer Res 1995; 55: 4065-8.*
29. Goeminne JC, Guillaume T, Salmon M, Machiels JP, D'Hondt V, Symann M. *Unreliability of carcinoembryonic antigen (CEA) reverse transcriptase-polymerase chain reaction (RT-PCR) in detecting contaminating breast cancer cells in peripheral blood stem cells due to induction of CEA by growth factors. Bone Marrow Transplant 1999; 24: 769-75.*
30. Gluck S, Ross AA, Layton TJ, Ostrander AB, Goldstein LC, Porter K, Ho AD. *Decrease in tumor cell contamination and progenitor cell yield in leukapheresis products after consecutive cycles of chemotherapy for breast cancer treatment. Biol Blood Marrow Transplant 1997; 3: 316-23.*
31. Bertolini F, Lanza A, Peccatori F, Zibera C, Gibelli N, Perotti C, Da Prada GA, Torretta L, Cocorocchio E, Martinelli G, Robustelli della Cuna G. *Hematopoietic progenitor cell collection and neoplastic cell contamination in breast cancer patients receiving chemotherapy plus granulocyte-colony stimulation factor (G-CSF) or -G-CSF alone for mobilization. Ann Oncol 1998; 9: 913-6.*
32. Passos-Coelho JL, Ross AA, Kahn DJ, Moss TJ, Davis JM, Huelskamp AM, Noga SJ, Davidson NE, Kennedy MJ. *Similar breast cancer cell contamination of single-day peripheral blood progenitor cell collections obtained after priming with hematopoietic growth factor alone or after cyclophosphamide followed by growth factor. J Clin Oncol 1996; 14: 2569-75.*
33. Schulze R, Schulze M, Wischnik A, Ehnle S, Doukas K, Behr W, Ehret W, Schlimok G. *Tumor cell contamination of peripheral blood stem cell transplants and bone marrow in high-risk breast cancer patients. Bone Marrow Transplant 1997; 19: 1223-8.*
34. Vredenburgh JJ, Silva O, Broadwater G, Berry D, DeSombre K, Tyer C, Petros WP, Peters WP, Bast RC Jr. *The significance of tumor contamination in the bone marrow from high risk primary breast cancer patients with high dose chemotherapy and hematopoietic support. Biol Blood Marrow Transplant 1997; 3: 91-7.*
35. Brockstein BE, Ross AA, Moss TJ, Kahn DG, Hollingsworth K, Williams SF. *Tumor cell contamination of bone marrow harvest products: clinical consequences in a cohort of advanced stage breast cancer patients undergoing high dose chemotherapy. J Hematother 1996; 5: 617-24.*
36. Pedrazzoli P, Battaglia M, Da Prada GA, Lanza A, Cuomo A, Bertolini F, Pavesi L, Robustelli della Cuna G. *Role of tumor cells contaminating the graft in breast cancer recurrence after high dose chemotherapy. Bone Marrow Transplant 1997; 20: 167-9.*
37. Cooper BW, Moss TJ, Ross AA, Ybanez J, Lazarus M. *Occult tumor contamination of hematopoietic stem cell products does not affect clinical outcome of autologous transplantation in patients with metastatic breast cancer. J Clin Oncol 1998; 16: 3509-17.*