Cumhuriyet Tıp Derg 2009; 31: 52-59
Cumhuriyet Med J 2009; 31: 52-59

Outcomes of bone marrow micrometastases in breast carcinoma

Meme kanserinde kemik iliği mikrometastazlarının sonuçları

Suat Kutun, Alper Çelik, Olga Koçkar, Abdullah Çetin

Department of General Surgery (S. Kutun, MD, General surgeon; A. Çetin, MD, General surgeon), Ankara Oncology Hospital, TR-06320 Ankara; Department of General Surgery (Assist. Prof. A. Çelik, MD), Gaziosmanpaşa University Faculty of Medicine, TR-60100 Tokat; Department of Radiology (O. Koçkar, MD, Radiologist), Isparta Maternity and Children's Hospital, TR-32200 Isparta

Abstract

Aim. The aim of this study was investigate the correlation of bone marrow micrometastases with conventional prognostic parameters (age, menopausal status, tumor size, and lymph node involvement), immunohistochemical markers and extensive intraductal component (EIC). **Methods.** Forty-nine consecutive histologically proven, operable breast cancer patients treated at Ankara Oncology Hospital (Ankara) were considered. All patients were preoperatively evaluated for the presence of distant metastases with chest X-ray, abdominal ultrasound, and whole body bone scan. Cases with distant metastases were not included in the study. Patients were treated by modified radical mastectomy or conservative breast surgery, and intraoperative bone marrow biopsy was obtained from sternum. Detection of cytokeratin-19 (CK-19) mRNA was performed by polymerase chain reaction (PCR) at bone marrow samples in each individual. Mastectomy specimens were sent for histopathological analysis of tumor size and grade, lymph node status, and EIC. Expression of estrogen receptor (ER), progesterone receptor (PR), c-erbB2, and p53 were analyzed by immunohistochemistry. **Results.** Mean age of the study subjects was 49.1 (min 20 and max 79). All patients had invasive ductal carcinoma proven histologically. Eighteen patients were premenopausal, 9 were perimenopausal, and the remaining 22 were postmenopausal. The tumor was located in the central area in 10, upper-outer quadrant in 13, lower-outer quadrant in 7, upper-inner quadrant in 8, and lower-inner quadrant in 11 cases. Tumor size was less than 2 cm in 8, 2 to 5 cm in 14, and larger than 5 cm in 27 patients. In 8 subjects axilla was free of lymphatic metastases, 1 to 4 nodal involvement was detected in 10, 4 to 9 nodal metastases was observed in 19, and more than 9 nodal metastases was detected in 12 women. ER was positive in 32, and PR was positive in 30 subjects. Expression of P53 was positive in 14, and c-erbB2 in 19 women. Six cases had grade I tumor, 18 had grade II, and the remaining 25 had grade III tumor. EIC was detected in 11 cases. Positive CK-19 expression was detected in 13 patients. Expression of bone marrow micrometastases significantly correlated with tumor size, more than 9 nodal metastases in axilla and presence of EIC. **Conclusion.** It is concluded that bone marrow micrometastases correlates with the tumor size, more than 9 lymph node metastases, and the presence of EIC in breast carcinoma patients.

Keywords: Breast cancer, bone marrow, micrometastases, mRNA, cytokeratin-19, PCR

Özet

Amaç. Bu çalışmada meme kanserli hastalarda kemik iliğindeki mikrometastazlar ile konvansiyonel prognostik parametreler (yaş, menopozal durum, tümör büyüklüğü ve lenf nodu tutulumu) ve immünhistokimyasal belirleyiciler ve ekstensif intraduktal komponent (EİK) arasındaki ilişki araştırıldı. **Yöntem.** Ankara Onkoloji Hastanesinde tedavi edilen meme kanseri histolojik tanısı konmuş, operabl, 49 kadın hasta başvuru sırası ile değerlendirildi. Tüm hastalarda akciğer grafisi, karın ultrasonografisi ve tüm vücut kemik taraması ile uzak organ metastazı varlığı araştırıldı. Uzak organ metastazı bulunan hastalar çalışmaya alınmadı. Hastalara modifiye radikal mastektomi veya meme koruyucu cerrahi uygulandı ve sternumdan intraoperatif kemik iliği biyopsisi alındı. Tüm hastalardan alınan örneklerden polimeraz zincir reaksiyonu (PCR) yöntemi ile sitokeratin-19 (CK-19) mRNA araştırılması yapıldı. Mastektomi materyalleri tümör çapı ve

derecesi ve lenf nodu durumu ile EİK varlığının araştırılması için histopatolojik değerlendirmeye gönderildi. Östrojen reseptörü (ER), progesteron reseptörü (PR), Cerb-B2 ve P53 ekspresyonu immünhistokimya yöntemi ile analiz edildi. **Bulgular.** Hastaların yaş ortalaması 49,1(en az 20, en çok 79) idi. Tüm hastalarda histolojik olarak tanı konmuş invaziv duktal karsinom mevcuttu. Onsekiz hasta premenopozal, 9 hasta perimenopozal ve geri kalan 22 hasta postmeonopozal idi. On hastada tümör santral kesimde yerleşik iken, 13 hastada üst-dış kadran, 7 hastada alt-dış kadran, 8 hastada üst-iç kadran ve 11 hastada alt-iç kadranda yerleşikti. Sekiz hastada tümör çapı 2 cm'den küçük iken, 14 hastada 2-5 cm aralığında ve 27 hastada da 5 cm'den büyüktü. Sekiz hastada koltuk altında lenf bezi metastazı (LBM) saptanmazken, 10 hastada 1-4 arası LBM, 19 hastada 4-9 arası ve 12 hastada 9'un üzerinde LBM saptandı. ER 32 hastada ve PR 30 hastada pozitifti. P53 ekspresyonu 14 hastada ve Cerb-B2 19 hastada pozitifti. Altı hastada grade 1, 18 hastada grade 2 ve geri kalan 25 hastada da grade 3 tümör saptandı. EİK 11 hastada görüldü. Pozitif CK-19 ekspresyonu 13 hastada görüldü. Kemik iliği mikrometastazları ile tümör çapı, 9 ve üzerinde lenf nodu tutulumu ve EİK arasında anlamlı ilişki vardı. **Sonuç.** Meme kanseri olgularında tümör çapının artması, 9 ve üzerinde lenf nodu tutulumu ve EİK varlığı kemik iliği mikrometastazı olasılığını artırır.

Anahtar kelimeler: meme kanseri, kemik iliği, mikrometastaz, mRNA, sitokeratin-19, PCR

Geliş tarihi/Received: March 8, 2009; **Kabul tarihi/Accepted:** March 30, 2009

Corresponding author:

Dr. Alper Çelik, Genel Cerrahi Anabilim Dalı, Gaziosmanpaşa Üniversitesi Tıp Fakültesi, TR-60100 Tokat. Email: doktoralper@hotmail.com

Introduction

Breast carcinoma is the most commonly diagnosed malignancies among women. Annual incidence approximates 180.000 in Europe and 182.000 in U.S. [1]. Cumulative risk of developing breast cancer in life period is 8 per 100 women, and each year 500.000 women die because of breast cancer [2]. Despite the evidence of large epidemiological data, a unique treatment option effective in all stages of the disease is still lacking. Factors effecting prognosis in breast cancer cannot be changed or corrected by the physician. Responsibility for the physician is to apply the appropriate treatment protocol due to the stage of the disease and the patient's requires. There is still a search for more definitive prognostic parameters in order to settle global standardization of treatment and follow-up protocols. From this point of view, we have investigated the correlation of bone marrow micrometastases with conventional prognostic parameters (age, menopausal status, tumor size, and lymph node involvement), immunohistochemical markers and extensive intraductal component (EIC).

Materials and methods

We prospectively analyzed 49 consecutive histologically proven, operable breast carcinoma patients treated at Ankara Oncology Hospital between 2002 and 2005. Ethical committee approval of Ankara Oncology Hospital was obtained before study set up, and written informed consent was taken from the patients. Patients with locally advanced tumors and with demonstrated metastases by whole body bone scan, chest X-ray or abdominal US were not included in to the study. All patients had invasive ductal carcinoma and underwent modified radical mastectomy or breast conserving surgery. Intraoperative bone marrow samples were taken from the sternal bone by 0.3-mm bone trochars and 2-3 g of bone marrow samples were obtained. After completion of mastectomy, axillary regions were separately clipped for distinct analysis on the specimens. Histopathological and immunohistochemical analyses were performed at the Pathology Laboratory of Ankara Oncology Hospital.

Histological and immunohistochemical analysis

Selected data of all patients including age, tumor size, tumor location, and menopausal status were recorded. The histological examination of the surgical specimens was performed on paraffin sections stained by hematoxylin and eosin. All tumors were invasive carcinomas. Histological grading was assessed according to modified Bloom-Richardson criteria [3]. EIC (invasive tumor in which 25% or more of the overall area involved by the invasive carcinoma is composed of ductal carcinoma in situ) was examined. Immunohistochemical studies were performed following standard avidin-biotin-peroxidase technique. Expressions of estrogen receptor (Clone 105+6F11, mouse monoclonal antibody, 1/50), progesterone receptor (Clone hpRa2+hpRa3, mouse monoclonal antibody, 1/50), P53 (Clone DO-7+BP53-12, mouse monoclonal antibody, 1/250), and c-erbB2 (Clone e2-4001+365, mouse monoclonal antibody, 1/250) were determined. These antibodies were purchased from Neomarkers (Fremont, CA, USA). Estrogen and progesterone receptor immunoreactions were judged as positive when more than 10% of tumor cells revealed positive nuclear staining. This cut-off point was 5% for P53 immunoreactivity. C-erbB2 immunoreactivity was assessed as 1+, a barely perceptible partial membrane staining detected in more than 10% of tumor cells; 2+, a weak to moderate complete membrane staining observed in more than 10% of tumor cells; and 3+, string complete membrane staining. Cytoplasmic staining or membrane staining in less than 10% of the tumor cells was categorized as negative.

RNA preparation and PCR Technique

Bone marrow samples were placed in normal saline solutions and sent to Duzen Labarotories Inc. (Ankara) for PCR analysis. Samples were kept in minus 20°C and were minced for analysis.

Annealing of probe. Total RNA extraction was performed using Rneasy Total RNA Kit (Qiagen Inc, Valencia, CA, USA). In order to complete the Annealing of probe procedure for RNA was filled with Rnase free distilled water to complete 100 µL and was kept in sterile RNase tube to dissolve. The solution was then heated at 65°C for 10 min on heater block. One µL of biotin probe (oligo) dT was added before losing its heat and thereafter, 5 µL of 20xSSC was added and stirred promptly. The solution was then kept in room conditions to cool.

Washing with SA-PMPs. The SAPMP (Streptavidin Paramagnetic Particles; Promega Corp., Madison, WI, USA) tube is kept on magnetic field until complete dissolution and precipitated. The supernatant is carefully taken and collected in a separate tube. SAPMP is washed with 0.1 mL 0.5xSSC (Sodium chloride-sodium citrate mixture) for three times, with attention to keep on magnetic field each time until particles are precipitated. SAPMPs solution is suspended with 100 µL of 0.5xSSC. Thirty µL of this solution is used for further analysis.

Precipitation. Extracts of the annealing of probe procedure was added to the washed 30 µL of SAPMP solution in an Eppendorf tube. The new composition was kept in room temperature for 10 min, stirred gently for 1 to 2 min and kept in magnetic stand. The supernatant was discharged and washed for 4 times with 0.1mL 0.1xSSC. The Eppendorf tube was gently flipped until particles were dissolved. The supernatant was again discharged.

Elution of mRNA. SAPMP palette was dissolved in 50 µL of RNase free water, kept on magnetic field, and is transferred to 1.5 µL RNase free Eppendorf tube. Fifty µL of the solution was transferred to SAPMP palette that was kept on magnetic field, and the steps mentioned above were repeated. The RNA solution was added to the same tube to complete 100 µL.

Final assessment. Leukocytes of the patients were used as negative controls for every PCR reactions. Each sample was subjected to electrophoresis with 1.5% agarose gels

stained with ethidium bromide. Samples of each patient were considered to have a positive score if any fragment showed a band of the expected size as positive control for CK-19 (Figure 1).

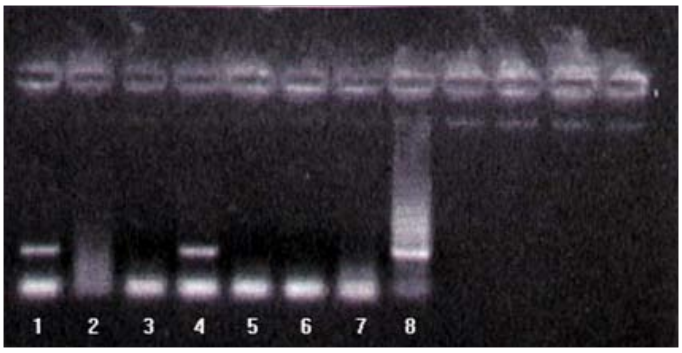

Figure 1. Each sample was subjected to electrophoresis with agarose gels. Samples of each patient were considered to have a positive score if any fragment showed a band of the expected size as positive control for CK-19.

Statistical analysis

The correlation between prognostic parameters and micrometastasis status was analyzed using Pearson chi-square test. In cases of positive correlation with quantitative expression power analysis of the test was performed. Correlation between age and micrometastatic expression was analyzed using t test. The SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. $P < 0.05$ was considered significant.

Results

Characteristics of patients

A total of 49 patients with unilateral breast cancer were evaluated. Mean age of the patients was 49.1 years (min 20 and max 79). All patients had invasive ductal carcinoma proven histologically. Eighteen patients (36.7%) were premenopausal, 9 (18.4%) were perimenopausal, and the remaining 22 (44.9%) were postmenopausal. Of the 49 patients, the tumor was located in the central area in 10 (20.4%), upper-outer quadrant in 13 (26.5%), lower-outer quadrant in 7 (14.3%), upper-inner quadrant in 8 (16.3%), and lower-inner quadrant in 11 (22.5%).

Tumor size was less than 2 cm in 8 (16.3%), 2 to 5 cm in 14 (28.6%), and larger than 5 cm in 27 patients (55.1%). In 8 subjects (16.3%) axilla was free of lymphatic metastases, 1 to 4 nodal involvement was detected in 10 (20.4%), 5 to 9 nodal metastases was observed in 19 (38.8%), and more than 9 nodal metastases was detected in 12 women (24.5%). ER and PR were positive in 30 (61.2%) and 29 (59.2%) subjects, respectively. Expressions of P53 and c-erbB2 were positive in 14 (28.6%) and 16 (32.6%) women. Six cases (12.2%) had grade I tumor, 18 (36.7%) had grade II, and the remaining 25 (51.0%) had grade III tumors. EIC was observed in 11 (22.4%) cases.

Micrometastatic correlation

The bone marrow samples obtained from each individual were subjected to PCR evaluation of CK-19 and a total of 13 (26.5%) patients had micrometastatic disease. The age of patients with micrometastatic disease was 48.7 years (min 20, max76), and those of intact bone marrow was 49.3 years (min 26, max 79). Micrometastatic expression did not correlate with age (p= 0.270). In a similar manner there were no significant difference in micrometastatic expression according to menopausal status (p=0.361).

Micrometastatic bone marrow expression linearly increased with tumor size. One (12.5%) of the 8 cases with T1 tumor had micrometastatic bone marrow involvement, 4 (28.6%) of 14 patients with T2 tumors, and 8 (29.6%) of 27 women with T3 tumors had micrometastases. The difference in expression of micrometastases due to tumor size was

significant (p=0.02).

CK-19 expression of bone marrow micrometastases also correlated with axillary nodal involvement. We detected 1 (11.1%) micrometastases in 9 patients without axillary nodal involvement. Two (20%) of 10 cases with 1-4 positive lymph nodes (PLN), 4 (21.0%) of the 19 subjects with 5-9 PLN and 6 (50%) of the 12 patients with more than 10 PLN also had bone marrow micrometastases. Though in subgroup analysis presence of more than 10 axillary PLN linearly correlated with micrometastatic expression (p=0.002), the difference in micrometastatic expression due to lymph nodes was not significant (p=0.429). CK-19 expression of bone marrow metastases according to tumor size and axillary nodal involvement is listed in Table 1 and their graphical expressions are shown in Figure 2.

Table 1. CK-19 expression according to tumor size and axillary lymph nodes.

		CK-19 Expression		Total
		Negative	Positive	
Tumor size*	T1	7	1	8
	T2	10	4	14
	T3	19	8	27
Number of positive axillary lymph node	None	7	1	8
	1-4	8	2	10
	5-9	15	4	19
	≥10**	6	6	12

*CK-19 expression significantly correlated with tumor size (p=0.02).
**Presence of 10 or more positive lymph nodes had a strong correlation with bone marrow micrometastasis (p=0.002).

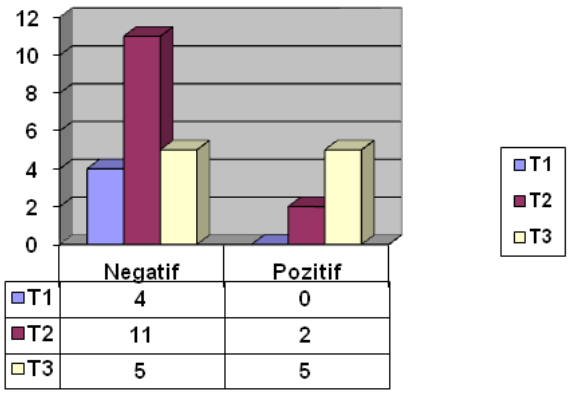

Figure 2. CK-19 expression of micrometastases according to tumor size.

Micrometastatic expression of CK-19 was detected in 5 (26.3%) of 19 cases with negative ER, and in 8 (26.6%) of 30 patients with positive ER and also in 4 (20%) of 20 subjects with negative PR and 9 (31.0%) of 29 cases with positive PR. C-erbB2 was positive in 16 (32.6%) patients. The difference in bone marrow micrometastases according to steroid receptor status was not significant (p=0.251).

Micrometastases were observed in 10 (30.3%) of 33 with negative c-erbB2 and in 3 (18.7%) of 16 with positive c-erbB2 expression. Though in subgroup analysis of c-erbB2 positive cases, the magnitude of c-erbB2 positivity did not correlate with the bone marrow micrometastases (p=0.551), there was a weak negative correlation between c-erbB2 expression and micrometastases (p=0.034).

P53 was positive in 28 (57.14%). Micrometastatic expression was observed in 7 of 28 with positive p53 and in 6 of 21 individuals with negative p53 expression. The difference in micrometastatic positivity due to p53 status was not significant (p=1.09). Table 2

shows CK-19 expression according to immunohistochemical parameters.

Table 2. CK-19 expression according to immunohistochemical parameters.

		CK-19 expression		Total
		Negative	Positive	
Estrogen receptor	Negative	14	5	19
	Positive	22	8	30
Progesterone receptor	Negative	16	4	20
	Positive	20	9	29
Cerb-B2	Negative	23	10	33
	Positive	13	3	16

Expression of bone marrow micrometastases significantly correlated with tumor size, more than 9 nodal metastases in axilla and presence of EIC. EIC was detected in 21 (42.8%) patients and micrometastatic bone marrow involvement was observed in 9 (42.8%) of them. The remaining 28 (57.1%) cases had no EIC, and bone marrow micrometastases were detected in 4 (14.3%) of these patients. Presence of EIC significantly correlated with bone marrow micrometastases (p=0.002).

Discussion

Bones are the most frequent site of distant metastases from breast cancer, and are usually the first presentation of metastatic disease [4]. During the progression of malignant process, the autonomous tumor cells increase in number and disseminate through vascular or lymphatic ways [5]. In the systemic circulation metastatic tumor cells are up-taken by the immune cells; therefore detection of circulating tumor cells is a hard procedure. The bone marrow serves as an appropriate environment for the disseminated malignant cells [6, 7]. Though, most of the tumor cells are filtered by the bone sinusoids and are eliminated, those who have the ability to invade and are not eliminated by the sinusoids adhere to the bone matrix and develop clinical metastases [8].

Determination of bone metastases verifies the appropriate stage and correct staging verifies appropriate treatment. There is an increasing demand towards detecting micrometastases in cancer patients. Whole body bone scan is still the procedure of choice for the detection of bone marrow metastases. Although it should be underlined that bone scan cannot verify metastases unless bone marrow matrix is destructed by the invading metastatic cells, there is no doubt that a proportion of subjects undergoing surgery with normal bone scans have underlying bone marrow micrometastases. From this point of view, we have tried to investigate the role of PCR as a possible screening tool for the staging of breast carcinoma.

Detection of metastatic cells in bone marrow by conventional hematological methods is a tough procedure and is associated with high failure rates [9, 10]. With the use of advanced techniques like immunohistochemistry or PCR, the possibility of detecting tumor cells increases [11-13]. Noguchi et al. [14] stated that a single metastatic cell can be detected by the use of RT-PCR. They compared immunohistochemistry with RT-PCR with respect to diagnosing nodal micrometastases in patients with breast cancer. They observed 9 micrometastases using immunohistochemistry and 15 using RT-PCR, which means that immunohistochemistry failed to demonstrate micrometastases in 6 cases, and concluded that RT-PCR is superior to immunohistochemistry in detecting micrometastatic disease (14). The superiority of PCR to immunohistochemistry has also been confirmed by Mattano et al. [15]. It should not be underestimated that immunohistochemistry is superior to conventional histological techniques and offers the possibility of visualizing cell morphology [16, 17]. Meanwhile it is a subjective method, and estimations are personal [18].

Clinical significance of bone marrow micrometastases has been the point to a number of investigations. Mansi et al. [19] have investigated the role of micrometastases in breast

carcinoma with antiserum precipitation and have detected a 26.4% rate among 307 patients. Within 81 cases with micrometastases, 60 patients have developed distant metastases, both in osseous and extraosseous sites. They have concluded that bone marrow micrometastases significantly predict those patients at risk for developing bone metastases. It also determines an increased risk for extraosseous spread, but this correlation is insignificant. They have further stated a positive correlation between tumor size, lymph node invasion, vascular invasion and bone marrow micrometastases [18]. In another study by Ikeda et al. [20], it is concluded that the presence of occult bone marrow metastasis was not only a predictor of bone recurrences but it was indicative of a high likelihood of recurrence in any organ such as liver, lung, regional lymph nodes or local skin. They also demonstrated that occult bone marrow metastases are significantly associated with lymph node metastases and lymphatic vessel invasion and that the prognosis of the patients with occult bone marrow metastasis is significantly poorer than that of patients without them. In our study, we have demonstrated a positive correlation of tumor size, lymphatic involvement and EIC with bone marrow micrometastases.

Although 85% of primary breast carcinoma patients, staged by conventional methods, have local disease at the time of surgery, up to 40% of them have tumor recurrence and a significant proportion of them die because of disseminated disease. In spite of recent developing techniques in radiotherapy and surgical procedures, these data has not changed in last 50 years.

It is presumed that, well-established modalities for the detection of metastatic disease, such as computed tomography (CT), magnetic resonance imaging (MRI) and bone scan, are all limited in their ability to detect distant disease at the time of initial diagnosis. Some indirect prognostic parameters such as tumor size, lymphatic vessel invasion or lymph node metastases can be used to detect high-risk patients for distant metastases who have local disease apparently; and these patients can be recommended for more aggressive adjuvant chemotherapy by using these techniques. But these parameters are prognostic indicators not exact evidences of metastases. It is necessary to find more definite parameters that show distant disease in solid organ tumors.

According to our study, it is possible to state that evaluation of micrometastases will be one of the standard staging criteria in the future. It is clear that breast surgeons will attempt to find out if the patients with micrometastases will be named as neo-adjuvant group or disseminated group. We conclude that micromeastatic breast carcinoma correlates with advanced tumor size, more than 9 positive lymph nodes and presence of EIC, however further prospective randomized studies with larger patients groups are needed.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgements

We would like to thank the subjects that participated in the study.

References

1. Boring CC, Squires TS, Tong T. Cancer Statistics, 1993. J Clin Cancer 1993; 43: 7-26.
2. Jatoi I. The natural history of breast cancer. Surg Clin North Am 1999; 79: 949-60.
3. Ozer E, Sis B, Ozen E, Sakizli M, Canda T, Sarioglu S. BRCA1, Cerb-B2, and H-ras gene expressions in young women with breast cancer. An Immunohistochemical study. Appl Immunohistochem Mol Morphol 2000; 8: 12-8.
4. Coombes RC, Powles TJ, Abbott M, De Rivas L, Ford HT, McCready VR, Neville AM, Gazet JC. Physical test for distant metastases in patients with breast cancer. J R Soc Med 1980; 73: 617-23.
5. Diel IJ, Kaufman 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

metastasis. J Clin Oncol 1992; 10: 1534-9.

6. Cote RJ, Rosen PP, Lesser ML, Old LJ, Osborne MP. Prediction of early relapse in patients with operable breast cancer by detection of occult bone micrometastases. J Clin Oncol 1991; 9: 1749-56.
7. Diel IJ, Kaufman M, Holle R, Von Minckwitz G, Solomayer EF, Kaul S, Bastert G. Micrometastatic breast cancer cells in bone marrow at primary surgery- prognostic value in comparison to nodal status. J Natl Cancer Inst 1996; 88: 1652-8.
8. Solomayer EF, Diel IJ, Wallwiener D, Bode S, Meyberg G, Sillem M, Gollan C, Kramer MD, Krainick U, Bastert G. Prognostic relevance of urokinase plasminogen activator detection in micrometastatic cells in the bone marrow of patients with primary breast cancer. Br J Cancer 1997; 76: 812-8.
9. Ridell B, Landsy K. Incidence and histopathology of metastases of mammary carcinoma in biopsies from the posterior iliac crest. Cancer 1979; 44: 1782-8.
10. Ingle JN, Tormey DC, Tan HK. The bone marrow examination in breast cancer. Cancer 1978; 41: 670-4.
11. Coombes RC, Dearnaley DP, Redding WH. Micrometastases in breast cancer. In Peeters H, eds Protides of the biological fluids. Vol 31 Oxford: Pergamon, 1983:317-23.
12. Dearnaley DP, Sloane JP, Ormerod MG, Steele K, Coombes RC, Clink HM, Powles TJ, Ford HT, Gazet JC, Neville AM. Increased detection of mammary carcinoma cells in marrow smears using antisera to ephitelia membrane antigen. Br J Cancer 1981; 44: 85-90.
13. Dearnaley DP, Ormerod MG, Sloane JP, Lumley H, Imrie S, Jones M, Coombes RC, Neville AM. Detection of isolated mammary carcinoma cells in marrow of patients with primary breast cancer. JR Soc Med 1983; 76: 359-64.
14. Noguchi S, Aihara T, Nakamori S, Motomura K, İnaji H, Imaoka S, Koyama H. The detection of breast carcinoma micrometastases in axillary lymph nodes by means of reverse transcriptase- polymerase chain reaction. Cancer 1994; 74: 1595-600.
15. Mattano LA, Moss TJ, Emerson SG. Sensitive detection of rare circulating neuroblastoma cells by the RT-PCR. Cancer Res 1992; 52: 4701-5.
16. Wells CA, Heryet A, Brochier HJ, Gatter KC, Mason DY. The immunocytochemical detection of axillary micrometastases in breast cancer. Br J Cancer 1984; 50: 193-7.
17. International (Ludwig) Breast Cancer Study Group. Prognostic importance of occult axillary lymph node micrometastases from breast cancer. Lancet 1990; 335: 1565-8.
18. Mascarel I, Bonichon F, Coindre JM, Trojani M. Prognostic significance of breast cancer axillary lymph node micrometastases assessed by two special techniques: reevaluation with longer follow-up. Br J Cancer 1992; 66: 523-7.
19. Mansi J, Berger U, Easton D, McDonnell T, Redding H, Gazet JC, McKinna A, Powles TJ, Coombes RC. Micrometastases in bone marrow in patients with primary breast cancer: evaluation as an early predictor of bone metastases. Br Med J 1987; 295: 1093-6.
20. Ikeda N, Y Miyoshi, K Motomura, Inaji H, Koyama H, Noguchi S. Prognostic significance of occult bone marrow micrometastases of breast cancer detected by quantitative polymerase chain reaction for cytokeratin 19 mRNA. Jpn J Cancer Res 2000; 91: 918-24.