Cukurova Medical Journal

Araştırma Makalesi / Research Article

Bone Marrow Micrometastasis Detected by Flow Cytometry is Associated Bone, Bone Marrow, Lung Macrometastasis in Breast Cancer

Göğüs Kanserinde Flow Sitometri ile Tespit Edilenen Kemik İliği Mikrometastazının Kemik, Kemik İliği, ve Akciğer Makrometastazları ile İlişkilidir

Salih Akın[1], Berna Bozkurt Duman[2], Berksoy Sahin[2]

[1]Çukurova University, Faculty of Medicine, Department of Internal Medicine. ADANA
[2]Çukurova University, Faculty of Medicine, Department of Medical Oncology, ADANA

Cukurova Medical Journal 2014;39(2): 305-314.

ABSTRACT

Purpose: The aim of the present study was to analyze deposits in bone marrow for determining micrometastases in breast cancer patients by flow cytometry. And its correlation of prognostic factors and macrometastasis at the first diagnosis.

Material and Methods: Bone marrow samples were obtained from 52 breast cancer patients and 16 control patients via aspiration from the iliac spine at the time of first diagnosis after the surgery. Epithelial cells were identified with anti-cytokeratin monoclonal antibody, and double-staining with propidium iodide and CD45using flow cytometry.

Results: In all, 2 (12.5%) of the 16 control patients and 11 (21%) of the 52 breast cancer patients had cytokeratin-18 positive cells in their bone marrow. A relationship between the presence of occult metastatic cells in bone marrow, and the presence/absence of lymph node metastases, tumor size, stage, menopausal status, hormone receptor status, histological grade, c-erb-B2 expression, tumor subtype, lymphovascular invasion, Ductal carcinoma in situ (DCIS) component, and gender was not observed. Significant positive relationships were observed between bone marrow micrometastasis, and age, and bone, bone marrow, lung, and liver metastases.

Conclusion: Bone marrow micrometastasis was associated with age, bone, bone marrow, lung, and liver metastases at the time of diagnosis..

Key Words: Bone marrow; breast cancer; cytokeratin; flow cytometry; metastasis.

ÖZET

Amaç: Bu çalışmanın amacı, flow sitometri ile göğüs kanseri hastalarında mikrometastazların belirlenmesi için kemik iliği analizi yapılması ve bulguların ilk tanıdaki prognostik faktorler ve makrometastazlar ile korelasyonur.

Materyal ve Metod: Kemik iliği örnekleri 52 göğüs kanserli hasta grubu ve 16 kontrol grubunun iliyak omurundan aspirasyonla alındı. Epitel hücreleri flow sitometrisi kullanılarak propidyum iyodür ve CD45 ile birlikte anti-sitokeratin monoklonal antikor ve ikili boyama ile tanımlandı.

Bulgular: Toplamda, 52 göğüs kanseri hastasının 11 (% 21)'inde ve 16 kontrol grubunun 2 (%12.5)'sinde kemik iliğinde sitokeratin 18-pozitif hücreleri vardı. Kemik iliğinde gizli metastatik hücrelerin varlığı, ve lenf nodu metastazının varlığı/yokluğu, tümörün boyutu, evresi, menopozal durumu, hormon reseptör durumu, histolojik derecesi, c-erb-B2 ifadesi, tümör altgrubu, lenfovasküler nöbet, Duktal karsinomanın tamamlayıcı bileşeni (DCIS), ve cinsiyet arasında bir ilişki gözlenmedi. Yaş, kemik, kemik iliği, akciğer ve karaciğer ile kemik iliği mikrometastazı arasında anlamlı pozitif bir ilişki olduğu gözlemlendi.

Sonuç: Tanı sırasında yaş, kemik, kemik iliği, akciğer ve karaciğer metastazıyla kemik iliği mikrometastazları ilişkiledirildi.

Anahtar Kelimeler: Kemik iliği; göğüs kanseri; sitokeratin; flow sitometri; metastaz.

INTRODUCTION

Disseminated tumor cells are regarded as a surrogate for early metastatic spread of disease. These cells can be detected in bone marrow aspirates, lymph nodes and peripheral blood, where we refer to them as circulating tumor cells. The risk of distant metastases is correlated with prognostic factors such as the size of the primary tumor, axillary lymph node involvement, and primary tumor markers in patients with operable breast cancer. Some factors, such as tumor size, HER2 positivity, hormone receptor status, lymphovascular invasion, and high histological grade, have been used to determine the need for systemic treatment and the risk of recurrence in patients with negative axillary lymph nodes[1,2]. New prognostic factors and novel techniques are needed to more accurately determine the prognosis. The present study aimed to determine the prevalence of bone marrow micrometastasis, in 52 stage I-IV breast cancer patients at the time of diagnosis. We investigated the relationship between the presence of cytokeratin-positive cells in bone marrow, and conventional clinicopathological prognostic factors such as age, menopausal status, tumor size, and lymph node involvement) and immunohistochemical markers.

MATERIAL and METHODS

This prospective study began in 2007 to 2009. The study included 40 stage I-III breast cancer patients, and 12 stage IV breast cancer patients. A total of 52 patients - 20 with negative axillary lymph node metastases, 20 with positive axillary lymph node metastases, and 12 with bone, bone marrow, liver, lung, or cerebral metastases-were included in the study. Bone marrow aspiration was performed to the patients after diagnosis and before the treatment. All patients had chest X-ray, abdominal ultrasound, and whole-body bone scans in order to investigate distant metastasis. The patients were informed about the procedure and each provided informed consent. The patients were staged according to American Joint Committee on Cancer (AJCC) 2002 criteria, based on tumor size (T), lymph node status (N), and distant metastases (M). Patient age, gender, tumor size, number of metastatic axillary lymph nodes, stage, tumor subtype, histologic grade, presence of lymphovascular invasion and carcinoma in situ, estrogen (ER) and progesterone receptor (PR) status, c-erb-B2 expression, menopausal status, and the locations of metastases were recorded.

Preparation of bone marrow

Bone marrow aspiration was performed under local anesthesia and sterile conditions and aspirate was obtained from the iliac spine. An incision was made using a scalpel before aspiration in order to prevent contamination from the skin, and the initial 0.5 cc of aspirated bone marrow was discarded. On average, 9 cc of bone marrow was aspirated from each patient and was placed into 2 different tubes that contained ethylene diamine tetraacetic acid (EDTA).The material was frozen with DMSO (Dimethyl Sulfoxide) and stored at -80°C. Before use the material was melted in hand and 10% PBS containing fetal bovine serum was added, and then centrifuged at 400 rpm for 10 min. The supernatant was removed and washed with 10% PBS containing fetal bovine serum at 400 G. This procedure was performed 3 times and DMSO was removed.

Flow cytometric analysis

After removal of DMSO, 70% alcohol was added dropwise to a portion of bone marrow material, and then was stained with FITC-conjugated cytokeratin 18 monoclonal antibody at 4°C (Abcam, Cambridge, USA). The antibody was diluted to 1/20 with PBS and 10 µL of antibody was added for each 100 µL of sample. After again

washing with PBS, the DNA content was stained with propidium iodide (PI) containing RNAse[8]. Another portion of the bone marrow that was not fixed was stained superficially with CD45 and intracellularly with CK18, and then analyzed using flow cytometry[9]. Intracellular staining was performed for cytokeratin 4, 6, 8, and 10, and superficial staining was performed for CD24, CD44, CD45, and EpCAM. At least 10^5 cells were analyzed in each patient. The samples were analyzed using BD FACSCalibur™ flow cytometry (Becton Dickinson, NJ, USA). PI negative-cytokeratin 18 positive and in the other group, CD45 negative-cytokeratin 18 positive cells were regarded as micrometastatic tumor cells. Non-specific staining and falsely stained debris were regarded as negative. Normal bone marrow samples obtained from 16 patients with leukemia and lymphoma in remission were included in the study as the control group. CD44, CD24, cytokeratin 4, 6, 8, and 10, and EpCAM monoclonal antibodies were analyzed using flow cytometry in the patients that had cytokeratin 18-positive bone marrow.

Statistical analysis

All data were analyzed using SPSS (Statistical Package for Social Sciences) for Windows v.12.0. Stepwise multivariate Cox regression was performed to analyze the relationship with other prognostic factors in patients with and without micrometastases. The chi-square test was employed to determine the relationship between micrometastasis and each tested variable. P values < 0.05 were regarded as statistically significant.

RESULTS

The study included 52 patients; The demographic, clinical, and tumoral characteristics of the patients are presented in Table 1.

There was bone marrow metastasis in 11 (22%) of 50 female patients but none in males. Median age of the patients without micrometastases in their bone marrow was 45 years (range: 27-82 years) Median tumor size was 4 cm patients with bone marrow micrometastases, whereas median tumor size was 2.5 cm (range: 0.5-10 cm) in the patients without bone marrow micrometastases. The median number of metastatic lymph nodes was 1 (range: 0-34) and versus a median number of metastatic lymph nodes of 2 (range: 0-16) in the patients without bone marrow micrometastases.

Among the 11 patients with bone marrow micrometastases, the primary tumor subtype was invasive ductal carcinoma in 10 (90%) and invasive lobular carcinoma in 1 (1%). Among the patients without bone marrow micrometastases, 33 (80%) had the invasive carcinoma tumor subtype, 3 (7.3%) had the invasive lobular carcinoma subtype, 1 (2.4%) had the mucinous carcinoma subtype, and 4 (10%) had other tumor subtypes. Among the patients with bone marrow micrometastases, 1 (9%) had stage I disease, 4 (36%) had stage II, 1 (9%) had stage III, and 5 (46%) patients had stage IV. Bone marrow micrometastases were observed in 1 of the 12 (8.3%) patients with stage I disease, 4 of the 20 (20%) with stage II, 1 of the 8 (12.5%) with stage III, and 5 of the 12 (41.6%) with stage IV. Median ER positivity was 30% (range: 0%-100%) in the patients with bone marrow micrometastases, while these value was 80% (range: 0%-100%) in those without bone marrow micrometastases.

Median PR positivity was 0% (range: 0%-95%) in the patients with bone marrow micrometastases, versus 30% (rang: 0%-100%), in the patients without bone marrow micrometastases. Bone marrow micrometastases were observed in 2 of the16 c-erb-B2-negative patients. In all, 3 of the 6 patients that were c-erb-B2 (+)-positive, 1 of the12 patients that were c-erb-B2 (++)-positive, and 5 of the 18 patients that were c-erb-B2 (+++)-positive had micrometastases in their bone marrow. In total, 2 of the 4 (50%) patients with histologic grade 1 tumors, 3 of the 22

patients with histologic grade 2 tumors (13.6%), and 6 of the 26 (23.1%) patients with histologic grade 3 tumors had micrometastases in their bone marrow. In total, 6 of the 32 (18.8%) patients with lymphovascular invasion and 5 of the 20 (25%) patients without lymphovascular invasion had bone marrow micrometastases. In all, 5 of the 25 (20%) patients that had a ductal carcinoma in situ (DCIS) component in their primary tumors and 6 of the 27 (22.2%) patients that did not had micrometastases in their bone marrow. Micrometastatic cells were identified using flow cytometry in 100% of the patients with bone marrow micrometastases that were identified immunohistochemically. Micrometastases were noted in 9 of the 50 (18%) patients without bone marrow metastases. Bone marrow micrometastases were observed in 3 of the 5 patients (60%) with liver metastases and in 8 of the 47 (17%) patients without liver metastases. Bone marrow metastases were observed in 1 of the 3 (33.3%) patients with cerebral metastases and in 10 of the 49 patients (20.4%) without cerebral metastases. Both patients with lung metastases (100%) and 9 of the 50 (18%) patients without lung metastases had bone marrow micrometastases. Bone marrow micrometastases were noted in 5 of the 10 (50%) patients with bone metastases and in 6 of the 42 (14.3%) without bone metastases.

According to multivariate analysis, the relationship between bone marrow micrometastases, and tumor size, gender, the number of metastatic lymph nodes, menopausal status, histologic grade, ER, PR, c-erb-b2 status, tumor subtype, stage, lymphovascular invasion, and a DCIS component was not statistically significant (p=0.455, p=0.092, p=0.843, p=0.144, p=0.247, p=0.1, p=0.155, p=0.147, p=0.377, p=0.227, p=0.591, and p=0.455, respectively)

The relationship between bone marrow micrometastasis, and age, bone marrow metastasis, bone metastasis, liver metastasis, and lung metastasis was statistically significant (p=0.036, p=0.005, p=0.013, p=0.025, and p=0.005, respectively), while its relationship with cerebral metastasis was not (p=0.595). The prevalence of bone marrow micrometastasis, with respect to clinical variables, is shown in Table 2.

DISCUSSION

Bone marrow micrometastasis (also named disseminated tumor cells (DTC) minimal residual disease(MRD), tumor cells isolated from bone marrow) can be defined as microscopic metastatic deposits of tumor cells that disseminated from the primary tumor to distant organs[11,13,14].

Bone marrow is considered to be the organ most frequently involved with micrometastatic cells that stem from the primary tumor. These cells may remain in G0 phase for years before they give rise to skeletal metastasis or pass into systemic circulation and cause distant metastasis[2].

Studies that have used immunocytochemical methods show that DTC in bone marrow is an independent indicator of total and disease-free survival, and is a prognostic factor indicating a poor prognosis[2,15-18]. On the other hand, other studies have not observed a relationship between DTC in bone marrow and prognosis[19,20].

As these cells are present in small numbers or individually in the lymph nodes, blood, and bone marrow, immunocytochemical, flow cytometric, and molecular methods have been developed to identify them[16,21-23]. Flow cytometry is superior to immunocytochemical and other molecular methods due to its ability to determine ploidy status, to differentiate apoptotic cells and dead cells, and to objectivity count many cells rapidly. Flow cytometry was used in the present study due to its ease of use in the daily practice.

The meta-analysis by Braun et al showed that micrometastasis ratio is correlated by tumor size (T) ($p<0.001$)[15]. The relationship between tumor size and bone marrow micrometastasis was not statistically significant according to multivariate analysis (p=0.092) in our study. The meta-analysis showed that.26.4% of patients with N0 nodes, 30%

of those with N1 nodes, 39.4% of those with N2 nodes, and 50% of those with N3 nodes had bone marrow micrometastases (p=0.001)[16]. In the present study, the relationship between the number of the metastatic lymph nodes and bone marrow micrometastasis was not statistically significant (p=0.843). Reported that the rate of bone marrow micrometastasis was 34.8% in their 20-35-year-old age group, 33.3% in their 36-50 year-old age group, 29.5% in their 51-65 year-old age group, and 27.8% in their patients >65 years of age (p=0.001)[16]. The present study shows that there was a statistically significant relationship between age and bone marrow micrometastasis, based on multivariate analysis (p=0.036).

The rate of bone marrow micrometastasis was 32.7% in premenopausal patients and 29.5% in postmenopausal patients (p=0,02) and bone marrow micrometastasis incidence rate of 34.5% in receptor-negative patients, versus 29.5% in receptor-positive patients (estrogen and/or progesterone) (p=0.003) in the metaanalyses reported by Braun et al[3]. When all the patients in the present study were analyzed, 4 of 28 (14.3%) premenopausal and 7 of 22 (31.9%) postmenopausal patients had bone marrow micrometastases. The relationship between menopausal status and bone marrow micrometastasis was not statistically significant (p=0.144).

The relationship between bone marrow micrometastasis and ER status was not statistically significant (p=0.1). Additionally, there wasn't a statistically significant relationship between progesterone receptor status and bone marrow micrometastasis (p=0.155).

C-erb-B2 over expression by micrometastatic cells in bone marrow is a predictor of poor outcome [18,24, 25]. In the present study 2 of 14 (14.2%) c-erb-B2-negative patients, 3 of 6 (50%) c-erb-B2 (+)-positive patients, 1 of 11 (9%) c-erb-B2 (++)-positive patients, and 5 of 18 (27.7%) c-erb-B2 (+++)-positive patients had bone marrow micrometastases when all the patients were analyzed; however, the relationship between c-erb-B2 and bone marrow micrometastasis was not statistically significant (p=0.147).

The rate of micrometastasis was 30.7% in the patients with invasive ductal carcinoma (IDC) subtype, 31.4% in patients with invasive lobular carcinoma (ILC) subtype, 24.8% in patients with mixed subtype, and 47.8% in patients with inflammatory subtype in the meta-analysis published by Braun et al. (p=0,08) [15]. When all of our patients were analyzed, 10 of 43 (23.2%) patients with IDC subtype and 1 of 4 (25%) with ILC had bone marrow micrometastases, whereas 3 patients with mixed subtype and 1 patient with mucinous carcinoma did not have bone marrow micrometastases. Based on the present study's multivariate analysis, the relationship between tumor subtype and bone marrow micrometastasis was not statistically significant (p=0.377).

The meta-analysis published by Braun et al. evaluated stage I-III patients and reported that the rate of micrometastasis increased as tumor stage increased[15]. Bidard et al. immunocytochemically investigated bone marrow micrometastasis in 138 patients with distant metastases and reported that bone marrow micrometastasis was observed in 59% of their patients[28]. In the present study the relationship between tumor stage and bone marrow micrometastasis was not significant, based on multivariate analysis (p=0.227)..

Braun et al.'s meta-analysis reported that the rate of bone marrow micrometastasis was 22.5%, 29.9%, and 34.5% in patients with histologic grade 1, grade 2 and grade 3 tumors, respectively (p<0.001). The relationship between histologic grade and bone marrow micrometastasis was not statistically significant in the present study (p=0.247).

Positive correlations were reported between bone marrow micrometastasis, and tumor size, axillary lymph node involvement, the presence of lymphovascular invasion, c-erb-B2 expression, and

ER/PR[26]. When we evaluated all of our patients, we observed bone marrow micrometastasis in 5 of 20 (25%) patients without lymphovascular invasion and 6 of 32 (18.75%) with lymphovascular invasion. The relationship between lymphovascular invasion and bone marrow micrometastasis was not statistically significant (p=0.591).

The presence of DCIS is strongly related to c-erb-B2 and p53 positivity in primary tumors[27,28]. Among the 12 metastatic (stage IV) patients, bone marrow micrometastases were observed in 3 of 5 (60%) patients with a DCIS component and in 2 of 7 (28.5%) patients without a DCIS component. When all patients were analyzed, 5 of 25 (20%) patients with a DCIS component and 6 of 27 (22.2%) patients without a DCIS component had bone marrow micrometastases. The relationship between DCIS and bone marrow micrometastasis was not statistically significant (p=0.591).;

Different studies were reported about the correlation between metastatic disease and micrometastasis[25,29]. Bidard et al. reported that among 138 patients with distant metastasis, 41 of 62 (66%) with liver metastases and 53 of 76 (69%) without liver metastases had bone marrow micrometastases (the difference between the groups was not statistically significant)[25]. A statistically significant relationship was observed between liver metastasis and bone marrow micrometastasis (p=0.025),but the relationship between cerebral metastasis and bone marrow micrometastasis was not statistically significant (p=0.595),No studies have investigated the relationship between lung metastasis and bone marrow micrometastasis. In the present study both patients with lung metastases (100%) and 9 of 50 (18%) patients without lung metastases had bone marrow micrometastases. We observed a statistically significant relationship between lung metastasis and bone marrow micrometastasis (p=0.005).

We noted bone marrow micrometastases in 21.1% of the patients in the present study. Braun et al. reported that the rate of bone marrow micrometastasis was 30.6% in their meta-analysis. This study observed that the patients with micrometastases had larger primary tumors, higher histologic grades, and more lymph node metastases, and that their tumors were mostly negative for hormone receptors. Total and breast cancer-specific survival among the patients with bone marrow micrometastasis were significantly shorter (univariate mortality rate: 2.15 and 2.44, respectively; p<0.001 for both)[15]. Bidard et al. reported that the rate of bone marrow micrometastasis was 59% in patients with distant metastases; however, they did not note a relationship with the prognosis[25].

Determination of early metastatic disease before it becomes manifest, elucidating the mechanisms that play a role in the dormancy phenomenon, and defining molecular mechanisms and interactions should provide more accurate and clinically beneficial data for use in the field of medical oncology. Development of new drugs that eradicate residual disease or control its growth, and use of antibody-based treatments, independent of the cell cycle, in combination with standard chemotherapy are promising therapeutic options.

Conflict of Interest

All authors have no financial affiliation or supports or conflict of interest to disclose. No conflict of interest.

Table 1. General characteristics of the patients.

	Parameters	**Number (%)**
Age groups	<50 >50	32 (61.5) 20 (38.5)
Menopausal status	Pre Post	23 (44) 27 (56)
Gender	Male Female	2 (4) 50 (96)
Tumor size	T_1 T_2 T_3 T_4	21 (40.5) 24 (46) 6 (11.5) 1 (2)
Lymph node metastasis	Absent Present	22 (42.5) 30 (57.5)
The number of metastatic lymph nodes	N_0 N_1 N_2 N_3	22 (42.5) 9 (17) 11 (21) 10 (19.5)
Stage	I II III IV	12 (23) 20 (38.5) 8 (15.5) 12 (23)
Tumor histopathology	İDK İLK Mucinous Other	43 (82.5) 4 (7.5) 1 (2) 4 (8)
Estrogen receptor (ER)	Negative Positive	12 (23) 40 (77)
Progesterone receptor (PR)	Negative Positive	18 (34.5) 34 (65.5)
c-erb-B2	0 1+ 2+ 3+	16 (31) 6 (11.5) 12 (23) 18 (34.5)
Grade	1 2 3	4 (8) 22 (42.5) 26 (49.5)
Lymphovascular invasion	Negative Positive	20 (38.5) 32 (61.5)
DCIS component	Absent Present	27 (52) 25 (48)
Rate of micrometastasis	LN (–) group LN (+) group Metastatic group	25% 5% 41.7%

Table 2. The prevalence of bone marrow micrometastasis according to clinical variables.

Clinical variable	Total number of patients	Patients with bone marrow micrometastasis	Patients without bone marrow micrometastasis	P
Age (years)				0.036*
20-35	7	1	6	
36-50	26	2	24	
51-65	15	6	9	
>65	4	3	1	
Tumor size				0.092
T1	21	2	19	
T2	24	8	18	
T3	6	0	6	
T4	1	1	0	
Lymph node metastasis				0.843
N0				
N1	22	5	17	
N2	9	1	8	
N3	12	2	10	
	11	3	8	
Menopausal status				0.144
Premenopausal				
Postmenopausal	28	4	24	
	22	7	15	
Histologic grade				0.247
1	4	2	2	
2	22	3	19	
3	26	3	23	
Estrogen receptor				0.1
Positive				
Negative	40	6	34	
	12	5	7	
Progesterone receptor				0.155
Positive				
Negative	35	4	31	
	17	7	10	
c-erb-b2				0.147
0	14	2	12	
+	6	3	3	
++	11	1	10	
+++	18	5	13	
Tumor subtype				0.377
IDC	43	10	33	
ILC	4	1	3	
Mucinous	1	0	1	
Mixed	3	0	0	
Stage				0.227
I	12	1	11	
II	20	4	16	
III	8	1	7	
IV	12	5	7	
Lymphovascular invasion				0.591
Positive	32	6	26	
Negative	20	5	15	
DCIS component				0.591
Positive	25	5	20	
Negative	27	6	21	
Gender				0.455
Male	2	0	2	
Female	50	11	39	

Bone marrow metastasis				0.005*
Positive	2	2	0	
Negative	50	11	39	
Bone metastasis				0.013*
Positive	10	5	5	
Negative	42	6	36	
Liver metastasis				0.025*
Positive	5	3	2	
Negative	47	8	39	
Lung metastasis				0.005*
Positive	2	2	0	
Negative	50	9	41	
Cerebral metastasis				0.595
Positive	3	1	2	
Negative	49	10	39	

*Statistically significant.
IDC: invasive ductal carcinoma
ILC: invasive lobular carcinoma

KAYNAKLAR

1. Balic M, Williams A, Dandachi N, Cote RJ. Micrometastasis: detection methods and clinical importance. Cancer Biomark. 2011;9:397-419.
2. Pantel K, Alix-Panabières C, Riethdorf S. Cancer micrometastases.Nat Rev Clin Oncol. 2009;6:339-51.
3. Mansi JL, Gogas H, Bliss JM. Outcome of primary-breast-cancer patients with micrometastases: a long-term follow-up study. Lancet 1999;354:197-202.
4. Diel IJ, Kaufmann M, Costa SD. Micrometastatic breast cancer cells in bone marrow at primary surgery: prognostic value in comparison with nodal status. J Natl Cancer Inst. 1996;88:1652-658.
5. Braun S, Kentenich C, Janni W. Lack of effect of adjuvant chemotherapy on the elimination of single dormant tumor cells in bone marrow of high-risk breast cancer patients. J Clin Oncol 2000;18:80-6.
6. Diel IJ, Solomayer EF, Costa SD. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. N Engl J Med. 1998;339:357-63.
7. Pantel K, Schlimok G, Angstwurm M, et al. Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. J Hematother. 1994;3:165-73.
8. O'Sullivan GC, Collins JK, O'Brien F, et al. Micrometastases in bone marrow of patients undergoing curative surgery for gastrointestinal cancer. Gastroenterology. 1995;109:1535-40.
9. Molino A, Colombatti M, Bonetti F, et al. A comparative analysis of three different techniques for the detection of breast cancer cells in bone marrow. Cancer. 1991;67:1033-6.
10. Pantel K, Woelfle U. Cancer micrometastasis: Detection, clinical relevance, and molecular description. In: ASCO educational book, Lisa Graves Publisher, USA, 2004;701-08.
11. Zhu L, Lam CK, Chow LWC. Sentinel lymph node biopsy or detection of micrometastasis in bone marrow: Which might be an alternative to axillary lymph node dissection in breast cancer patients? Asian Journal of Surgery. 2004;27:279-83.
12. Dearnaley D P, Sloane JP, Ormerod MG, Steele K, Coombes RC, Clink HM. Increased detection of mammary carcinoma cells in marrow smears using antisera to epithelial membrane antigen. British Journal of Cancer. 1981;44:85-90.
13. Timar J, Csuka O, Orosz Z, Jeney A, Kopper L. Molecular pathology of tumor metastasis: II. Molecular staging and differential diagnosis. Pathology Oncology Research. 2002;8:204-19.
14. Guinebretiere JM, Contesso G. "Micrometastases": The pathologist's point of view. Bulletin du Cancer. 2001;88:549-50, 555-60.
15. Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC. A pooled analysis of bone marrow

micrometastasis in breast cancer. N Engl J Med. 2005;353: 793-802.

16. Braun S, Pantel K, Muller P. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. N Engl J Med. 2000;342:525-33.

17. Harbeck N. Tumour cell detection in the bone marrow of breast cancer patients at primary therapy: results of a 3-year median follow-up. Br J Cancer 1991;69:566–71.

18. Benoy IH, Elst H, Philips M. Prognostic significance of disseminated tumor cells as detected by quantitative real-time reverse-transcriptase polymerase chain reaction in patients with breast cancer. Clinical Breast Cancer. 2006;7(2):146-52.

19. Masuda TA, Kataoka A, Ohno S, et al. Detection of occult cancer cells in peripheral blood and bone marrow by quantitative RT-PCR assay for cytokeratin-7 in breast cancer patients. International Journal of Oncology. 2005;26:721–30.

20. Gebauer G, Fehm T. Micrometastases in axillary lymph nodes and bone marrow of lymph node-negative breast cancer patients: Prognostic relevance after 10 years. Anticancer Research. 2003;23:4319-24.

21. Landys K, Persson S, Kovarik J, Hultborn R, Holmberg E. Prognostic value of bone marrow biopsy in operable breast cancer patients at the time of initial diagnosis: results of a 20-year median follow-up. Breast Cancer Res Treat. 1998;49:27-33.

22. Funke I, Fries S, Rolle M. Comparative analyses of bone marrow micrometastases in breast and gastric cancer. Int J Cancer. 1996;65:755-61.

23. Pierga JY, Bonneton C, Magdelenat H, et al. Real-time quantitative PCR determination of urokinase-type plasminogen activator receptor (uPAR) expression of isolated micrometastatic cells from bone marrow of breast cancer patients. Int J Cancer 2005;114:291-98.

24. Emens LA, Reilly RT, Jaffee EM. Breast Cancer vaccines: maximizing cancer treatment by tapping into host immunity. Endocrine-Related Cancer. 2005;12:1–17.

25. Bidard F, Vincent-Salomon A, Sigal-Zafrani B, et al. Prognosis of women with Stage IV breast cancer depends on detection of circulating tumor cells rather than disseminated tumor cells. Annals of Oncology. 2008;19(3):496-500.

26. Naume B, Borgen E, Kvalheim G, et al. Detection of isolated tumor cells in bone marrow in early-stage breast carcinoma patients: comparison with preoperative clinical parameters and primary tumor characteristics. Clin Cancer Res. 2001;7:4122-29.

27. Jung YS, Lee KJ. Clinical Significance of Bone Marrow Micrometastasis Detected by Nested RT-PCR for Keratin-19 in Breast Cancer Patients. Jpn J Clin Oncol. 2003;33:167–72.

28. Donegan WL. Tumor-Related Prognostic Factors for Breast Cancer CA Cancer J Clin. 1997;47:28-51.

29. Magire D, O'Sullivan GC, McNamara B. Bone marrow micrometastases in patient with brain metastases from epithelial cell tumours. Q J Med. 2000;93:611-15.

Yazışma Adresi / Address for Correspondence:
Dr. Berna Bozkurt Duman
Cukurova University Faculty of Medicine
Department of Medical Oncology
Tel:+90322 338 60 60
Fax :+90322 338 70 10
E-mail: berboz@hotmail.com

geliş tarihi/received :11.11.2013
kabul tarihi/accepted:09.12.2013