Featured Article

Isolated Tumor Cells in Bone Marrow Three Years after Diagnosis in Disease-Free Breast Cancer Patients Predict Unfavorable Clinical Outcome

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ABSTRACT

Purpose: The aim of the study was to explore the value of analyzing bone marrow (BM) for the presence of isolated tumor cell(s) (ITCs) in disease-free breast cancer patients 3 years after diagnosis.

Experimental Design: ITCs in BM at operation was found to be an independent prognostic factor in 817 breast cancer patients. Among these, 356 disease-free patients were analyzed with a second BM after 3 years follow-up (median 40 months, SD 3 months, range 29–52). ITC was detected by immunocytochemistry with anticytokeratine antibodies (AE1/AE3).

Results: The population consisted of 70% T1 and 72% node-negative patients. ITC in BM was detected in 15%. At a median of 25 months since the second BM aspiration (66 months since diagnosis), 32 patients had developed relapse, 12 local and 20 systemic. Of the patients with ITC in BM, 21% relapsed compared with 7% of the ITC-negative patients (P < 0.001). Ten patients died of breast cancer. Survival analyses showed that ITC in BM predicted reduced distant disease-free survival (DDFS) and breast cancer specific survival (BCSS; P < 0.001, log-rank test). Uni- and multivariate analyses of the prognostic value of N, T, estrogen receptor/progesterone receptor, and BM status, histological grade, vascular invasion, p53-, c-erb-B2-, and cathepsin D expression were performed. BM status was the only independent prognostic factor for both DDFS and BCSS, whereas c-erbB-2 and N status were independent for BCSS and vascular invasion and T status for DDFS.

Conclusions: ITC in BM 3 years after diagnosis in disease-free breast cancer patients is an independent prognostic factor. Detection of residual disease by BM analysis at follow-up may unravel insufficient adjuvant treatment. The clinical implications should be further explored.

INTRODUCTION

Several prospective clinical studies with >3000 breast cancer patients have shown that the presence of isolated tumor cell(s) (ITC) in bone marrow (BM) at the time of operation is associated with an unfavorable outcome (1–7). In the largest trials, detection of ITCs is a strong and independent prognostic factor. However, the clinical value of ITC detection in node-negative patients varies (5–7).

The knowledge about “the natural history” of ITCs in BM is limited, but it is known that subclinically disseminated tumor cells can be affected by adjuvant therapy in breast cancer (8, 9). Furthermore, remaining epithelial cells after primary treatment can potentially be a useful marker for subsequent systemic relapse (10, 11). Therefore, to explore the value of monitoring the presence of ITCs in BM is the first step in the attempt to make possible testing of secondary adjuvant treatment changes. A second bone marrow aspiration was performed in 421 breast cancer patients about 3 years (median 40 months) after the primary operation. All these patients had previously been included in our reported study of the clinical significance of ITC detection in BM at primary surgery (7, 12). The presence of ITCs in BM 3 years after primary surgery was compared with the clinical outcome at median 66 months (range 37–92 months) after diagnosis and median 25 months (range 1–57 months) after the second BM aspiration.

MATERIALS AND METHODS

Patients. In 421 breast cancer patients, after written informed consent, a second BM aspiration was performed in local anesthesia from the two posterior iliac crests median 40 (SD 3) months (range 29–52) after the primary operation. All these patients were included in our study to explore the value of detecting ITCs in BM at the time of operation (7, 12), and those who were alive and had acceptable performance status were asked to participate in the current study. The original study population (n = 817) consisted of 63% node-negative and 34% node-positive patients. Sixty one percent had T1, 31% T2, and 6% T3–4 tumors; 24% grade 1, 49% grade 2, and 25% had grade 3 tumors; 76% were hormone-receptor positive and 20% were receptor negative. The patients were treated for their cancer according to the Norwegian Guidelines (7). Briefly, node-negative patients with pT1 grade 2–3 tumors and node-positive patients received the following: (a) chemotherapy [cyclophos-
Mononuclear cells were collected and cytospins were prepared previously (12). After separation by density centrifugation, 10 ml BM was aspirated from each of the posterior iliac crests bilaterally, 10 ml/site. At the follow-up operation, a total of 40 ml of BM was aspirated from anterior and posterior organs at distant sites. A new primary tumor. Systemic relapse was recorded as recurrence in contralateral breast and/or regional lymph nodes, ipsilateral breast, and contralateral breast cancer. No information was available to differ between a relapse in contralateral breast and recurrence in thoracic wall, regional lymph nodes, ipsilateral breast, and contralateral breast cancer. No information was available to differ between a relapse in contralateral breast and a new primary tumor. Systemic relapse was recorded as recurrence at distant sites.

Preparation of the Bone Marrow. At the primary operation, a total of 40 ml of BM was aspirated from anterior and posterior iliac crests bilaterally, 10 ml/site. At the follow-up aspiration, 10 ml BM was aspirated from each of the posterior crests, totally 20 ml. The BM was processed as described previously (12). After separation by density centrifugation, mononuclear cells were collected and cytospins were prepared (5 × 10^5 mononuclear cells/slide).

Immunocytochemical Staining. The immunocytochemical staining has been described previously (12). Briefly, four slides (2 × 10^6 BM mononuclear cells) were incubated with the anticytokeratin monoclonal antibodies AE1 and AE3 (Sanbio, Uden, the Netherlands), and the same number of slides was incubated with an isotype-specific irrelevant control monoclonal antibodies. The visualization step included the alkaline phosphatase/antialkaline phosphatase technique, and the slides were counterstained with hematoxylin to visualize nuclear morphology. BM samples from 98 healthy donors were also analyzed following the same procedures. In 50 samples, four slides, and in 48 samples, two slides were immunostained with both AE1/AE3 and control antibody. Four of the 98 BMs had one or more positive cells detected, without similar cells in the negative control.

Detection of ITC. The cytospins prepared after the first aspiration were manually screened by light microscopy using the ×10 lens. All immunostained cells were closely evaluated by one pathologist (E. B.). According to published guidelines (13), immunostained cells present in clusters or with nuclear size clearly enlarged as compared with surrounding hematopoietic cells were scored as ITCs. Also cells lacking these features could be scored as ITCs if recognizable hematopoietic cell characteristics were absent; typically these cells had either strong and/or irregular cytoplasmic staining partially covering the nucleus. In addition, immunocytochemical positive cells categorized into a morphology group called “un-interpretable” according to the published guidelines were also recorded as positive, as this category contains cells associated with poor outcome (14). The cytospins prepared after the second aspiration were screened by an automatic device (MDS 1, Applied Imaging; Ref. 15). The slides were then reviewed by one of the authors (G. W.), and the cells were categorized according to recommended guidelines together with a pathologist (E. B.). In doubtful cases, a second pathologist (J. M. N.) was consulted and consensus was obtained. The presence of positive cells classified as tumor cells both in AE1/AE3-stained slides and in the corresponding negative controls resulted in exclusion of the sample from diagnostic conclusion (21 samples in the first BM examination, 22 samples in the second). The results from the BM analyses were blinded to the patients.

Analysis of Primary Tumor and Axillary Lymph Nodes. The primary tumor and axillary lymph nodes collected during surgery were processed on a routine diagnostic basis. Histological tumor type, tumor size, and nodal involvement were analyzed and the disease was staged according to the tumor-node-metastasis (TNM) system (Union Internationale Contre le Cancer 1997). Tumor grading was performed according to Elston and Ellis (16), and tumor slides were screened for vascular invasion. Small axillary lymph nodes were embedded undivided in paraffin; of larger nodes, only a half of the node was embedded, if possible sectioned longitudinally through the hilar plane. One (sometimes two) H&E section of each lymph node was screened for metastases by ordinary light microscopy. Immunostaining was performed using mouse monoclonal antibodies against ER and PgR (clones 6F11 and 1A6, respectively; Novocastra, Newcastle on Tyne, United Kingdom), p53 protein (clone DO-1, catalogue no. SC-126; Santa Cruz Biotechnology, Santa Cruz, CA), erb-B2 (clone CB11; Bio Genex, SanRamon, CA), and rabbit polyclonal antichemopxin D antibodies (catalogue no.18-0109, Zymed, San Fransisco, CA). Automated immunostaining systems were used, Ventana Medical System, Inc (Tucson, AZ) for c-erbB-2 and BioGenex for the other markers. Immunopositivity was recorded if ≥10% (ER, PgR, cathepsin D, c-erbB2) or ≥5% (p53) of the tumor cells were immunostained. In addition, cathepsin D positivity required well-defined immunolabeled secretory granules, whereas c-erbB2-positivity required distinct membranous staining.

Statistical Analysis. Breast cancer specific survival (BCSS) was measured from the date of surgery to breast cancer-related death or, otherwise, censored at the time of the last follow-up visit or at noncancer-related death. Locoregional and systemic relapse were measured in the same way. Metastases to the skeleton, liver, lungs, or central nervous system were recorded as systemic relapse. Kaplan-Meier survival curves for time to distant recurrences and breast cancer-specific death were constructed. P values were computed by log-rank test. Cox proportional hazards regression analysis was used for univariate and multivariate (stepwise backward elimination) analyses of prognostic impact of relevant variables. The Pearson x² test and linear by linear test were used to compare categorical variables. For statistical analysis the SPSS (Version 10.1; SPSS, Inc, Chicago, IL) software was used.

RESULTS
The correlation between clinical outcome and detection of ITCs in BM among the 817 patients analyzed at the time of operation has been reported (7). The criteria for an ITC-positive result have later been modified to include not only cells with
morphology compatible with a tumor cell but also cells categorized into an un-interpretable cell group (14). This is because the presence of cells categorized as un-interpretable also is associated with reduced survival (14). Including this cell group as a positive result, the number of ITC-positive patients increased from originally reported 13.2% to 22.5% (14). A second BM aspiration at 3 years (median 40 months, SD 3 months, range 29–52) was performed in 421 of these patients. Sixteen of the patients had premalignant breast cancer and 27 had diagnosed recurrence of the disease at the time of the second BM aspiration, 17 local and 10 systemic relapse. The second BM analysis was noninterpretable because of a positive result in both the specific test and the negative control in 22 patients. Excluding these patients from the survival analysis, 356 disease-free breast cancer patients remained for further analyses.

**Patient Characteristics and ITC Detection.** The median age at diagnosis was 57 years. The study population consisted of 70% with T1 tumors and 72% with node-negative disease. Eighty percent of the patients were hormone receptor positive, defined as being either ER and/or PgR positive. Further clinicopathological characteristics are presented in Table 1. The BM analysis at 3 years revealed 53 positive samples (15%). Detection of ITCs correlated to the nodal status (P = 0.003) and systemic adjuvant treatment (chemotherapy, CMF; P = 0.015, endocrine therapy, tamoxifen; P = 0.047), but not to other clinicopathological characteristics (Table 1).

**Detection of ITCs and Clinical Outcome.** At median 66 months from diagnosis (range 37–92 months; 25 months since the 3 year BM aspiration, range 1–57), 32 patients had developed relapse, 12 with local relapses (including contralateral cancer) and 20 with systemic relapses. Of the recurrences defined as local, six were localized to the contralateral breast, three to the thoracic wall, one to the axilla, one to the supraclavicular nodes and one to the ipsilateral breast. The systemic relapses included 8 patients with visceral metastases only, 3 with skeletal metastases only and nine with both visceral and skeletal metastases. Among the patients with ITC-positive BM at 3 years 21% (11 of 53 patients) experienced relapse, opposed to 7% (21 of 303 patients) in the ITC-negative group (P = 0.001, Pearson χ² test). The difference was significant for systemic relapse (P = 0.001) but not for local relapse (P = 0.317).

The Kaplan-Meier survival analyses show significantly reduced distant disease-free survival (DDFS) in patients with ITC-positive BM at 3 years (P < 0.001, log-rank test; Fig. 1A). Local relapse-free survival was not associated with the presence or absence of ITCs in BM (data not shown). Separate analyses of the node positive (n = 93) and node negative (n = 256) patients, demonstrate that ITC only predicts reduced DDFS in the node-positive patients (Fig. 1, C and E).

**Detection of ITCs and Survival.** During the observation time of median 66 months from diagnosis, 10 of the 356 patients died of breast cancer. BCSS was markedly decreased in the ITC-positive group (P < 0.001, log-rank test), but the poor prognosis was only confined to the node-positive patients (Fig. 1, B, D, and F).

**Detection of ITCs and Survival According to Systemic Adjuvant Treatment.** A total of 143 patients received adjuvant systemic treatment. In this group, the presence of ITCs in BM correlated to both reduced DDFS (P = 0.001, log-rank test) and BCSS (P = 0.001, log-rank test). No significant difference in survival was detected between ITC-positive- and ITC-negative patients without systemic treatment (P = 0.255 and P = 0.082, respectively). For hormone receptor-positive patients receiving tamoxifen, remaining cancer cells after several years on treatment may indicate insufficient therapy. Therefore, DDFS and BCSS were analyzed in tamoxifen-treated receptor-positive patients (n = 116), with comparison to the BM status. As shown in Fig. 2, a
reduction in both DDFS and BCSS were observed among the ITC-positive patients ($P = 0.015$ and $P = 0.004$, respectively).

**Uni- and Multivariate Analyses.** Detection of ITCs, nodal status, T status, histological grade, hormone receptor status, vascular invasion, p53, c-erb-B2, and cathepsin D-expression were all tested as prognostic factors in univariate analyses. Also, the ITC results from the BM analysis performed at diagnosis were tested. ITC detection at follow-up and at diagnosis, N status, and c-erb-B2 expression were significantly associated with BCSS. ITC detection at follow up, N status, T status, and vascular invasion were associated with DDFS (Table 2). All these factors were tested in multivariate analyses. BM status was the only independent prognostic factor for both DDFS and BCSS, whereas c-erb-B2 and N status were independent for BCSS and vascular invasion and T status for DDFS. (Table 3).

**Survival Analyses Based on Results from BM Examinations at Diagnosis and at 3 Years.** Among the 356 patients, 21 were excluded from the combined analysis of the BM results at diagnosis and at 3 years follow-up because of non-interpretable results in the first BM analysis. At diagnosis, 21% of the patients had ITC-positive BM. On the basis of the results from both the BM analysis at diagnosis and at 3 years, the following groups were created: (a) positive for ITCs at both examinations, +/+ ($n = 17$); (b) first BM positive, second BM negative, +/− ($n = 54$); (c) first BM-negative, second BM-positive: −/+ ($n = 33$); and (d) both BMs negative, −/− ($n = 33$).
ITCs in BM at 3-Year Follow-Up in Breast Cancer

The frequency of local relapses did not differ between the groups, but the rate of distant metastases was significantly higher in the +/+ group, 29%, as compared with the other groups (≤6%; Table 4; \(P = 0.004\), Pearson \(\chi^2\)). As shown in the survival analyses, the +/+ group experienced a markedly reduced DDFS and BCSS (\(P < 0.001\), log-rank test; Fig. 3).

DISCUSSION

To our knowledge, this study represents the most extensive report of repeated BM aspirations in recurrence-free breast cancer patients. After 66 months of follow-up, the presence of ITCs in BM significantly predicts reduced distant disease-free survival and breast cancer-specific survival. In multivariate analyses, the detection of ITCs is the only independent prognostic factor for both BCSS and DDFS. When analyzing the data separately for the node-negative and the node-positive patients, the significance was only confined to the node-positive group. The time point for the second BM analysis (median 40 months) was chosen partly for practical reasons (available resources in the laboratory) and partly as a result of clinical considerations. A substantial number of breast cancer patients experience late relapses, which opens for the detection of ITCs at later time points in follow-up for selection of these patients.

The patient population asked to participate in the study (at operation) had overall early breast cancer with high frequency of \(T_1\) tumors (58%) and \(N_0\) stage (63%; Ref. 12). This is reflected in a lower frequency of ITCs in BM and a reduced relapse rate in these patients, as compared with other study populations (4–7). From this early stage study population, the absence of relapse 3 years after diagnosis selects a very good prognosis group for the second BM analysis. In accordance, 72% of the patients had \(N_0\) stage, and 80% were ER/PgR positive. This can partially explain the lower frequency of positive BMs (15%) in our study as compared with Janni et al. (11), who reported a rate of 28% positives in BM analyses performed 19 months after diagnosis. In addition, median time for the BM analysis was 19 months earlier than in our study. This indicates that Janni et al. have included a larger proportion of high-risk patients experiencing relapse within 3 years. Nevertheless, we confirm the results from their study. The presence of ITCs in BM after end of therapy identifies patients at risk for future systemic relapse and reduced breast cancer-specific survival. Similarly, we also found that patients with ITCs in BM both at primary surgery and at follow-up experienced the most unfavorable clinical outcome, followed by those with only one

**Table 2** The prognostic significance of ITC\(^+\) detection (BM status) and primary tumor characteristics in univariate analyses.*

<table>
<thead>
<tr>
<th></th>
<th>DDFS</th>
<th></th>
<th></th>
<th>BCSS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR(^c)</td>
<td>95% CI</td>
<td>(P)</td>
<td>RR(^c)</td>
<td>95% CI</td>
</tr>
<tr>
<td>BM status at FU</td>
<td>4.3</td>
<td>1.8–10.6</td>
<td>0.001</td>
<td>9.8</td>
<td>2.8–34.7</td>
</tr>
<tr>
<td>BM status at diagnosis</td>
<td>2.1</td>
<td>0.8–5.4</td>
<td>0.113</td>
<td>4.5</td>
<td>1.3–15.9</td>
</tr>
<tr>
<td>N status</td>
<td>3.0</td>
<td>1.3–7.3</td>
<td>0.013</td>
<td>6.9</td>
<td>1.8–26.9</td>
</tr>
<tr>
<td>T status(^d)</td>
<td>2.5(^d)</td>
<td>1.0–6.2</td>
<td>0.044</td>
<td>3.2(^d)</td>
<td>0.9–11.2</td>
</tr>
<tr>
<td>c-erb-B2</td>
<td>2.2</td>
<td>0.5–9.59</td>
<td>0.297</td>
<td>4.8</td>
<td>1.0–23.3</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>3.6</td>
<td>1.2–9.7</td>
<td>0.021</td>
<td>2.1</td>
<td>0.4–10.0</td>
</tr>
</tbody>
</table>

\* ITC, isolated tumor cell; BM, bone marrow; DDFS, distant disease-free survival; BCSS, breast cancer specific survival; RR, relative risk; CI, clearance interval; FU, follow-up.

\* Cox regression. Only factors of significance either for DDFS or BCSS are presented.

\* Relative risk. Positive versus negative.

\* \(T_{2–4}\) versus \(T_1\).

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**Table 3** Multivariate survival analyses of prognostic factors (\(n = 256\)*)

<table>
<thead>
<tr>
<th></th>
<th>DDFS(^a)</th>
<th></th>
<th></th>
<th>BCSS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR(^c)</td>
<td>95% CI</td>
<td>(P)</td>
<td>RR(^c)</td>
<td>95% CI</td>
</tr>
<tr>
<td>BM status at FU</td>
<td>3.1</td>
<td>1.1–8.4</td>
<td>0.026</td>
<td>7.5</td>
<td>1.7–32.2</td>
</tr>
<tr>
<td>N status</td>
<td>NS(^d)</td>
<td>NS</td>
<td>NS</td>
<td>5.5</td>
<td>1.1–26.3</td>
</tr>
<tr>
<td>T status(^e)</td>
<td>2.8(^e)</td>
<td>1.1–7.3</td>
<td>0.039</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>c-erb-B2</td>
<td>NS</td>
<td>NS</td>
<td>14.5</td>
<td>2.6–81.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>3.6</td>
<td>1.3–10.3</td>
<td>0.017</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\* Only patients with complete datasets were included. The analyses were performed by Cox regression. Only factors of significance are presented.

\* DDFS, distant disease-free survival; BCSS, breast cancer specific survival; RR, relative risk; CI, clearance interval; BM, bone marrow; FU, follow-up.

\* Relative risk. Positive versus negative.

\* NS, not significant. The parameter was eliminated from the multivariate analysis and no RR, CI or \(P\) value computed.

\* \(T_{2–4}\) versus \(T_1\).

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**Table 4** Relapses analyzed according to the detection of isolated tumor cell at time of operation bone marrow 1 (BM1) and at 3-year follow-up (BM2).

<table>
<thead>
<tr>
<th></th>
<th>Relapse free no.</th>
<th>Local relapse no.</th>
<th>Systemic relapse no.</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM1/BM2(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–/+</td>
<td>231 (93.1)</td>
<td>6 (2.6)</td>
<td>10 (4.3)</td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>54 (92.6)</td>
<td>2 (3.7)</td>
<td>2 (3.7)</td>
<td></td>
</tr>
<tr>
<td>–/+</td>
<td>33 (87.9)</td>
<td>2 (6.1)</td>
<td>2 (6.1)</td>
<td></td>
</tr>
<tr>
<td>+/+</td>
<td>17 (70.6)</td>
<td>0</td>
<td>5 (29.4)</td>
<td></td>
</tr>
</tbody>
</table>

\* BM aspirates were analyzed for isolated tumor cell, positive = +, negative = –.
of the two analyses being positive (Table 4, Fig. 2). The development of overt metastases in breast cancer patients is highly unpredictable. Patients can experience early and aggressive relapses, whereas others have their first symptoms of metastases >10 years after primary diagnosis. One reason for this is the level of remaining tumor load after primary treatment. The presence of ITCs at both primary surgery and at follow-up may reflect a higher load of nonapoptotic ITCs present, with a higher probability for disease progression. A positive BM status only at diagnosis can be explained by eradication of ITCs by the primary treatment and/or that ITCs in these cases were apoptotic or false positives. Consequently, a better prognosis should be observed, as reported. A switch from BM negative to BM positive at follow-up indicates an increase in tumor cell load from negative or under the detection limit to above the detection limit. It is reasonable to believe that these patients have a slow disease progression. The follow-up after the second BM may therefore be too short for final conclusions about the relapse frequency. For the same reason, calculations of the positive predictive value of the BM analysis are premature. Thus far, the BM analysis at 3 years only detect 34% of all relapses (37% of the systemic). Again, longer observation time is needed for more certain conclusions. However, it is probable that the sensitivity and specificity can be increased if larger volumes of BM (increased possibility for detection of ITCs) are analyzed, combined with characterization of the cells for their malignant potential (increased specificity).

To use detection of ITCs as a surrogate marker for effect of adjuvant chemotherapy, BM analysis needs to be performed immediately after the completion of therapy. This was not practical when the present study was designed, but will be addressed in another ongoing study from our group. However, late BM analysis can be of interest as a tool for monitoring residual cancer cells in patients receiving endocrine treatment. A total of 116 hormone receptor positive patients received adjuvant tamoxifen in the current study. In this group, a clear reduction in DDFS and BCSS were observed in the BM-positive patients (Fig. 2). Considering the reported benefit of endocrine-treatment changes at late time points in adjuvant treatment (extended treatment with letrozole after tamoxifen or switch to exemestane after 2–3 years of tamoxifen; Refs. 17 and 18), BM analysis may be a candidate method for possible selection of patients profiting from such late endocrine treatment switches. Because of the low number of events thus far reported, one should, however, be cautious about drawing certain conclusions of the role of a late BM analysis. Therefore, the use of repeated BM aspirations in follow-up should be further tested within clinical studies. If BM analysis in the future can be used for consideration of secondary treatment alteration, characterization of the ITCs in BM opens possibilities for exploring tailored treatment decisions. Indeed, the expression of c-erb-B2, p53, and urokinase-type plasminogen activator receptor on ITCs has been reported (19–21).

Our results and those reported by Janni et al. (11) are in contrast with what was reported by Mansi et al. in 1989 (22). Eighteen months after surgery, ITCs were detected in only 2 of 82 breast cancer patients. This difference can be explained by methodological differences (11). Furthermore, Molino et al. (23) analyzed 100 pairs of BMs, without showing any prognostic value of repeated BM analyses. To our knowledge, the antibodies used in their study have not been tested in other clinical trials, and the specificity of these can be questioned. Furthermore, their BM analyses at diagnosis did not correlate to clinical outcome (24). In other studies, repeated BM examinations have mostly been performed in high-risk and metastatic breast cancer patients receiving high dose chemotherapy and/or immunotherapy (10, 25, 26). As shown in the present study, prediction of outcome by ITC-detection in follow-up is not only applicable to high-risk patients not responding to chemotherapy but also to relatively low-risk patients.

The presence of ITCs predicts poor outcome most clearly in the node-negative patients, and this patient group may probably be the most interesting to include in studies of BM analysis at follow-up in the future. An explanation for the more uncertain value of this analysis in the node negative patients may be the limited numbers of clinical events reported in this selected low-risk node-negative group. Only 4% (10 of 256) of the patients experienced systemic relapse opposed to 11% (10 of 93) among the node positives (data not shown). Supporting this, none of the many prognostic factors tested reached significance in the univariate subgroup analyses of node negative (data not shown), in huge contrast to the results from the study of all patients at the time of diagnosis (7). Including in the analysis, patients with local relapse but not systemic before the 3-year...
BM, the ITC detection was associated with increased systemic relapse rate also in the node-negative patients \( (P = 0.057) \). This indicates that node negatives in general have low risk of relapse and low frequency of clinically relevant ITCs present. But in node-negative subgroups where remaining tumor cells exist locally after primary treatment, BM analysis may still detect patients at risk for systemic relapse.

This study confirms the potential use of ITC detection in BM in follow-up after primary therapy in breast cancer. Although detailed primary tumor analyses in the future possibly can result in a better selection of therapy for the individual patient, adjuvant treatment failures must be expected. Analysis for ITCs in BM in at-risk patients, for example node-positive patients or BM-positive patients at diagnosis, can help detect such failures and give an opportunity for designing secondary adjuvant rescue treatment studies.

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