

DISSEMINATED TUMOUR CELLS IN BONE MARROW: AN INDEPENDENT PROGNOSTIC FACTOR IN BREAST CANCER

Gayathri P¹, Shantaraman K², Radhika A.R³

Received : 02/08/2026
Received in revised form : 22/08/2026
Accepted : 05/09/2026

Keywords:

Breast neoplasms; Bone marrow; Disseminated tumour cells; Micrometastasis; Prognostic factors; Cytokeratin 18.

Corresponding Author:

Dr. Gayathri P,

Email: gaibaski@gmail.com

DOI: 10.47009/jamp.2026.8.5.105

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2026; 8 (5); 669-674



¹Associate Professor, Department of Pathology, Government Vellore Medical college, Vellore, Tamil Nadu, India

²Dean, Madras Medical College, Chennai, Tamil Nadu, India

³Professor, Department of Pathology, Government Tiruvannamalai Medical College, Tiruvannamalai, Tamil Nadu, India

ABSTRACT

Background: Breast cancer is one of the most common malignancies affecting women worldwide and remains a major cause of cancer-related mortality. The prognosis of breast cancer is traditionally assessed using clinical stage, tumour size, lymph node involvement, histological grade, lymphovascular invasion, resection margin status and hormone receptor profile. However, these conventional prognostic parameters do not completely predict the clinical course of the disease. Approximately 20–30% of patients considered to have favourable prognostic features may subsequently develop relapse or recurrence during treatment, after completion of treatment or within five years, whereas some patients with initially unfavourable prognostic characteristics may survive for more than 10 years. The presence of disseminated tumour cells (DTCs) in bone marrow may precede the development of clinically detectable marrow metastasis by months or years and may therefore represent an important marker of minimal residual disease. Aim of present study is to determine the incidence of disseminated tumour cells in the bone marrow of patients with breast carcinoma and to evaluate their association with established clinicopathological prognostic factors. **Materials and Methods:** This cross-sectional study was conducted over a period of two years from 2009 to 2011. Of 1432 women who underwent clinical evaluation for breast lumps, 1385 underwent fine-needle aspiration cytology, of whom 132 had cytological evidence of ductal carcinoma. Fifty-one patients with biopsy- and/or mastectomy-proven breast carcinoma, without radiological evidence of bone metastasis or lymphatic spread, were selected according to predefined inclusion and exclusion criteria. Bone marrow aspirates were obtained from both posterior superior iliac crests, with approximately 5 mL of pooled marrow collected in EDTA tubes. Cytological smears were evaluated using standard cytological criteria, and disseminated tumour cells were further assessed by cytokeratin 18 (CK18) immunohistochemistry. Clinical, operative, gross and histopathological parameters, including tumour size, lymph node status, vascular permeation, histological grade, oestrogen receptor (ER), progesterone receptor (PR) and HER2/neu status, were recorded. Statistical analysis was performed using the Chi-square test, with $p < 0.05$ considered statistically significant. **Result:** Among the 51 patients included in the study, 17 (33.3%) demonstrated DTCs in marrow concentrate smears, whereas DTCs were identified in only two (3.92%) marrow clot sections. Of the 17 patients identified as DTC-positive by routine staining, 13 (76.47%) showed positivity for CK18. The presence of marrow DTCs demonstrated significant associations with tumour size ($p = 0.014$), lymph node involvement ($p = 0.036$), vascular permeation ($p = 0.001$), histological grade ($p = 0.0195$), ER status ($p = 0.004$) and PR status ($p = 0.004$). HER2/neu status did not demonstrate a statistically significant association with marrow DTCs ($p = 0.46$). The frequency of DTC positivity increased with increasing tumour size, higher lymph node status, increasing histological grade and the presence of vascular permeation. **Conclusion:** The detection of disseminated tumour cells in bone marrow was significantly associated with several established adverse prognostic characteristics of breast carcinoma. The increasing frequency of DTCs with tumour size, nodal involvement, vascular permeation

and histological grade suggests that bone marrow DTC assessment may provide additional information regarding minimal residual disease and risk of disease progression. Bone marrow DTCs may therefore have potential as an independent prognostic marker in breast cancer, although larger studies with long-term clinical follow-up are required to establish their independent predictive value.

INTRODUCTION

Breast cancer is one of the most common malignancies among women worldwide and represents a major cause of cancer-related morbidity and mortality.^[1] In the United States, breast cancer constitutes the most common cancer among women, accounting for approximately 32% of female cancers.^[2] In India, breast cancer has historically ranked second among cancers affecting women, after cervical cancer, and its epidemiological profile differs from that observed in developed countries. The peak incidence in developed countries occurs after 50 years of age, whereas in India the peak occurrence is reported after 40 years. The age-standardized incidence rate of breast cancer in India has been reported to range from 9 to 32 per 100,000 women.^[3]

Prognostic assessment in breast cancer is based on several established clinicopathological parameters. These include clinical stage, histological grade, nuclear grade, tumour size, lymphatic permeation, vascular invasion, lymph node involvement, resection margin status and hormone receptor expression.^[4] Despite advances in diagnosis and treatment and the availability of several established prognostic indicators, these parameters do not completely explain the subsequent clinical behaviour of breast cancer. Approximately 20–30% of patients with apparently favourable prognostic characteristics may develop relapse or recurrence during treatment, following completion of treatment or within five years, whereas some patients with initially unfavourable prognostic features may survive for more than a decade.^[4]

These observations have led to the search for additional prognostic markers capable of identifying patients at risk of recurrence who may not be adequately categorized by conventional staging systems. Among the proposed markers, the presence of disseminated tumour cells in the bone marrow has attracted considerable attention. Bone marrow micrometastases may precede the recognition of clinically apparent or macroscopic marrow metastasis by months or even years.^[5] These isolated tumour cells or small aggregates are generally not detectable by conventional serum tumour markers or routine radiological imaging.^[6]

The presence of tumour cells disseminated through the haematogenous route and detectable in bone marrow has been associated with an increased risk of metastatic relapse and death.^[5] The detection of these cells may therefore provide information regarding minimal residual disease that is not captured by

conventional tumour staging. As treatment strategies increasingly move towards individualized therapy directed against residual tumour burden, assessment of biological markers such as disseminated tumour cells may assume increasing importance.^[7,8]

Immunocytochemical techniques using epithelial markers, particularly cytokeratins, have facilitated the detection of isolated epithelial tumour cells in bone marrow. Cytokeratin 18 is expressed by epithelial cells and can assist in identifying disseminated epithelial tumour cells among marrow cells. Standardized cytological and immunocytochemical criteria have also been developed for the detection and evaluation of isolated tumour cells in bone marrow and peripheral blood.^[4,9,10]

The present study was therefore undertaken to evaluate the occurrence of disseminated tumour cells in the bone marrow of patients with breast carcinoma without clinically or radiologically evident distant metastasis and to determine their relationship with established clinicopathological prognostic parameters.

The primary objective of the study was to determine the incidence of disseminated tumour cells in the bone marrow of patients with breast carcinoma. The secondary objective was to assess the relationship between marrow DTC positivity and established prognostic factors, including tumour size, lymph node involvement, vascular permeation, histological grade, ER status, PR status and HER2/neu receptor status.

MATERIALS AND METHODS

This cross-sectional study was conducted over a period of two years, from 2009 to 2011. Women presenting to the General Surgery outpatient department with complaints of a breast lump were evaluated clinically and investigated according to standard diagnostic protocols. Among the 1432 women who underwent clinical evaluation, 1385 were subjected to fine-needle aspiration cytology. Of these, 132 patients demonstrated cytological evidence of ductal carcinoma of the breast. Fifty-one patients were subsequently selected for inclusion in the present study according to the predefined eligibility criteria.

Patients with malignancy confirmed by FNAC, biopsy and/or mastectomy, but without radiological evidence of bony lesions or lymphatic spread, were included. Patients with carcinoma in situ, proven distant metastasis, radiologically suspicious bone

lesions, previous chemotherapy or radiotherapy, or a history of another malignancy were excluded.

Bone Marrow Aspiration: Bone marrow aspiration was performed in all 51 patients from both posterior superior iliac crests. Approximately 5 mL of marrow obtained from the two sites was pooled and collected in EDTA tubes. Cytospin preparations were made and smears were prepared on positively charged glass slides. Three to six slides per patient were stained using Romanowsky stain. An additional three to six slides were air-dried, fixed in methanol and allowed to dry overnight at room temperature before immunohistochemical evaluation.

Cytokeratin 18 Immunohistochemistry: The prepared slides were subjected to cytokeratin 18 immunohistochemical staining. Antigen retrieval was performed using a steam-based epitope retrieval method with Tris-EDTA buffer at pH 9. Endogenous peroxidase activity was blocked using hydrogen peroxide. Following blocking, the slides were incubated with the primary antibody for 30 minutes, followed by incubation with Polyexcel Target Binder for 12 minutes. The chromogenic reaction was developed using DAB chromogen for five minutes, followed by counterstaining with haematoxylin for five minutes.

Criteria for Identification of Disseminated Tumour Cells: Bone marrow smears were evaluated for DTCs using established cytological characteristics. The criteria included the presence of cell clusters, large cell size, characteristic cell-border patterns, enlarged nuclei, a high nuclear-to-cytoplasmic ratio, irregular nuclear membranes and prominent nucleoli. CK18 positivity was characterized by strong or irregular cytoplasmic staining with cytokeratin filaments and a granular or stippled nuclear appearance.^[4,9,10]

Histopathological Evaluation: Mastectomy specimens were received in the pathology department and examined using standard histopathological protocols. Clinical, operative, gross and microscopic findings were recorded. Tumour prognostic parameters, including tumour size, lymph node status, lymphovascular permeation, skin involvement, resection margins and hormone receptor status, were documented.

All 51 patients had infiltrating ductal carcinoma of the breast, not otherwise specified. Histological grading was performed according to the modified Bloom-Richardson system as described by Elston and Ellis.^[11]

The recorded clinicopathological parameters were tabulated according to the presence or absence of marrow DTCs. The relationship between DTC status and tumour size, lymph node involvement, vascular permeation, histological grade, ER status, PR status and HER2/neu receptor status was evaluated using the Chi-square test. A p-value <0.05 was considered statistically significant.

RESULTS

All patients with cytologically proven breast malignancy underwent surgical treatment, and the final tumour histology was evaluated. TNM staging was performed, and 51 patients with N0/M0 status at selection were included for analysis. Among these 51 patients, 17 (33.3%) were positive for DTCs in marrow concentrate smears. When marrow clot sections were evaluated, DTCs were identified in two patients (3.92%). Of the 17 cases diagnosed as DTC-positive by routine staining, 13 (76.47%) demonstrated CK18 positivity, thereby providing immunocytochemical confirmation.

Surgical specimens were available for all 51 patients. Simple mastectomy was performed in 16 patients, modified radical mastectomy in 34 patients and segmental mastectomy in one patient. Histologically, all patients had infiltrating ductal carcinoma of the breast, not otherwise specified. Tumour size, lymph node status, vascular permeation, histological grade and hormone receptor status were subsequently compared with marrow DTC status.

The relationship between tumour characteristics and marrow DTC status is summarized in Table 1. Tumour size demonstrated a statistically significant association with DTC positivity ($p=0.014$). The proportion of DTC-positive patients increased with increasing tumour size, with the highest proportion observed among tumours infiltrating the skin and chest wall.

Lymph node involvement was also significantly associated with marrow DTC positivity ($p=0.036$). DTC positivity increased progressively from patients without nodal involvement to those with increasing numbers of involved lymph nodes. Among patients with N0 disease, one of 19 patients was DTC-positive, compared with six of 17 patients with involvement of one to three nodes and six of 15 patients with involvement of four to nine nodes.

Vascular permeation showed the strongest association with marrow DTC status ($p=0.001$). Twelve of the 17 patients with vascular permeation were DTC-positive, whereas only one of 34 patients without vascular permeation demonstrated DTC positivity.

Histological grade was significantly associated with DTC status ($p=0.0195$). DTC positivity increased progressively from grade I to grade III tumours. One of 17 grade I tumours, five of 20 grade II tumours and seven of 14 grade III tumours were DTC-positive.

A significant association was observed between ER status and DTC positivity ($p=0.004$). All 13 DTC-positive patients were ER-negative, whereas none of the 16 ER-positive tumours demonstrated DTC positivity. PR status showed a similar statistically significant relationship ($p=0.004$), with 12 of 34 PR-negative tumours demonstrating DTC positivity compared with one of 17 PR-positive tumours.

HER2/neu status was not significantly associated with DTC positivity ($p=0.46$). Among HER2/neu-negative tumours, nine of 31 patients were DTC-

positive, whereas four of 20 HER2/neu-positive tumours demonstrated DTC positivity.

Table 1: Relationship between tumour behaviour and marrow disseminated tumour cells

Tumour characteristic	Category	DTC positive	DTC negative	Total	p-value
Tumour size	<2 cm	1	12	13	0.014
	2–5 cm	1	10	11	
	>5 cm	4	11	15	
	Infiltrating skin/chest wall	7	5	12	
Lymph node involvement	No nodes	1	18	19	0.036
	1–3 nodes	6	11	17	
	4–9 nodes	6	9	15	
	>10 nodes	0	0	0	
Vascular permeation	Present	12	5	17	0.001
	Absent	1	33	34	
Histological grade	Grade I	1	16	17	0.0195
	Grade II	5	15	20	
	Grade III	7	7	14	
Estrogen receptor	Negative	13	22	35	0.004
	Positive	0	16	16	
Progesterone receptor	Negative	12	22	34	0.004
	Positive	1	16	17	
HER2/neu	Negative	9	22	31	0.46
	Positive	4	16	20	

Statistical significance was considered at $p<0.05$. Data reproduced from the original study.



Figure 1: Photomicroscopic picture of bone marrow smear showing disseminated tumour cells. CK18 Positive. (IHC, X100)

DISCUSSION

Over the past several decades, improvements in awareness, diagnostic techniques and therapeutic options have resulted in earlier and more accurate diagnosis of breast cancer. Nevertheless, the expected reduction in morbidity and mortality has not been achieved uniformly.^[12] Metastatic disease remains the major cause of death among patients with breast carcinoma.^[13] Although several established clinicopathological parameters are routinely used for prognostic assessment, these parameters cannot completely predict treatment response, recurrence or long-term survival.

One possible explanation is the presence of minimal residual disease that remains undetected at the time of primary diagnosis. Conventional staging systems primarily evaluate the characteristics of the primary tumour and regional lymph nodes and do not necessarily identify isolated tumour cells that have already disseminated through the circulation. The

development of recurrence may therefore depend, at least in part, on the presence of occult micrometastatic disease at the time of primary diagnosis.^[13]

The present study evaluated the clinical and pathological relevance of detecting isolated tumour cells in the bone marrow of patients with breast carcinoma at the time of primary diagnosis. The findings demonstrate significant relationships between DTC positivity and several established adverse prognostic features.

The study demonstrated an increase in the incidence of marrow DTCs with increasing age, with a significant association between patient age and DTC positivity ($p=0.037$). The incidence was also higher among postmenopausal patients ($p=0.024$). These findings were comparable with observations reported by Braun et al,^[5] and Diel et al,^[14] although they differed from the findings of Pierga et al,^[15] who reported a predominance of DTCs among premenopausal patients.

Lymph node status showed a significant association with marrow DTC positivity ($p=0.036$). Among the 51 patients, 19 (36%) had N0 disease, of whom one (5.6%) had marrow DTCs. Seventeen patients (34%) had N1 disease, with six (35.3%) demonstrating DTC positivity, while 15 patients (30%) had N2 disease, of whom six (40%) were DTC-positive. No patients with N3 disease were included. Thus, the frequency of DTCs increased with increasing nodal involvement. This concordance between axillary nodal disease and marrow dissemination suggests a relationship between regional tumour burden and systemic dissemination.

Tumour size was similarly associated with marrow DTC status ($p=0.014$). DTCs were identified in 8.3% of T1 tumours, 9.1% of T2 tumours, 26.7% of T3 tumours and 58.3% of T4 tumours. The progressive

increase in DTC frequency with increasing tumour size indicates a greater probability of haematogenous dissemination in patients with larger primary tumours. Similar observations have been reported by Braun et al,^[5] Wiedswang et al,^[16] Naume et al,^[17] and Diel et al.^[14]

Histological grade also demonstrated a significant association with DTC status ($p=0.0195$). Among the tumours, 32% were grade I, 40% were grade II and 28% were grade III. DTC positivity was observed in 10% of grade I, 24% of grade II and 66% of grade III tumours. The marked increase in DTC positivity with increasing histological grade supports the relationship between aggressive tumour biology and the capacity for systemic dissemination. Similar findings have been reported by Braun et al,^[5] Diel et al,^[14] and Naume et al.^[17]

Hormone receptor status also showed important associations with DTC detection. Thirty-five patients were ER-negative, of whom 34.4% demonstrated DTCs, whereas none of the 16 ER-positive patients was DTC-positive ($p=0.004$). A similar relationship was observed with PR status, with DTCs being more frequent among PR-negative tumours ($p=0.004$). In contrast, HER2/neu status did not demonstrate a statistically significant association with DTC positivity ($p=0.46$).

The predominance of DTCs among ER- and PR-negative tumours may reflect differences in tumour biology and metastatic potential. Previous studies have also examined the relationship between hormone receptor status and isolated tumour cells in bone marrow.^[15-17] The present findings suggest that hormone receptor-negative tumours may have a greater tendency for dissemination and warrant further investigation in larger cohorts.

Vascular permeation demonstrated the strongest association with marrow DTC positivity in the present study ($p=0.001$). Of the 17 patients with obvious vascular permeation, 12 (70.6%) were DTC-positive. In contrast, only one patient without obvious vascular permeation was DTC-positive. This observation is biologically plausible because vascular invasion provides a direct route for tumour cells to enter the circulation and disseminate to distant sites. Comparable findings have been reported by Pierga et al,^[15] and Naume et al.^[17]

Taken together, the findings suggest that DTC positivity is closely associated with several conventional indicators of aggressive breast cancer behaviour, including larger tumour size, higher nodal stage, higher histological grade and vascular permeation. The combination of tumour size, hormone receptor status, HER2/neu status, lymph node involvement, tumour grade, vascular permeation and marrow DTC status may therefore assist in identifying patients at increased risk of disease progression.

Patients with node-positive disease (N1 or higher), tumour size greater than 2 cm (T2 or higher), hormone receptor negativity and DTC-positive bone marrow may constitute a particularly high-risk group.

The detection of DTCs may provide information regarding occult systemic disease that is not captured by conventional clinical or radiological staging.

Previous studies have emphasized the prognostic relevance of isolated tumour cells in the bone marrow of patients with early breast cancer. Braun et al. demonstrated the prognostic importance of bone marrow micrometastases in a pooled analysis of breast cancer patients.^[5] Diel et al. reported the prognostic value of micrometastatic breast cancer cells in bone marrow in comparison with nodal status.^[14] Similarly, Pierga et al., Wiedswang et al. and Naume et al. reported significant associations between isolated tumour cells and clinical or pathological characteristics of breast cancer.^[15-17]

The detection of DTCs may therefore have potential implications for risk stratification and individualized management. The identification of minimal residual disease could become particularly relevant as treatment approaches increasingly focus on the biological characteristics of individual tumours and the eradication of residual disease.^[7,8]

However, the findings of the present study should be interpreted in light of its limitations. The study included only 51 patients and was conducted over a relatively short period. Consequently, the statistical power and generalizability of the observations are limited. Furthermore, the proportion of patients with marrow DTCs was lower than that reported in several other studies, potentially reflecting differences in age distribution, menopausal status and regional, socioeconomic or racial characteristics of the study population.

Most importantly, although the present study demonstrated significant associations between DTCs and several established prognostic factors, long-term outcome data were not available to directly establish recurrence-free or overall survival. Therefore, the designation of marrow DTCs as an “independent prognostic factor” should be interpreted cautiously and requires confirmation through larger prospective studies incorporating long-term follow-up and multivariable survival analysis.

CONCLUSION

The present study demonstrates that disseminated tumour cells in the bone marrow are associated with several established prognostic characteristics of breast carcinoma. Marrow DTCs were more frequently detected in patients with larger tumours, greater lymph node involvement, vascular permeation and higher histological grade. DTC positivity was also significantly associated with ER- and PR-negative tumours, whereas HER2/neu status did not show a significant association.

The incidence of marrow DTCs was particularly high among patients with T4 tumours, N2 disease, grade III tumours and vascular permeation. These findings support the potential role of bone marrow DTC assessment in identifying patients with occult

systemic tumour dissemination and in categorizing breast cancer patients into groups with differing risks of disease progression.

Bone marrow DTC detection may therefore provide prognostic information complementary to conventional clinicopathological staging. However, the present findings are based on a relatively small sample and do not establish independent prognostic significance through long-term survival analysis. Larger prospective studies with standardized DTC detection techniques and prolonged clinical follow-up are required before routine marrow DTC assessment can be recommended as an independent prognostic tool.

Further investigation in the Indian population is warranted, particularly with regard to regional, racial and socioeconomic differences in breast cancer biology and outcomes. The relationship between hormone receptor expression and isolated tumour cell dissemination also merits further study.

REFERENCES

- Hortobagyi GN, de la Garza Salazar J, Pritchard K, Amadori D, Haidinger R, Hudis CA, et al. The global breast cancer burden: Variations in epidemiology and survival. *Clin Breast Cancer* 2005;6:391-401.
- Chlebowski RT, Manson JE, Anderson GL, Cauley TA, Aragaki AK, Stefanick ML, et al. Estrogen plus progestin and breast cancer incidence and mortality in the Women's Health Initiative Observational Study. *J Natl Cancer Inst* 2013;105:526-535.
- Siddiqi M, Sen U, Mondal SS, Patel DD, Yeole BB, Jussawala DJ, et al. Cancer statistics from non-ICMR registries: Population based registries. CRAB (Cancer Registry Abstract) Newsletter of the National Cancer Registry Project of India 2001;47-59.
- Vincent-Salomon A, Bidard FC, Pierga JY. Bone marrow micrometastasis in breast cancer: review of detection methods, prognostic impact and biological issues. *J Clin Pathol* 2008;61:570-576.
- Braun S, Vogl FD, Naume B, Janni W, Osborne MP, Coombes RC, et al. A pooled analysis of bone marrow micrometastasis in breast cancer. *N Engl J Med* 2005;353:793-802.
- Zach O, Lutz D. Tumor cell detection in peripheral blood and bone marrow. *Curr Opin Oncol* 2006;18:48-56.
- Hartkopf AD, Wallwiener M, Fehm TN, Hahn M, Walter CB, Gruber I, et al. Disseminated tumor cells from the bone marrow of patients with nonmetastatic primary breast cancer are predictive of loco regional relapse. *Ann Oncol* 2015;26:1155-60.
- Hartkopf AD, Taran FA, Wallwiener M, Hahn M, Becker S, Solomayer EF, et al. Prognostic relevance of disseminated tumor cells from the bone marrow of early stage breast cancer patients—results from a large single-centre analysis. *Eur J Cancer* 2014;50:2550-2559.
- Borgen E, Naume B, Nesland JM, Kvalheim G, Beiske K, Fodstad O, et al. Standardization of the immunocytochemical detection of cancer cells in BM and blood. Establishment of objective criteria for the evaluation of immunostained cells. The European ISHAGE Working Group for Standardization of Tumor Cell Detection. *Cytotherapy* 1999;1:377-88.
- Borgen E, Pantel K, Schlimok G, Muller P, Otte M, Renolen A, et al. A European interlaboratory testing of three well-known procedures for immunocytochemical detection of epithelial cells in bone marrow. Results from analysis of normal bone marrow. *Cytometry B Clin Cytom* 2006;70:400-9.
- Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. *Histopathology* 1991;19:403-410.
- Bilimoria MM, Morrow M. The women at increased risk for breast cancer: Evaluation and management strategies. *CA Cancer J Clin* 1995;45:263-78.
- Alix-Panabières C, Vendrell JP, Pellé O, Rebillard X, Riethdorf S, Müller V, et al. Detection and characterization of putative metastatic precursor cells in cancer patients. *Clin Chem* 2007;53:537-39.
- Diel IJ, Kaufmann M, Costa SD, Holle R, von Minckwitz G, Solomayer EF, et al. Micrometastatic Breast Cancer Cells in Bone Marrow at Primary Surgery: Prognostic Value in Comparison With Nodal Status. *J Natl Cancer Inst* 1996;88:1652-1658.
- Pierga JY, Bonneton C, Vincent-Salomon A, Cremoux P, Nos C, Blin N, Pouillart P, Thiery JP, Magdelénat H. Clinical Significance of Immunocytochemical Detection of Tumor Cells Using Digital Microscopy in Peripheral Blood and Bone Marrow of Breast Cancer Patients. *Clin Cancer Res* 2004;10:1392-1400.
- Weidswang G, Borgen E, Karesen R, Kvalheim G, Nesland JM, Qvist H, et al. Detection of Isolated Tumour Cells in Bone Marrow is an independent prognostic factor in Breast Cancer. *J Clin Oncol* 2003;21:3469-3478.
- Naume B, Borgen E, Kvalheim G, Kåresen R, Qvist H, Sauer T, 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;7:4122-4129.