Clinical Cancer Research

Persistence of Disseminated Tumor Cells in the Bone Marrow of Breast Cancer Patients Predicts Increased Risk for Relapse—A European Pooled Analysis

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Abstract

Background: The prognostic significance of disseminated tumor cells (DTC) in bone marrow (BM) of breast cancer patients at the time of primary diagnosis has been confirmed by a large pooled analysis. In view of the lack of early indicators for secondary adjuvant treatment, we here evaluated whether the persistence of DTCs after adjuvant therapy increases the risk of subsequent relapse and death.

Patients and Methods: Individual patient data from 676 women with primary diagnosis of early breast cancer stages I–III from 3 follow-up studies were pooled. During clinical follow-up, patients underwent BM aspiration (BMA) to determine the presence of DTC. Tumor cells were detected by the standardized immunoassays. Univariate and multivariable proportional hazards models were estimated to assess the prognostic significance of DTC for disease-free survival (DFS) and overall survival (OS).

Results: Patients were followed for a median of 89 months. BMA was performed at median 37 months after diagnosis of breast cancer. At follow-up BMA, 15.5% of patients had DTCs. The presence of DTC was an independent indicator of poor prognosis for DFS, distant DFS (DDFS), cancer-specific survival, and OS during the first 5 years following cancer diagnosis (log-rank test $P < 0.001$ values for all investigated endpoints).

Conclusion: Among breast cancer patients, persistent DTCs during follow-up significantly predicted the increased risk for subsequent relapse and death. Analysis of DTC might serve as a clinically useful monitoring tool and should be tested as an indicator for secondary adjuvant treatment intervention within clinical trials. *Clin Cancer Res; 17(9); 2967–76. ©2011 AACR.*

Introduction

Disseminated tumor cells (DTC) can be detected in the bone marrow (BM) up to 30% to 40% of breast cancer patients (1–5). The strong independent prognostic significance of DTCs at the time of primary diagnosis has already been confirmed by a large pooled analysis including more than 4,700 breast cancer patients (1). On the basis of these results, it was hypothesized that DTCs reflect the presence of minimal residual disease (MRD) and may be the precursor of subsequent metastatic disease (6). The success of adjuvant therapy is based on the ability to eradicate MRD before it becomes clinically evident. Currently, no diagnostic tools are available to monitor treatment response after the completion of adjuvant treatment and identify patients with the need for secondary adjuvant therapy due to persistent tumor cell load. Reevaluation of BM status may be a promising procedure because the presence of DTC is a possible surrogate marker for persistent MRD. To date, only few small studies indicated that a positive BM status during follow-up may be associated with worse outcome (7, 8).

To elucidate the role of persistent DTCs in a larger cohort, clinical follow-up data of 676 patients from 3 academic breast cancer centers were pooled. The aim of the analysis was to assess the prevalence of tumor cells in BM of early breast cancer patients during clinical follow-up and evaluate the clinical significance of the BM status for the individual residual risk after primary treatment of breast cancer.

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Translational Relevance

Adjuvant treatment in breast cancer cannot be monitored in an individual patient. Increasing evidence suggests that the presence of isolated tumor cells in the bone marrow (BM) of breast cancer patients at the time of primary diagnosis not only indicates the presence of minimal residual but also predicts an increased risk for relapse. Our findings suggest that BM aspirations may serve as a future monitoring tool during the follow-up of breast cancer patients.

Our data imply that there is a clinical potential for monitoring treatment efficacy and residual risk in a postoperative follow-up situation, which should be further explored in well-designed randomized clinical trials. Among the options for both trial and research hypotheses are the following: (i) utilization of persistent disseminated tumor cells (DTC) for adapting adjuvant treatment to modulated individual residual risk; (ii) phenotyping of DTCs for addressing the differential impact of tumor cell biology; and (iii) profiling DTCs for targeted therapy development.

Patients and Methods

Data collection

Three academic breast cancer units in Oslo (Norway), Munich (Germany), and Tuebingen (Germany), which had previously investigated DTCs during follow-up of breast cancer patients, contributed individual patient data for analysis. Earlier data of parts of the patient cohorts (Oslo and Munich) have been published with shorter follow-up (7, 8). For this analysis, the follow-up was limited to 10 years. The study was approved by the Internal Review Boards (Germany) and the Regional Ethic Committee (Norway).

Patients

Patients were eligible if they had completed surgery for invasive breast cancer (stages pT1–4, pN0–3, and M0) and agreed to undergo BM aspiration (BMA), after prior written informed consent, during clinical follow-up without evidence of relapse.

The tumor stage at primary diagnosis was classified according to the revised AJCC (American Joint Committee on Cancer) tumor node metastasis (TNM) classification (9), and histopathological grading of the primary tumors was performed according to Elston and Ellis (Oslo cases; ref. 10) or the Bloom–Richardson system (Munich and Tuebingen cases; ref. 11).

Primary surgery consisted of either breast conservation (55.6%) or mastectomy (44.4%) leading to R_0 resection in all cases. Axillary dissection was performed in all patients, except for 7 women having cN0, where the surgeon decided not to do the procedure (age/other conditions). Radiotherapy was done according to the respective national guidelines.

In Oslo, systemic treatment followed the Norwegian guidelines 1995 to 1998 and was given to pT2pN0G2–3 or pN+ patients (5). Chemotherapy [CMF (cyclophosphamide, methotrexate, and fluorouracil)] was administered if age was less than 55 years or if age 55 to 65 years and negative hormone receptor (HR) status. HR-positive patients received tamoxifen for 5 years. In the German centers (Munich and Tuebingen), systemic treatment followed the St. Gallen Treatment Recommendations 1998 and 2001 (12, 13).

BM preparation and immunocytochemistry

We have previously published our semiquantitative assay for BM preparation (14–17). Tumor cell isolation and detection were done on the basis of consensus recommendations (18, 19). In summary, 5 to 20 mL of BM was aspirated and processed within 24 hours. Immunostaining of cytospins from the BM preparations using the pan-anticytokeratin mAbs (monoclonal antibodies) A45-B/B3 (Munich and Tuebingen) or AE1 and AE3 (Oslo) and controls has been described in detail elsewhere (8). The cytospins were screened for DTCs manually or by an automatic device (MDS 1, Applied Imaging) by skilled pathologists. The determination of the presence of DTC was based on consensus criteria (19, 20).

Following the same procedures, the specificity of the method has been determined. In Oslo, BM slides from 98 healthy donors were analyzed with both AE1/AE3 and control antibody. Four of 98 BMs had 1 or more positive cell detected, without similar cells in the negative control. In Tuebingen, among 100 patients without evidence of malignant disease, 1 patient was detected positive. In Munich, as previously published, BM aspirates from 191 patients with nonmalignant disease were also analyzed in a blinded fashion, before the final histopathological result was disclosed. In 2 patients in this group—1 with a chronic benign inflammation of the breast and the other with a benign cystadenoma of the ovary–specifically stained cytokeratin-positive cells were detected. Therefore, the mean overall false-positive rate in the 3 institutions was 1.8%.

Follow-up and patient evaluation

Patients were followed at the hospitals' outpatient departments or by family physicians/private gynecologists, at 3 to 12 months interval, and included clinical examination (each visit), mammography (yearly), and (if present) symptoms-driven examinations. Information on disease recurrence was obtained from the patient records. Deaths (including cause) were verified with the regional Cancer Registries (Germany) or the National Mortality Registry (Norway).

Statistical analysis

The association of DTCs in BM with patient characteristics was tested by the chi-squared test. For survival

analysis, breast-cancer–related death, death due to any cause, distant metastasis, and any disease recurrence were separately investigated. If no endpoint was reached, data were censored at last follow-up.

For patients surviving more than 10 years, the follow-up data were censored at 120 months after diagnosis (35 patients, none with events after 120 months). Survival time was measured from the time of surgery to the time of death or first evidence of recurrence. As BM aspiration was an eligibility criterion for this study, left truncation was used to correct for the fact that patients could have died before having had a chance to determine their DTC status. Thus, in the statistical analysis, patients came under observation with regard to the endpoint of interest starting only from the time of BM aspiration.

Meta-analysis techniques were used to compute a summary estimate of the HR and 95% CI for recurrence or death with DTCs as the sole variable on the basis of the effect estimates of each study calculated from the individual patient data. The Q test was done to assess heterogeneity between studies (21).

For univariate significance of DTCs, Kaplan–Meier curves were plotted (22) and the log-rank statistic calculated. Incidence rates and mortality were calculated as the number of disease recurrences or deaths per 1,000 person years. Mortality ratios, incidence–rate ratios, and 95% CI were estimated. Univariate results for all covariates are given in the Supplementary Table.

Cox proportional hazards regression was used to evaluate the simultaneous effect of factors potentially influencing survival (23). The categorical variables were tested for trend across strata. If separate categories did not improve the fit of the model, a linear trend was preferred. A test for interaction between pairs of variables in the final models was done, and the effect of each variable assessed with the Wald test and described by the HR with a 95% CI. All estimates were stratified according to study center, and all reported *P* values are 2-sided.

The initial model included age at diagnosis, menopausal status, grade, histological tumor type, HR-, pT- and pN-status, and whether a patient had received adjuvant therapy (endocrine and/or cytotoxic). Subjects with any missing values were excluded from modeling. The final model was developed by dropping each variable, in turn, and conducting a likelihood ratio test to compare the full and the nested models. A significance level of 0.10 was used as cutoff to exclude a variable from the model. Finally, the variable of DTCs was added to the model to test the resultant model against that without the variable.

As we observed that curves on the Kaplan–Meier graphs dispersed during the first year of follow-up and then showed less divergence, the assumption of proportional hazards was not met over the entire follow-up period. We therefore opted for a piecewise Cox model with a cutoff point set at 5 years. For both the first and second intervals, separate Cox models were fit. The proportional hazards assumption was formally tested for each interval and separate regression estimates are given (24).

Results

Prevalence of DTCs in BM during follow-up

Individual patient data from a total of 676 histologically confirmed invasive breast cancer patients from 3 centers were included in this study (Table 1). The median age was 56 years. BMA was done at a median time of 37 months after primary diagnosis. Overall, 105 patients (15.5%) had DTCs during follow-up. There was no association of persistent DTCs with clinicopathological characteristics (Table 2). The detection rate of DTC decreased to some extent over time. While DTCs were found in 21% of patients who underwent BMA in the first year of follow-up, DTCs were detected in only 6% of patients who had BMA done after the 4th year of follow-up (trend not significant; Table 3).

Meta-analysis

The meta-analytic HR was 4.87 (CI: 2.04–11.61) for overall survival (OS) and 3.10 (CI: 1.70–5.65) for disease-free survival (DFS) during the first 5 years of follow-up. For these endpoints, the individual HRs calculated for each single study ranged from 4.33 to 5.33 and from 1.58 to 4.30, respectively. The 95% CIs were significant for all but the smallest study (Tuebingen) and showed considerable overlap, indicating a similar effect of DTCs in all studies. The Q test for statistical heterogeneity showed no significant interstudy variation among the estimated HRs ($P = 0.976$ for OS and $P = 0.510$ for DFS).

Follow-up BM status and DFS

Median follow-up time was 89 months from diagnosis. Seventy-one patients (10.5%) relapsed: 54 with distant metastasis (76.1%) and 17 with locoregional relapse (23.9%). DTCs were detectable in 19 patients (35.2%) with distant metastasis and in 1 patient (5.9%) with locoregional relapse.

DFS and distant DFS (DDFS) were significantly shorter in patients with DTCs compared with patients with no DTC (log-rank test: $P = 0.002$ and $P < 0.001$, respectively; Fig. 1). The piecewise model revealed that the difference was significant only during the first 5-year interval of follow-up. There was no survival disadvantage for patients with DTCs during the follow-up period from 6 to 10 years.

In the multivariable model, DTC remained an independent indicator of poor prognosis for both endpoints during the first 5-year interval of follow-up (Table 4).

Follow-up BM status and OS

Overall, 47 patients (7.0%) died during follow-up. In 30 women (63.8%), death was related to breast cancer. Of these, 12 patients (40.0%) had DTCs in BM. Both OS and cancer-specific survival (CCS) were significantly shorter in

Table 1. Baseline patient characteristics by center

Variable	All patients	Study center Oslo	Munich	Tuebingen	P^a
Breast cancer patients					
Number (%)	676	356 (52.7)	198 (29.3)	122 (18.1)	
Year of primary diagnosis	1988–2004	1995–1998	1988–2002	1999–2004	
Age at diagnosis–-mean ± SD, in years	55.7 ± 9.8	57.3 ± 10.0	54.2 ± 9.4	53.8 ± 9.5	<0.001
(median, range)	(56, 28–85)	(56, 28–85)	(55, 33–77)	(54, 32–75)	
BMA					
Year of BMA	1994–2005	1998–2002	1994–2003	2001–2005	
Time from primary diagnosis to BMA–-mean ± SD (in months)	31.3 ± 15.9	39.8 ± 2.9	27.2 ± 21.8	12.9 ± 5.3	<0.001
(median, range)	(37, 4–104)	(40, 29–52)	(20, 5–104)	(13, 4–41)	
Prevalence of DTCs in BM (%)	15.5	14.9	13.6	20.5	0.230
Follow-up					
Latest follow-up (year)	2008	2005	2008	2007	
Time from diagnosis to end of follow-up–-mean ± SD (in months)	77.3 ± 32.7	100.9 ± 13.7	57.8 ± 30.2	40.0 ± 16.5	<0.001
(median, range)	(89, 9–120)	(102, 43–120)	(57, 9–120)	(37, 11–103)	
Time from BMA to end of follow-up–-mean ± SD (in months)	46.0 ± 23.4	61.1 ± 13.5	30.6 ± 22.3	27.0 ± 16.9	<0.001
(median, range)	(50, 1–92)	(61, 4–86)	(28, 1–92)	(25, 1–77)	

[a]Kruskal–Wallis test.

patients with DTCs compared with patients with no DTC (log-rank test: $P < 0.001$). In the piecewise model, the survival difference was significant only during the first 5-year interval of follow-up (Fig. 1). In the multivariable model, DTC remained an independent indicator of poor prognosis for both survival endpoints during the first 5 years of follow-up (Table 4).

Follow-up BM status in patients with adjuvant therapy

To specifically investigate the prognostic ability of DTCs after adjuvant therapy, patients who had received any kind of adjuvant therapy (endocrine and/or cytotoxic therapy) were selected for separate analysis. In this patient group, DTC was a predictor of poor outcome for all 4 endpoints (*P* values of log-rank test for OS, CSS, and DDFS: <0.001, DFS: 0.002; Fig. 1). In univariate models, the prognostic difference was significant for all endpoints during the first 5 years of follow-up but did not reach statistical significance after 5 years. In multivariable models, DTC remained an independent indicator of poor prognosis for all survival and DFS endpoints during the first 5-year interval of follow-up (Table 5).

Other subgroup analyses

In patients who did not receive adjuvant therapy, outcome was poorer in the group of patients with DTC (incidence rate ratio ranging from 1.60 for DFS to 3.53 for CSS), but the difference was not statistically significant (data not shown). Note that only few events were observed in this subgroup.

Information on vessel invasion was available of 574 patients. Although significant in univariate analysis ($P = 0.018$ for overall and $P = 0.004$ for DFS), vessel invasion was not significant in multivariable analysis, neither in the whole patient sample nor in the subgroup of patients with adjuvant therapy (data not shown).

Discussion

This pooled analysis showed that DTC detected in BM of breast cancer patients during relapse-free follow-up is an independent prognostic factor for adverse patient outcome. The negative prognostic effect was seen in each of the 3 contributing studies alone. There was no statistical heterogeneity between studies. The use of pooled individual patient data, which is acknowledged as a reliable mode to carry out meta-analysis of survival data, allowed to both standardize inclusion criteria and investigate the effect of changing treatments over time on patient outcome. The prognostic impact of persistent DTC was valid for the first 5-year interval of follow-up after primary breast cancer diagnosis. This effect was also observed in patients who underwent BMA at the time of diagnosis (1). These findings, and particularly the fact that the poor prognosis was confirmed in our subgroup of patients with persistent DTC

Table 2. Prevalence of DTC in BM by clinical variables

Variables	All patients ($N = 676$)	Patients with DTC ($N = 105$)	Patients without DTC ($N = 571$)	*P*
Patient age groups—number (%)				0.479
20–35 y	19	2 (10.5)	17 (89.5)	
36–50 y	178	33 (18.5)	145 (81.5)	
51–65 y	373	52 (13.9)	321 (86.1)	
>65 y	106	18 (17.0)	88 (83.0)	
Menopausal status—number (%)				0.959
Premenopausal	256	40 (15.6)	216 (84.4)	
Postmenopausal	420	65 (15.5)	355 (84.5)	
Tumor size—number (%)				0.462
≤0.5 cm (stage pT1a)	59	10 (17.0)	49 (83.0)	
>0.5–1 cm (stage pT1b)	135	24 (17.8)	111 (82.2)	
>1–2 cm (stage pT1c)	294	38 (12.9)	256 (87.1)	
>2 cm (stages pT2–pT4)	179	31 (17.3)	148 (82.7)	
pTx	9			
Tumor grade				0.388
1	125	15 (12.0)	110 (88.0)	
2	363	56 (15.4)	307 (84.6)	
3	138	25 (18.1)	113 (81.9)	
Unknown[b]	50			
Lymph node metastasis—no. (%)				0.502
No metastases (stage pN0)	435	61 (14.0)	374 (86.0)	
1–3 metastases (stage pN1)	142	26 (18.3)	116 (81.7)	
4–9 metastases (stage pN2)	52	7 (13.5)	45 (86.5)	
≥10 metastases (stage pN3)	40	8 (20.0)	32 (80.0)	
pNx	7			
Histological type—no. (%)				0.103[a]
Ductal	460	64 (13.9)	396 (86.1)	
Lobular	132	26 (19.7)	106 (80.3)	
Mixed ductal lobular or other	84	15 (17.9)	69 (82.1)	
Receptor status—no. (%)				0.187
No receptor positive	122	24 (19.7)	98 (80.3)	
Any receptor positive	532	79 (14.8)	453 (85.2)	
Unknown[b]	22			
Vessel invasion—no. (%)				0.225
No invasion	531	75 (14.1)	456 (85.9)	
Invasion	43	9 (20.9)	34 (79.1)	
Unknown[b]	102			
Systemic therapy—no. (%)				0.217
No systemic (neo-)adjuvant therapy	327	44 (13.5)	283 (86.5)	
Endocrine therapy only	149	24 (16.1)	125 (83.9)	
Cytotoxic therapy only	141	23 (16.3)	118 (82.7)	
Combined endocrine-cytotoxic therapy	58	14 (24.1)	44 (75.9)	
Unknown	1			

[a]Comparing ductal carcinoma with lobular carcinoma.

[b]Patients were excluded from multivariable analysis because of missing data; hence, prevalence of positive BM findings is not given.

after adjuvant therapy, indicate that DTC is a marker of disease recurrence and persistent DTC at follow-up may serve as a surrogate marker for treatment failure in the adjuvant setting.

Prevalence of DTC during follow-up was 15.5%, which is overall lower than those 30% reported across studies investigating the DTC prevalence at primary diagnosis (1). Apart from disease stage–related causes, this might

Table 3. BM status by time of aspiration after primary breast cancer diagnosis

Time period after primary diagnosis	All patients	Positive BM findings	Negative BM findings	P^a
4–12 months	95	20 (21.1)	75 (78.9)	
13–24 months	136	22 (16.2)	114 (83.8)	
25–36 months	67	12 (17.9)	55 (82.1)	
37–48 months	344	49 (14.2)	295 (85.8)	
49+ months	34	2 (5.9)	32 (94.1)	0.246
Total	676	105 (15.5)	571 (84.5)	

[a] Fisher's exact test.

be due to a selection bias inherent in this particular study, although patients were required to be free of relapse for enrollment in the individual trials. One might assume that many patients with DTCs at time of primary diagnosis, and thus at high risk of recurrence, may have relapsed before they could be included into this study. This might also explain the lack of association between DTC detected during follow-up and clinical variables, such as advanced

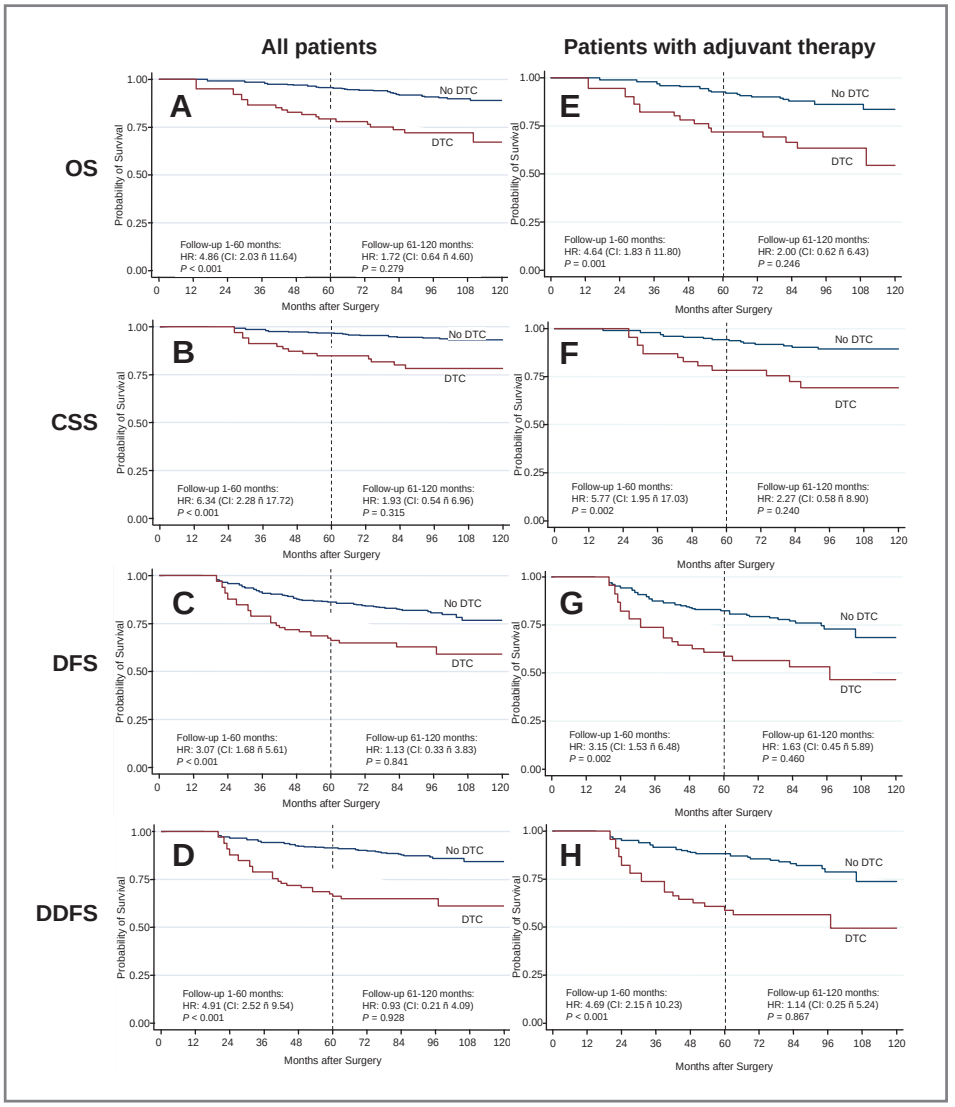

Figure 1. A–H, Kaplan–Meier plots of long-term survival and outcome according to the presence or absence of DTC in BM. Vertical dotted lines indicate the cutoff point at 5 years of follow-up used in the piecewise Cox regression modeling. A–D, all patients in the study. E–H, patients receiving adjuvant systemic treatment.

tumor stage and lymph node involvement. The assumption of early drop out of high risk patients is further supported by the fact that the prevalence of DTC to some extent decreased with increasing time intervals between primary breast cancer diagnosis and BMA during follow-up. On the other hand, from a clinical point of view, it is of value to observe the significant impact of DTC on patient outcome during the first year of follow-up.

Although DTC status at diagnosis is a strong prognostic marker, a substantial number of DTC-positive patient never recur (1). Performing BMA during follow-up could improve the prognostic accuracy, as the fate of patients with DTC presence at diagnosis would be expected to be affected by the DTC status at follow-up. In this study, the BM status of the patients at time of primary diagnosis is not available in the entire patient group for comparison. However, in a previous analysis of the Oslo cohort, patients with the presence of DTC at both diagnosis and follow-up had a very poor prognosis compared with those that turned DTC negative at follow-up (8).

Importantly, the information obtained from molecular analyses of the primary tumor has improved the prognostication and tailoring of adjuvant treatment in early breast cancer (25–27), but no routine method for modification of the treatment exists during follow-up. Our results indicate that detection of persistent DTC can identify patients who are at risk of relapse because of inadequate initial treatment. As repetitive BMAs were not carried out in this study, individual changes in DTC over time and the optimal time point of BMA cannot be estimated. Repeated BMAs over time could also provide insight into the fate of DTC in patients not suffering from relapse. We are also aware of the possibility of a false negative result when performing only 1 BMA. Multiple testing or more sensitive techniques combined with the analysis of DTC markers for malignancy might be able to improve the prognostic accuracy in the future (6).

Even though positive BM status was an indicator of early disease recurrence, far from all patients with DTC-experienced relapse. The presence of dormant persistent tumor cells has been reported as long as 15 years after primary diagnosis in otherwise tumor-free patients. However, the significance and characteristics of these cells remain unclear (28–30). In our study, outcome of patients with persistent DTC and more than 5 years of recurrence-free follow-up was not significantly worsened. Possibly, still larger studies and more events at later follow-up are needed for a better estimate of 10-year survival rates. It may also be of a great interest to extend the research beyond the presence and characteristics of DTC, and consider the stromal factors and their influence on DTC survival and their metastatic outgrowth.

In principle, patients with persistent DTCs during or after adjuvant therapy would be interesting candidates for clinical trials investigating the impact of treatment changes. Studies to properly address the switches in therapeutic regimens as a function of persistent DTC have been initiated or are under way. In the Norwegian SATT study (NBCG9), about 1,100 breast cancer patients receiving a taxane-free, antracycline-containing adjuvant chemotherapy regimen have been analyzed for DTC in BM at 1 year after surgery. Patients with detectable DTCs received taxane-containing chemotherapy as secondary adjuvant treatment and further monitoring of DTC and clinical outcome. Follow-up in this study is still ongoing. Bisphosphonates or endocrine therapy represent interesting candidates for cell-cycle–independent intervention, given the dormant state of persistent DTC in a substantial number of patients (31, 32). In a small pilot study reported by Rack and colleagues, 31 patients with persistent DTCs were treated with zoledronic acid. All patients but 4 were free of DTC 6 months after the end of zoledronate therapy. The reduction in cell numbers between first and second aspiration was statistically significant ($P < 0.0001$; ref. 33). In addition, new biological treatments and newer agents for antiresorptive bone treatment are candidate DTC intervention strategies to be explored in next-generation randomized clinical trials.

Because of the greater feasibility of peripheral blood sampling as compared with BM, many research groups are currently assessing circulating tumor cells (CTC) in clinical studies and have established CTC screening as a monitoring tool in the metastatic setting (34, 35). In contrast to patients with metastatic disease, much less information about the prognostic relevance of CTC in patients with early-stage disease is available (36). Studies using the CellSearch System or reverse transcriptase-PCR–based techniques have reported CTC persistence as an indicator of unfavorable outcome after completion of chemotherapy (37–40). However, available studies comparing CTC and DTC analysis so far show only partially overlapping results, and the prognostic information from DTC seems to be higher than that from CTC (41–43). The difference between CTC and DTC results might be explained by methodological and/or sensitivity-level issues. It is also likely that CTC and DTC provide complementary information. Parallel analysis of CTC and DTC by using the same methodology should be encouraged in future studies.

In conclusion, the results of this study further strengthen the prognostic significance and the clinical impact of DTC in BM of breast cancer patients. Our data imply that there is a clinical potential for monitoring treatment efficacy, which should be further explored in well-designed randomized clinical trials. Among the options for both trial and research hypotheses are the following: (i) utilization of persistent DTCs for adapting adjuvant treatment to modulated individual residual risk; (ii) phenotyping of DTCs for addressing the differential impact of tumor cell biology; and (iii) profiling DTCs for targeted therapy development. In this respect, we see a clinical potential of MRD indicators that by far outperforms that of established parameters currently used in breast cancer diagnosis and treatment.

Table 4. Multivariable HRs for OS and DFS in different time intervals, adjusted for center

	HR (95% CI)	*P* (Wald)	HR (95% CI)	*P* (Wald)
Overall survival	0–5 y follow-up	(*N* = 515)	5–10 y follow-up	(*N* = 367)
DTC positive vs. negative	4.33 (1.65–11.39)	0.003	—	
N stage[a]	2.67 (1.63–4.36)	<0.001*	2.19 (1.43–3.34)	<0.001*
Hormone receptor expression positive vs. negative for any receptor	0.18 (0.07–0.46)	<0.001	—	
Cancer-specific survival	0–5 y follow-up	(*N* = 515)	5–10 y follow-up	(*N* = 367)
DTC positive vs. negative	6.11 (1.97–18.93)	0.002	—	
N stage[a]	2.96 (1.67–5.25)	<0.001*	2.88 (1.70–4.87)	<0.001*
Hormone receptor expression positive vs. negative for any receptor	0.12 (0.04–0.38)	<0.001	—	
Disease-free survival	0–5 y follow-up	(*N* = 513)	5–10 y follow-up	(*N* = 338)
DTC positive vs. negative	2.50 (1.27–4.93)	0.008	—	
N stage[a]	2.17 (1.57–3.00)	<0.001*	1.73 (1.08–2.79)	0.023
Hormone receptor expression positive vs. negative for any receptor	0.28 (0.14–0.53)	<0.001	—	
Age group[b]	—		0.41 (0.23–0.72)	0.002
Distant disease-free survival	0–5 y follow-up	(*N* = 513)	5–10 y follow-up	(*N* = 338)
DTC positive vs. negative	3.45 (1.67–7.10)	0.001	—	
N stage[a]	2.36 (1.64–3.38)	<0.001*	1.73 (1.02–2.94)	0.041
Hormone receptor expression positive vs. negative for any receptor	0.28 (0.14–0.59)	0.001		
Age group[b]	—		0.43 (0.23–0.82)	0.010

[a]N stage: categories of N0, N1, N2, and N3.
[b]Age group: categories of 20–35 y, 36–50 y, 51–65 y, and 66+ y.
*, *P* value for trend test across categories.

Table 5. Multivariable HRs for survival endpoints in patients with adjuvant therapy in different time intervals, adjusted for center

	HR (95% CI)	*P* (Wald)	HR (95% CI)	*P* (Wald)
Overall survival	0–5 y follow-up	(*N* = 265)	5–10 y follow-up	(*N* = 162)
DTC positive vs. negative	3.91 (1.39–10.99)	0.010	—	
N stage[a]	2.68 (1.47–4.90)	0.001*	5.23 (2.30–11.91)	<0.001*
Hormone receptor expression positive vs. negative for any receptor	0.18 (0.06–0.51)	0.001	—	
Age group[b]	—		3.36 (1.25–9.01)	0.016
Cancer-specific survival	0–5 y follow-up	(*N* = 265)	5–10 y follow-up	(*N* = 162)
DTC positive vs. negative	4.62 (1.44–14.85)	0.010	—	
N stage[a]	2.95 (1.48–5.88)	0.002*	3.89 (1.86–8.14)	<0.001*
Hormone receptor expression positive vs. negative for any receptor	0.15 (0.05–0.50)	0.002	—	
Disease-free survival	0–5 y follow-up	(*N* = 264)	5–10 y follow-up**	(*N* = 148)
DTC positive vs. negative	2.37 (1.06–5.32)	0.036	—	
N stage[a]	3.35 (2.05–5.46)	<0.001*	—	
Hormone receptor expression positive vs. negative for any receptor	0.35 (0.15–0.80)	0.012	—	
Distant disease-free survival	0–5 y follow-up	(*N* = 264)	5–10 y follow-up**	(*N* = 148)
DTC positive vs. negative	3.16 (1.35–7.40)	0.008	—	
N stage[a]	2.96 (1.79–4.89)	<0.001*	—	
Hormone receptor expression positive vs. negative for any receptor	0.35 (0.14–0.87)	0.023	—	

[a]N stage: categories of N0, N1, N2, and N3.
[b]Age group: categories of 20–35 y, 36–50 y, 51–65 y, and 66+ y.
*, *P* value for trend test across categories.
**, None of the tested variables were significant for DFS during the interval 5–10 y of follow-up.

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No potential conflicts of interest were disclosed.

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