

## ORIGINAL ARTICLE

# A Pooled Analysis of Bone Marrow Micrometastasis in Breast Cancer

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## ABSTRACT

**BACKGROUND**

We assessed the prognostic significance of the presence of micrometastasis in the bone marrow at the time of diagnosis of breast cancer by means of a pooled analysis.

**METHODS**

We combined individual patient data from nine studies involving 4703 patients with stage I, II, or III breast cancer. We evaluated patient outcomes over a 10-year follow-up period (median, 5.2 years), using a multivariable piecewise Cox regression model.

**RESULTS**

Micrometastasis was detected in 30.6 percent of the patients. As compared with women without bone marrow micrometastasis, patients with bone marrow micrometastasis had larger tumors and tumors with a higher histologic grade and more often had lymph-node metastases and hormone receptor-negative tumors ( $P < 0.001$  for all variables). The presence of micrometastasis was a significant prognostic factor with respect to poor overall survival and breast-cancer-specific survival (univariate mortality ratios, 2.15 and 2.44, respectively;  $P < 0.001$  for both outcomes) and poor disease-free survival and distant-disease-free survival during the 10-year observation period (incidence-rate ratios, 2.13 and 2.33, respectively;  $P < 0.001$  for both outcomes). In the multivariable analysis, micrometastasis was an independent predictor of a poor outcome. In the univariate subgroup analysis, breast-cancer-specific survival among patients with micrometastasis was significantly shortened ( $P < 0.001$  for all comparisons) among those receiving adjuvant endocrine treatment (mortality ratio, 3.22) or cytotoxic therapy (mortality ratio, 2.32) and among patients who had tumors no larger than 2 cm in diameter without lymph-node metastasis and who did not receive systemic adjuvant therapy (mortality ratio, 3.65).

**CONCLUSIONS**

The presence of micrometastasis in the bone marrow at the time of diagnosis of breast cancer is associated with a poor prognosis.

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**D**ATA FROM EXPERIMENTS IN ANIMALS<sup>1</sup> performed in the 1960s and from more recent immunocytochemical<sup>2,3</sup> and molecular<sup>4,5</sup> studies suggest that lymph-node involvement does not accurately predict hematogenous dissemination of cancer cells, nor is hematogenous dissemination necessarily associated with lymph-node involvement.<sup>6,7</sup> During the past two decades, several studies have assessed the prevalence and prognostic value of hematogenous dissemination of tumor cells in patients with node-positive and node-negative breast cancer.<sup>3,8-15</sup> The influence of the presence of micrometastasis in the bone marrow on prognosis has been shown in patients with identical stages of breast cancer, as defined by tumor size, histologic grade, presence or absence of lymph-node metastasis, and expression of hormone receptors.<sup>3,9-13</sup> However, the clinical usefulness of finding such micrometastasis is limited by the low statistical power of published studies and the lack of clinical trials specifically investigating the predictive role of bone marrow micrometastasis. To date, only two small studies have reported the outcome of patients with bone marrow micrometastasis<sup>10,12</sup> well beyond a median observation time of five years. In this study, we investigated the long-term outcome of patients with and those without bone marrow micrometastasis. We also explored the effect of bone marrow micrometastasis on prognosis in clinically relevant subgroups. To accomplish these goals, we analyzed pooled data from nine independent studies with updated follow-up data and numbers of patients; these studies involved 4703 patients with stage I, II, or III breast cancer who were treated in Augsburg and Munich, Germany (two independent studies that were initially published together),<sup>11</sup> Paris,<sup>8</sup> Oslo,<sup>9</sup> Rostock, Germany,<sup>3</sup> New York,<sup>15</sup> Erlangen, Germany,<sup>10</sup> Heidelberg, Germany,<sup>13</sup> and London.<sup>12</sup> (These studies are referred to hereinafter by the names of the cities.)

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## METHODS

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### DATA COLLECTION

The National Library of Medicine of the National Center for Biotechnology Information ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) was searched for studies related to bone marrow micrometastasis and survival of patients with breast cancer. Six such studies were identified.<sup>3,10-13</sup> Furthermore, on the basis of personal contact, we knew of three studies that were in

the process of manuscript preparation<sup>15</sup> or submission<sup>8,9</sup> at the time we were collecting our data. We defined eligible patients as women with complete baseline clinical records, data on follow-up examinations, and histologically confirmed and completely removed primary stage I, II, or III breast cancer, with information on tumor size and the presence or absence of axillary lymph-node metastasis. We further required documented validation of the immunoassays used to detect micrometastasis. Patients were excluded from the analysis if they had in situ carcinomas only, if they had either distant metastases or local recurrence within 3 months after diagnosis, or both, or if the duration of follow-up was less than 12 months at the time of data collection.

We asked the principal investigators of the nine studies to submit the original data collected for each patient.<sup>3,8-13,15</sup> Owing to differences in criteria for inclusion and exclusion, the numbers of patients in the original publications may differ from those reported here. Survival results differ if the follow-up information was updated after publication.

We asked the collaborating groups to code data that had been rendered anonymous in a standardized fashion for inclusion in a database. In a signed letter, all principal investigators stated that local institutional review boards had agreed to the bone marrow–aspiration procedure and the study and that all patients whose data were submitted had agreed to bone marrow aspiration and statistical analysis in accordance with international regulations regarding data safety.

### BONE MARROW ASPIRATION AND IMMUNOCYTOCHEMISTRY

The criteria for designating a case positive for the presence of micrometastasis and the details of the immunocytochemical assays used by the contributing groups have been described in detail elsewhere.<sup>3,8-16</sup>

### STATISTICAL ANALYSIS

We tested for associations between the presence of bone marrow micrometastasis and the baseline characteristics of patients, as well as established prognostic factors, using the chi-square test. Categorical variables with more than two categories were analyzed for trend.

Hazard ratios and 95 percent confidence intervals for recurrence or death with micrometastasis as the sole variable were calculated for each of the

**Table 1. Baseline Characteristics of the Patients and Study Variables, According to Study Center (City) and Technical Variables.\***

Variable	Cytokeratin-Based Detection Assay						Mucin-Based Detection Assay			
	Augsburg	Munich	Paris	Oslo	Rostock	New York	Erlangen	Heidelberg	London	
<b>Patient characteristic</b>										
Patients with breast cancer										
No.	917	431	270	758	507	371	410	721	318	
Mean ( $\pm$ SD) age at diagnosis (yr)	58 $\pm$ 13	56 $\pm$ 12	53 $\pm$ 11	58 $\pm$ 12	55 $\pm$ 13	54 $\pm$ 13	54 $\pm$ 12	53 $\pm$ 12	58 $\pm$ 12	
Enrollment period	1993–2000	1994–2002	1998–2001	1995–1998	1985–1998	1985–1991	1988–1994	1985–1994	1980–1986	
<b>Bone marrow micrometastasis</b>										
Prevalence (%)	32.4	21.4	38.2	12.4	32.2	33.4	42.4	43.3	24.8	
Median no. detected (range)†	3 (1–1223)‡	3 (1–1223)‡	3 (1–400)	1 (1–8525)	3 (1–300)	NA	NA	NA	NA	
Follow-up (mo)										
Maximum	98	102	49	85	143	147	160	128	120	
Median	52	55	35	54	41	94	120	73	178	
No. of control subjects without cancer	221‡	221‡	NA	98	17	45	20	21	NA	
Positive immunostaining (%)	1	1	NA	4	0	0	0	0	NA	
<b>Study variables</b>										
No. of aspiration sites	2	2	2	4	2	2–3	3	2	8	
Method of slide preparation	Cytospin	Cytospin	Cytospin	Cytospin	Smear	Cytospin and smear	Smear	Smear	Smear	
No. of cells screened per patient	2 $\times$ 10 <sup>6</sup>	2 $\times$ 10 <sup>6</sup>	3 $\times$ 10 <sup>6</sup>	2 $\times$ 10 <sup>6</sup>	2–4 $\times$ 10 <sup>6</sup>	1 $\times$ 10 <sup>6</sup>	25 Smears	4 Smears, each with 10 <sup>6</sup> cells	10–25 Smears	
Target antigen	Cytokeratin	Cytokeratin	Cytokeratin	Cytokeratin	Cytokeratin	Cytokeratin	EMA, cytokeratin	TAG-12	EMA	
Detection antibody (clone)	A45-B/B3	A45-B/B3	A45-B/B3	AE1/AE3	5D3	AE1/AE3	E29, 5D3	2E11	E29	
Labeling system	APAAP	APAAP	APAAP	APAAP	ABC-POX	APAAP	Indirect AP	ABC-AP	Indirect AP	

\* Data for the studies (identified by the cities where patients were treated) came from the following: data for Augsburg and Munich from Braun et al.,<sup>11</sup> data for Paris from Pierga et al.,<sup>8</sup> data for Oslo from Wiedswang et al.,<sup>9</sup> data for Rostock from Gerber et al.,<sup>3</sup> data for New York from Wong et al.,<sup>15</sup> data for Erlangen from Gebauer et al.,<sup>10</sup> data for Heidelberg from Diel et al.,<sup>13</sup> and data for London from Mansi et al.<sup>12</sup> Differences between the published data and those shown in the table are due to updating of information for the pooled analysis. NA denotes not assessed, EMA epithelial membrane antigen, TAG-12 tumor-associated glycoprotein-12, APAAP alkaline phosphatase–antialkaline phosphatase, ABC-POX avidin–biotin complex–peroxidase, and AP alkaline phosphatase.

† The values shown are the median numbers of cells detected among all patients with bone marrow micrometastasis (ranges are shown in parentheses).

‡ A combined protocol was used in a study of 552 patients with breast cancer<sup>11</sup> and 221 controls without cancer.

nine studies by means of meta-analysis (with the use of the random-effects model based on individual patient data). The Q-test was performed to assess interstudy heterogeneity.<sup>17</sup> For the sensitivity analysis, meta-analytic hazard ratios and confidence intervals were computed with the omission of one study at a time.

For the survival analysis, we considered in separate analyses the following primary end points: death due to any cause; death due to causes related to breast cancer (i.e., metastasis-dependent organ failure or progression of breast cancer); distant or local disease recurrence, or both; and distant metastasis. Survival intervals were measured from the time of surgery and bone marrow aspiration to the time of death or of the first clinical or radiographic evidence of disease recurrence. Incidence rates and mortality were calculated as the number of disease recurrences or deaths per 1000 person-years; mortality ratios, incidence-rate ratios, and 95 percent confidence intervals were estimated.

For patients surviving 10 years or more (412 patients), the follow-up data were censored after 120 months. Data for women in whom the envisaged end point was not reached were censored as of the last follow-up. We constructed Kaplan–Meier curves<sup>18</sup> and used the log-rank test<sup>19</sup> to determine the univariate significance of the study variables.

We used a Cox proportional-hazards regression model to examine simultaneously the effects of multiple covariates on survival.<sup>20</sup> In all models, the categorical variables were tested for trend and the proportional-hazards assumption was assessed. 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 performed. The effect of each variable in these models was assessed with the use of the Wald test and described by the hazard ratio, with a 95 percent confidence interval. All estimates were stratified according to study center, and all reported P values are two-sided.

The initial model included age at diagnosis, menopausal status, tumor size and grade, and information on lymph-node metastases as well as hormone-receptor expression. Since progesterone-receptor expression was not routinely assessed in all participating centers, a binary variable was created to indicate that at least one hormone receptor was positive. Subjects with missing values for this hormone-receptor variable or for tumor grade were excluded from modeling. The final model was de-

veloped by dropping each variable in turn from the model and conducting a likelihood-ratio test to compare the full and the nested models. We used a significance level of 0.05 as the cutoff to exclude a variable from the model. Finally, the variable of bone marrow micrometastasis (present vs. absent) was added to the model in order to test the resultant model against that without the variable.

On the basis of the observation that curves on the Kaplan–Meier graphs dispersed during the first years of follow-up and then showed less divergence, the assumption of proportional hazards for the final model was not met over the entire follow-up period. We therefore opted for a piecewise Cox model,<sup>21</sup> with a cutoff point set at five years for overall survival and breast-cancer-specific survival and at four years for disease-free survival and distant-disease-free survival. We fit separate Cox models for both the first and second intervals. The proportional-hazards assumption was formally tested for each interval,<sup>22</sup> and separate regression estimates are given.

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## RESULTS

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### PREVALENCE OF BONE MARROW MICROMETASTASIS

Table 1 gives a summary of the original studies, the patients enrolled in them, and the technical variables used in the studies. A total of 4703 patients with invasive breast cancer were included in our analysis. Bone marrow micrometastasis was present in 1438 patients (30.6 percent). As compared with women without bone marrow micrometastasis, patients with bone marrow micrometastasis had larger tumors, tumors with a higher histologic grade, more frequent lymph-node metastasis, and more hormone-receptor-negative tumors (Table 2).

### SENSITIVITY ANALYSIS

In the meta-analysis, using a random-effects model, we found a hazard ratio of 2.26 (95 percent confidence interval, 1.72 to 2.97;  $P < 0.001$ ) for death from any cause and for any disease recurrence associated with the presence of micrometastasis. For these survival end points, the hazard ratios calculated in eight studies ranged from 1.36 to 4.04 and from 1.23 to 3.16, respectively; in the ninth study (Paris), the hazard ratios were 8.58 and 8.23, respectively. For each outcome, the 95 percent confidence intervals were significant in all but two studies (Munich and New York) and showed considerable

**Table 2. Prevalence of Bone Marrow Micrometastasis According to Clinical Variables.**

Variable	All Patients (N=4703)	Patients with Bone Marrow Micrometastasis (N=1438)	Patients without Bone Marrow Micrometastasis (N=3265)	P Value
Age — no. (%)				0.001*
20–35 yr	224	78 (34.8)	146 (65.2)	
36–50 yr	1454	484 (33.3)	970 (66.7)	
51–65 yr	1980	585 (29.5)	1395 (70.5)	
>65 yr	1045	291 (27.8)	754 (72.2)	
Menopausal status — no. (%)				0.02
Premenopausal	1579	517 (32.7)	1062 (67.3)	
Postmenopausal	3124	921 (29.5)	2203 (70.5)	
Tumor size — no. (%)				<0.001*
<2 cm (stage pT1a, b, or c)	2507	633 (25.2)	1874 (74.8)	
2–5 cm (stage pT2)	1706	568 (33.3)	1138 (66.7)	
>5 cm (stage pT3)	263	100 (38.0)	163 (62.0)	
Infiltration of skin or chest wall (stage pT4a, b, c, or d)	227	137 (60.4)	90 (39.6)	
Tumor grade				<0.001*
1	693	156 (22.5)	537 (77.5)	
2	2141	641 (29.9)	1500 (70.1)	
3	1462	504 (34.5)	958 (65.5)	
Unknown†	407			
Lymph-node metastasis — no. (%)				0.001*
No metastasis (stage pN0)	2725	719 (26.4)	2006 (73.6)	
1–3 metastases (stage pN1)	1101	330 (30.0)	771 (70.0)	
4–9 metastases (stage pN2)	469	185 (39.4)	284 (60.6)	
≥10 metastases (stage pN3)	408	204 (50.0)	204 (50.0)	
Receptor status — no. (%)				0.003
No receptor positive	923	318 (34.5)	605 (65.5)	
Any receptor positive	3326	979 (29.4)	2347 (70.6)	
Unknown†	454			
Histologic type — no. (%)‡				0.08
Ductal	3605	1108 (30.7)	2497 (69.3)	
Lobular	646	203 (31.4)	443 (68.6)	
Mixed ductal–lobular	116	33 (28.4)	83 (71.6)	
Inflammatory	46	22 (47.8)	24 (52.2)	
Other	290			
Predefined patient subgroups — no. (%)				
Patients with stage pT1N0 and no systemic adjuvant therapy	1036	229 (22.1)	807 (77.9)	
Remaining patients	3667	1209 (33.0)	2458 (67.0)	<0.001
Hormonal therapy only	1499	445 (29.7)	1054 (70.3)	
Cytotoxic therapy only	1596	611 (38.3)	985 (61.7)	
Combined hormonal–cytotoxic therapy	119	19 (16.0)	100 (84.0)	
No therapy	1489	363 (24.4)	1126 (75.6)	<0.001

\* The P value is for trend.

† Patients with missing data were excluded from the multivariable analysis.

‡ P=0.013 for the comparison of inflammatory histologic type with ductal or lobular histologic type or with mixed ductal–lobular histologic type.

overlap, indicating a similar effect of micrometastasis on outcome in all nine studies. The Q-test for statistical heterogeneity showed significant interstudy variation among the estimated hazard ratios ( $P=0.007$  for death from any cause;  $P<0.001$  for disease recurrence), which was further investigated by sensitivity analysis. The exclusion of any one study did not markedly change the estimates of the hazard ratios or confidence intervals found in the meta-analysis (for details, see the Supplementary Appendix, available with the full text of this article at [www.nejm.org](http://www.nejm.org)). However, we found that the large Heidelberg study (hazard ratio, 4.04) had the most influence on the outcome of death from any cause. The omission of this study resulted in a marginally lower but still significant hazard ratio (2.02; 95 percent confidence interval, 1.62 to 2.77;  $P=0.18$  according to the Q-test) for death from any cause.

#### SURVIVAL

In the pooled data, the median follow-up time among survivors was 62 months. Of 889 patients who died during follow-up, 667 (75.0 percent) died from breast cancer and 222 (25.0 percent) from other causes; 76.9 percent of all deaths occurred during the first five years. Both the overall rate of death and the rate of death from breast cancer among patients with micrometastasis were significantly higher than the rate of death among patients without micrometastasis in bone marrow (Fig. 1A and 1B). The presence of micrometastasis remained a significant prognostic factor with respect to survival when we controlled for tumor size, grade, lymph-node metastasis, and hormone-receptor expression in the multivariable analysis. In the piecewise multivariable analysis, hazard ratios for death from any cause and death from breast cancer among patients with micrometastasis, as compared with those among patients without micrometastasis, were significantly increased during the first five years of follow-up and thereafter (Table 3).

#### RECURRENCE OF DISEASE

During the follow-up period, breast cancer recurred in 1192 patients (25.3 percent). Of these, 969 patients (81.3 percent) had a recurrence only in the form of distant disease, whereas 447 patients (37.5 percent) had a local relapse (in the breast or the chest wall) or a recurrence in regional lymph nodes (alone or in combination with distant metastases); 80.9 percent of all recurrences occurred within the first four years. Both the disease-free in-

terval and the distant-disease-free interval (Fig. 1C and 1D) were significantly shorter among patients with micrometastasis ( $P<0.001$  for all comparisons, by the log-rank test); for these two end points, piecewise multivariable Cox regression modeling showed that the presence of micrometastasis was a significant predictor of recurrence only during the first four years of follow-up (Table 3).

#### SUBGROUP ANALYSES

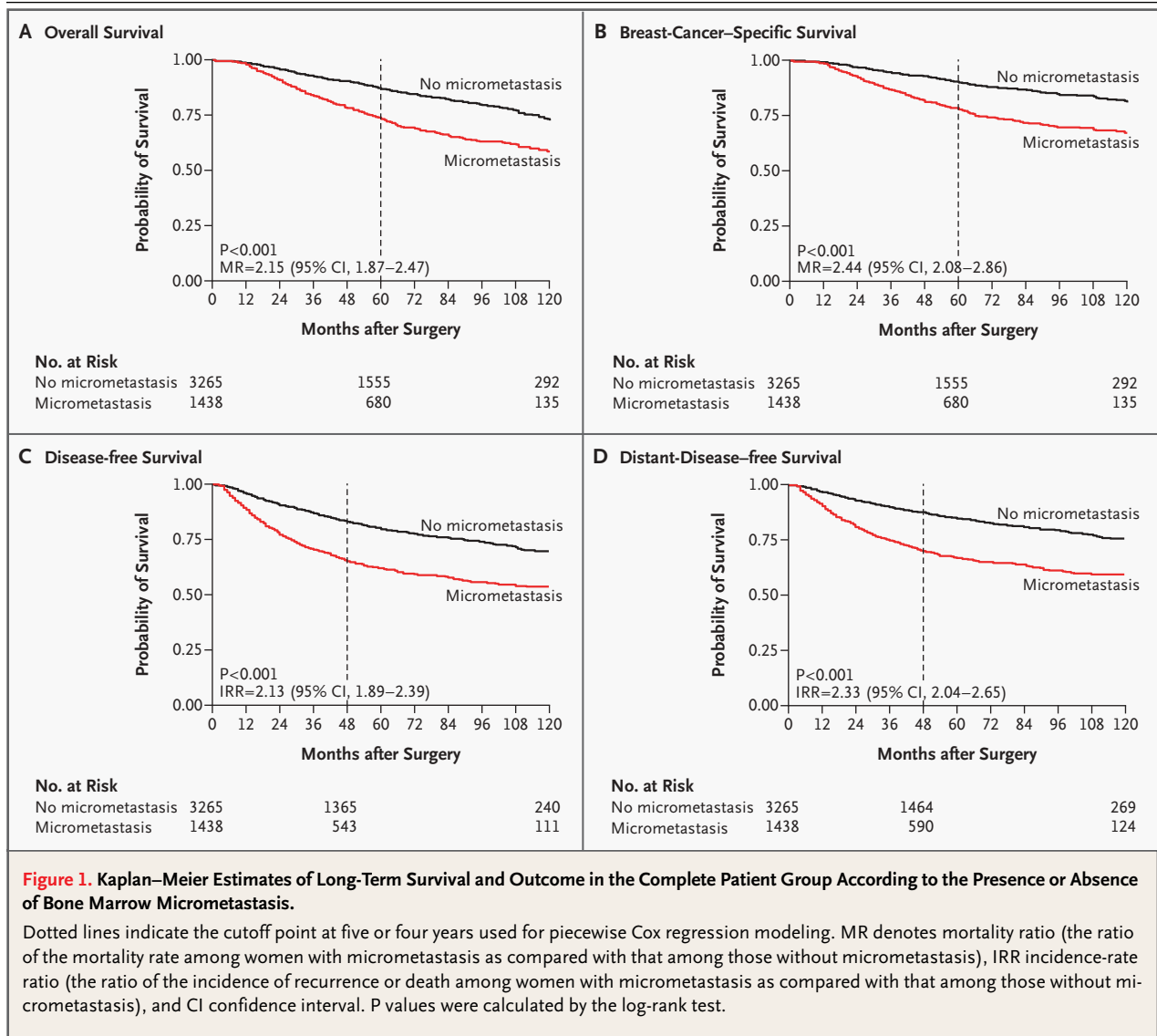
We analyzed subgroups of patients who had received endocrine treatment alone or chemotherapy alone and patients considered to be at low risk who had tumors no larger than 2 cm (pT1N0) and no lymph-node metastasis who did not receive systemic adjuvant therapy. Patients in the endocrine-therapy and chemotherapy subgroups had significantly poorer outcomes for all investigated end points if micrometastasis was present, as compared with patients in these subgroups in whom micrometastasis was absent (Fig. 2). Remarkably, among 1036 patients in the low-risk subgroup, the presence of micrometastasis was associated with an increase by a factor of 3.65 (95 percent confidence interval, 1.94 to 6.89;  $P<0.001$ ) in mortality from breast cancer and a factor of 2.00 (95 percent confidence interval, 1.20 to 3.35;  $P=0.007$ ) in the risk of distant metastasis during the first five years, as compared with patients in whom micrometastasis in the bone marrow was absent (Fig. 2).

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#### DISCUSSION

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This pooled analysis of data on 4703 patients with breast cancer who were enrolled in nine clinical studies found strong evidence of the independent, adverse prognostic significance of the presence of bone marrow micrometastasis at the time of the initial diagnosis of operable breast cancer. Interstudy heterogeneity was influenced by a single large study, but it introduced no significant bias with respect to overall survival or disease-free survival. Further sources of heterogeneity were differences in patients' characteristics and in the immunoassays used to detect micrometastasis. Stratification according to center and the inclusion of patients' characteristics in the regression models took these sources of heterogeneity into account. Variability in treatment over time was overcome by conducting a pooled analysis of data on individual patients. The use of these data allowed us to standardize inclusion and exclusion criteria and to update the



numbers of patients and follow-up information after the appearance of the original published reports. Others have suggested that the ideal way to perform a meta-analysis of survival data is to use individual patient data.<sup>23,24</sup>

In the multivariable analysis, the presence of micrometastasis was associated with the highest estimates of relative risk for each end point during the first follow-up interval of five years (for death from any cause and death from breast cancer) and four years (disease recurrence and distant metastasis) (Table 3). A plausible explanation for the failure to demonstrate a significant association between micrometastasis and recurrence or distant metastasis during the second interval (i.e., years 5 to 10

of follow-up) is that the presence of micrometastasis is associated with the recurrence of breast cancer before the second interval of follow-up, thereby selecting out patients at risk for recurrence during the second interval.

Not all bone marrow cells that stain with an anticytokeratin antibody or with antibodies against polymorphic epithelial mucins (the technical definition of micrometastatic cells) can be unequivocally or uncritically defined as malignant.<sup>6,7</sup> Convincing molecular data, however, point to numerous signs of malignancy in cytokeratin-positive cells.<sup>5,25–27</sup>

We did not identify a subgroup of patients in whom micrometastasis appeared to be prognos-

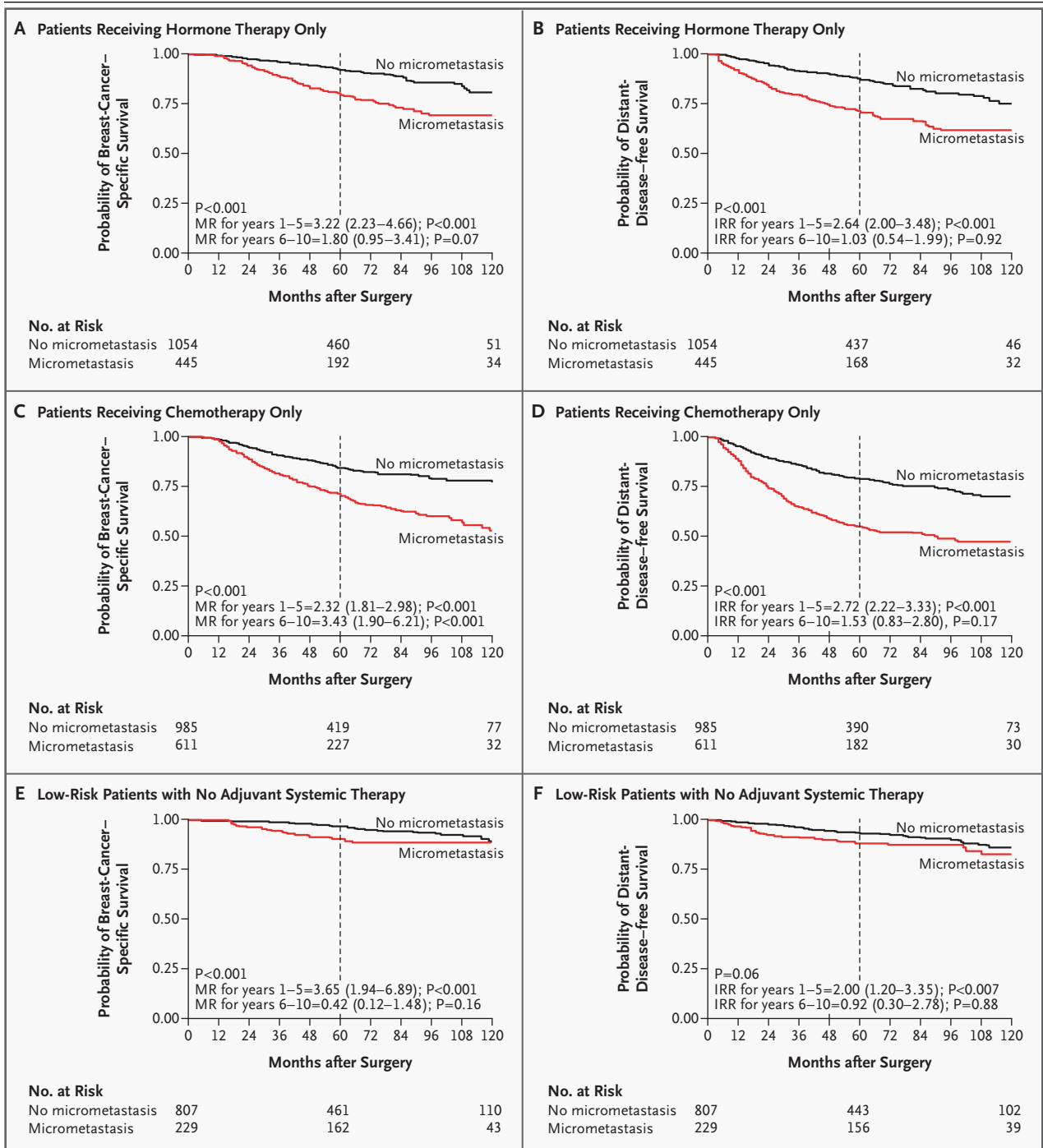
**Table 3. Multivariable Hazard Ratios for Death from Any Cause, Death from Breast Cancer, Disease Recurrence, and Distant Metastasis at Different Follow-up Intervals (Adjusted for the Study Center).\***

Outcome	Follow-up Interval Yr 0–5 (N=3974)		Follow-up Interval Yr 6–10 (N=1674)	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
<b>Death from any cause</b>				
Bone marrow micrometastasis (positive vs. negative)	1.81 (1.51–2.16)	<0.001	1.58 (1.12–2.22)	0.009
Tumor size (T1 vs. T2 vs. T3 and T4)	1.70 (1.50–1.94)†	<0.001	—	
Lymph-node metastasis (N0 vs. N1 vs. N2 vs. N3)	1.63 (1.50–1.77)†	<0.001	1.88 (1.61–2.21)†	<0.001
Tumor grade (G3 vs. G1 and G2)	1.72 (1.44–2.06)	<0.001	—	
Hormone-receptor expression (positive vs. negative for any receptor)	0.56 (0.47–0.68)	<0.001	—	
<b>Death from breast cancer</b>				
Bone marrow micrometastasis (positive vs. negative)	1.93 (1.58–2.36)	<0.001	1.63 (1.07–2.47)	0.02
Tumor size (T1 vs. T2 vs. T3 and T4)	1.67 (1.44–1.94)†	<0.001	—	
Lymph-node metastasis (N0 vs. N1 vs. N2 vs. N3)	1.71 (1.55–1.89)†	<0.001	1.98 (1.64–2.40)†	<0.001
Tumor grade (G3 vs. G1 and G2)	1.75 (1.43–2.15)	<0.001	—	
Hormone-receptor expression (positive vs. negative)	0.50 (0.41–0.61)	<0.001	—	
<b>Disease recurrence</b>				
Bone marrow micrometastasis (positive vs. negative)	1.85 (1.59–2.14)	<0.001	—	
Tumor size (T1 vs. T2 vs. T3 and T4)	1.52 (1.37–1.70)†	<0.001	1.38 (1.09–1.74)†	0.008
Lymph-node metastasis (N0 vs. N1 vs. N2 vs. N3)	1.62 (1.50–1.74)†	<0.001	—‡	
Tumor grade (G3 vs. G1 and G2)	1.53 (1.32–1.78)	<0.001	—	
Hormone-receptor expression (positive vs. negative)	0.61 (0.52–0.71)	<0.001	—	
<b>Distant metastasis</b>				
Bone marrow micrometastasis (positive vs. negative)	2.03 (1.72–2.39)	<0.001	—	
Tumor size (T1 vs. T2 vs. T3 and T4)	1.62 (1.43–1.82)†	<0.001	1.34 (1.06–1.70)†	0.02
Lymph-node metastasis (N0 vs. N1 vs. N2 vs. N3)	1.70 (1.57–1.84)†	<0.001	1.78 (1.52–2.07)†	<0.001
Tumor grade (G3 vs. G1 and G2)	1.65 (1.40–1.94)	<0.001	1.43 (1.02–1.99)	0.04
Hormone-receptor expression (positive vs. negative)	0.64 (0.54–0.76)	<0.001	—	

\* CI denotes confidence interval. A separate model was fit for the second follow-up interval; dashes indicate that no risk estimates are available for variables that dropped from the final model according to the selection process.

† The hazard ratio represents the linear trend across categories.

‡ No linear trend was observed across categories; “lymph-node metastasis” was an independent prognostic factor only for patients with N2 and N3 disease with hazard ratios of 2.01 (95 percent CI, 1.28 to 3.18) and 3.30 (95 percent CI, 2.04 to 5.34), but not for patients with N1 disease (hazard ratio, 0.85; 95 percent CI, 0.57 to 1.28) as compared with patients with N0 disease; separate categories therefore improved the fit of the model.



**Figure 2.** Kaplan–Meier Estimates of Breast-Cancer–Specific and Distant-Disease–free Survival among Predefined Patient Subgroups According to the Presence or Absence of Bone Marrow Micrometastasis.

Dotted lines indicate the cutoff point at five or four years used for piecewise Cox regression modeling. MR denotes mortality ratio (the ratio of the mortality rate among women with micrometastasis as compared with that among those without micrometastasis), IRR incidence-rate ratio (the ratio of the incidence of recurrence or death among women with micrometastasis as compared with that among those without micrometastasis), and CI confidence interval. P values were calculated by the log-rank test.

tically irrelevant. The data presented here may therefore help in planning clinical trials aimed at determining whether the presence or absence of micrometastasis suffices for a decision on the need for therapy and to predict the outcome of treatment in certain subgroups. In our study, the group of 807 patients with tumors no larger than 2 cm in diameter and without lymph-node metastasis, who had no detectable micrometastasis and who did not receive systemic adjuvant treatment, had a 94 percent five-year survival (Fig. 2) and might be considered cured. Treatment stratification based on the presence or absence of micrometastasis may

therefore be useful in trials of systemic adjuvant therapy in patients with pT1N0 tumors and bone marrow micrometastasis.

In summary, our data support the prognostic value of the presence of bone marrow micrometastasis and could be useful in the design of trials of the adjuvant treatment of breast cancer.

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#### APPENDIX

In addition to the authors, the following investigators, as members of the Pooled Analysis Study Group, contributed to this study: *Ullevål University Hospital, Oslo* — R. Kåresen; *Norwegian Radium Hospital, Oslo* — E. Borgen, J. Nesland, G. Kvalheim; *Central Hospital, Augsburg, Germany* — A. Wischnik, D. Steinfeld, P. Mueller, C. Schulz; *Ludwig-Maximilians University, Munich, Germany* — K. Friese, A. Krause, H. Sommer, B. Rack; *City Hospital, Duesseldorf, Germany* — W. Jaeger; *Cornell Medical Center, New York* — C. Potter; *St. George's Hospital, London* — J. Mansi; *Institute of Cancer Research, Sutton, United Kingdom* — J. Homewood; *Institut Curie, Paris* — H. Magdalénat, J.-P. Thiery.

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