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Papers

Bone Marrow Micrometastases in Primary Breast Cancer: Prognostic Significance After 6 Years' Follow-up

J.L. Mansi, D. Easton, U. Berger, J.-C. Gazet, H.T. Ford, D. Dearnaley and R.C. Coombes

Using an antiserum to epithelial membrane antigen we have screened multiple bone marrow aspirates from 350 patients with primary breast cancer taken at the time of initial surgery. 89 (25%) patients were found to have micrometastases and their presence was related to pathological size ($P < 0.01$), the presence of peritumoral vascular invasion ($P < 0.001$), and positive lymph nodes ($P < 0.005$) but not menopausal status. At a median follow-up of 76 months (range 34–108) 107 patients had relapsed with distant metastases. 48% (43 of 89) of these patients had micrometastases initially compared with 25% (64 of 261) who did not ($P < 0.005$). The test predicts for relapse in bone ($P < 0.01$) and other distant sites excluding bone ($P < 0.001$) and is associated with a shorter overall survival ($P < 0.005$). We conclude that the detection of micrometastases signals a high likelihood of early relapse and decreased survival in breast cancer.
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INTRODUCTION

DESPITE APPARENTLY curative surgery, over 50% of women with breast cancer relapse and die within 5 years of diagnosis. Our earlier publications were aimed at discovering whether multiple bone marrow aspirates taken at the time of surgery were a worthwhile technique in the estimation of prognosis [1, 2] and we are now presenting the long-term results.

PATIENTS AND METHODS

Patients

From July 1981 to August 1986 350 patients, median age 59 years, (26–85 years) with primary breast cancer had multiple marrow aspirates taken under general anaesthetic just prior to initial surgery. Each patient was screened for metastatic disease as described previously [2]. Surgery consisted of either mastectomy, wide local excision or excision biopsy depending on the size and site of the tumour and the patient's age. Oestrogen receptor status was assessed on the original tumour by the dextran-coated charcoal method and considered to be positive if it contained $\geq$15fmol/mg cytosol protein. Pathological size, histological subtype, the presence or absence of peritumoral vascular invasion, and lymph node status were assessed for each patient. Adjuvant therapy was given to 159 patients according to various treatment protocols.

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Preparation, staining and analysis

Approximately 16 ml bone marrow was taken from a total of eight sites and prepared and stained as described in detail by Dearnaley *et al.* [3] using anti-epithelial membrane antigen (anti-EMA) [4]. Cells were considered to be tumour cells provided they could be identified as such morphologically.

Follow-up

Patients were reviewed at 3-monthly intervals and had a clinical examination, together with routine blood tests including liver function tests and chest X-ray. A bone scan was done at 6-monthly intervals for 3 years and then annually. Liver ultrasound was done if the liver function tests were abnormal or hepatomegaly was noted. A skeletal survey was done if the bone scan was abnormal or the patient complained of bone pain. At relapse (defined according to criteria of the International Union Against Cancer [UICC]) [5] all patients were fully staged to detect disease at other sites [6].

Statistical analysis

χ^2 tests were used to compare the presence or absence of micrometastases with other prognostic variables. Standard survival analyses [7] were used to determine the effect of micrometastases on the relapse-free interval and overall survival. Cox regression analysis was used to assess the effect of micrometastases on survival and relapse free survival allowing for other prognostic factors.

RESULTS

Bone marrow micrometastases were found in 89 (25%) patients with primary breast cancer at the time of initial surgery. The presence or absence of micrometastases in relation to various prognostic factors is shown in Table 1. At a median follow-up

Table 1. Relation of micrometastases to prognostic factors

	Total	Micrometastases Present	Micrometastases Absent	P (χ^2)
T stage				
T0–T2	254	48 (19)	206 (81)	
				0.001
T3–T4	96	41 (43)	55 (57)	(15.216)
Pathological size (cm)				
<2	108	16 (15)	92 (85)	
2–5	204	63 (31)	141 (69)	0.01 (trend)
>5	15	4 (27)	11 (73)	(6.724)
Missing	23	6	17	
Nodal status				
−ve	170	32 (19)	138 (81)	
+ve	150	49 (33)	101 (67)	0.005 (8.052)
Not done	30	8	22	
Vascular invasion				
Not seen	208	33 (16)	175 (84)	
				0.001
+ve	142	56 (39)	86 (61)	(13.046)
Oestrogen receptor				
−ve	108	36 (33)	72 (67)	
+ve	224	51 (23)	173 (77)	0.041 (4.194)
Not done	18	2	16	
Menopausal status				
Pre	92	25 (27)	67 (73)	
				0.655
Post	258	64 (25)	194 (75)	(0.2)

No. (%).

Table 2. Cox regression analysis of time to first metastasis

Level	Coefficient	S.E.	Hazard ratio (95% CL)
Pathological size			
<2 cm	—	—	1.0
2–5 cm	0.66	0.27	1.94 (1.14–3.28)
5 cm+	1.09	0.38	2.96 (1.41–6.26)
Lymph nodes			
Negative	—	—	1.0
Positive	0.90	0.25	2.45 (1.51–4.01)
Vascular invasion			
Absent	—	—	1.0
Present	0.70	0.24	2.01 (1.26–3.22)
Micrometastases			
Absent	—	—	1.0
Present	0.25	0.23	1.28 (0.81–2.02)

50 individuals with no information on pathological size or lymph node involvement are excluded from this analysis (which is therefore based on 91 relapses in 300 individuals).
Test for effect of micrometastases after adjusting for other factors (based on 2 × log likelihood difference): $\chi^2 = 0.63$.

of 76 (range: 34–108) months there were 140 relapses of which 107 were at distant sites. Of the 89 patients with micrometastases, 43 (48%) relapsed compared with only 64 (25%) of the 261 patients without micrometastases (log rank $P < 0.005$) (Fig. 1).

Of particular interest is that 15 of 32 (47%) patients with negative lymph nodes but a positive bone marrow relapsed compared with only 29 of 138 (21%) of patients with both negative lymph nodes and bone marrow.

Patients with micrometastases had a higher risk of relapse in bone ($P < 0.01$) and of relapse in distant sites other than bone ($P < 0.005$). The difference in relapse-free survival was also observed both in patients who received adjuvant therapy ($P < 0.005$) and those who did not ($P < 0.025$).

Cox regression analysis of time to first metastasis is shown in Table 2. After allowing for pathological size, lymph nodes and vascular invasion, the effect of micrometastases was reduced and was no longer significant (hazard ratio 1.28, compared with 2.04 when considered as a single factor). Lymph node positivity, pathological size and the presence of peritumoral vascular invasion were all stronger risk factors for relapse.

There were 92 deaths, 37 occurring in the patients with micrometastases (log rank $P < 0.005$) (Fig. 2). Again, after allowing for other prognostic factors using Cox regression analysis, the effect of micrometastases as an independent factor was reduced (hazard ratio 1.62, compared with 1.98 as a single factor) (Table 3).

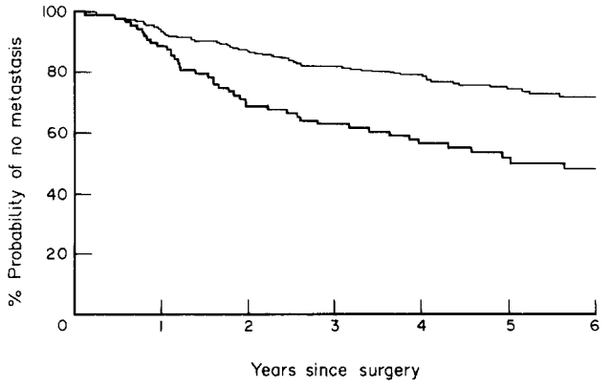

Fig. 1. Disease-free survival according to presence or absence of micrometastases at time of initial surgery ($P < 0.005$). lower trace = micrometastases, upper trace = no micrometastases.

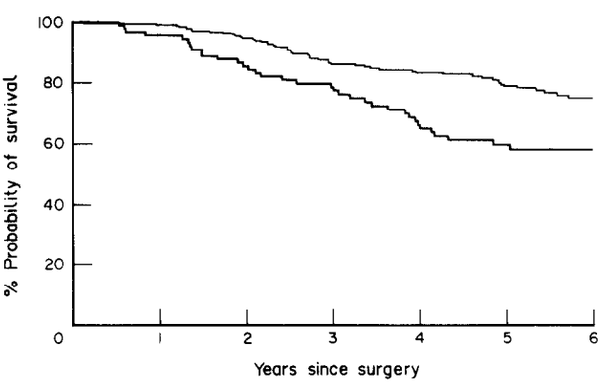

Fig. 2. Overall survival according to presence or absence of micrometastases at time of initial surgery ($P < 0.005$). lower trace = micrometastases, upper trace = no micrometastases.

Table 3. Cox regression analysis of survival

Level	Coefficient	S.E.	Hazard ratio (95% CL)
Pathological size			
<2 cm	—	—	1.0
2–5 cm	0.80	0.46	2.23 (0.90–5.48)
5 cm+	1.01	0.66	2.74 (0.75–10.01)
Lymph nodes			
Negative	—	—	1.0
Positive	1.05	0.43	2.85 (1.23–1.89)
Vascular invasion			
Absent	—	—	1.0
Present	0.91	0.41	2.48 (1.11–5.55)
Micrometastases			
Absent	—	—	1.0
Present	0.48	0.37	1.62 (0.78–3.34)

50 individuals with no information on pathological size or lymph node involvement are excluded from this analysis (which is therefore based on 74 deaths in 300 individuals).

There was no effect of the presence of micrometastases at presentation on overall survival once relapse had occurred ($P > 0.1$).

DISCUSSION

The long-term follow-up of those patients with micrometastases confirms our earlier findings, namely that their presence predicts for a shorter disease-free interval and overall survival.

Other centres have repeated our work using a cocktail of antibodies [8–11]. They have confirmed that these cells can be detected using immunocytochemistry although the incidence is variable ranging from 9.5% (9/95) to 35% (18/51). The relation to other prognostic factors is similar in the study of Diel *et al.* but Cote *et al.* and Schlimok *et al.* found no association with pathological size, peritumoral vascular invasion or lymph node status.

These differences may be related to the variety of antibodies used and the inherent heterogeneity of breast cancer cells. Osborne *et al.* [12] evaluated the sensitivity of their technique using an established breast cancer cell line (MCF-7) mixed with normal bone marrow at various dilutions and found that there is a 95% chance of detecting one cancer cell in 2×10^6 cells. Similarly, we looked at the place of flow cytometry and an automated technique to try and increase the efficiency of bone marrow screening [13] but concluded that manual evaluation was the most sensitive. However, the patient numbers are much smaller in other studies and the recruitment of larger numbers will be awaited with interest.

Longer follow-up now shows that the presence of these cells predicts for recurrence at all distant sites rather than just bone as was previously reported [2]. Harbeck *et al.* [14] have shown a clear relationship between the development of bone metastases and micrometastases whereas the preliminary results from Cote *et al.* [15] suggested that the site of relapse is not specific. Overall survival is shorter in patients with micrometastases, a finding which has become more significant with longer follow-up.

We have repeated bone marrow sampling in a group of patients after initial surgery but before relapse, and in patients who have developed disseminated disease to determine the fate of micrometastases [16]. Interestingly, we found that after surgery micrometastases were not detected, even in patients who had them documented beforehand, and that the prevalence increased to 19% in patients with locally recurrent disease, 30% in patients with disease at distant sites other than bone, and 100% in patients with radiologically proven bony disease. This would suggest that the detection of these cells at presentation is of considerable importance and that although present in small numbers they almost certainly possess metastatic potential. Although hitherto it has been impossible to isolate and grow these cells some progress has been made in detecting malignant cells in histologically normal bone marrow using long term culture techniques [17, 18]. These findings emphasise the need to try and eradicate these cells at presentation whilst the tumour load is small.

In this series, adjuvant therapy did not affect the disease-free interval although the prognosis was worse for patients who had micrometastases whether or not they received adjuvant treatment. The effects of such varied adjuvant therapies are, however, too small to be reliably detected and it is therefore impossible to determine whether adjuvant therapies are more or less effective in patients with micrometastases. The higher incidence of relapse in patients with micrometastases but with negative lymph nodes may help to further define a group who would benefit from adjuvant therapy.

Although the presence of micrometastases is a strong prognostic factor its independent effect after considering nodal involvement, tumour size and vascular invasion is moderate, and therefore currently of limited clinical value. More precise methods of detection of micrometastases may, however, provide a stronger prognostic factor of practical clinical value.

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Prognostic Factors in Axillary Lymph Node-negative (pN−) Breast Carcinomas

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Axillary lymph node-negative (pN−) breast carcinomas (n = 281) were analysed histoquantitatively for two mitotic indexes (MAI, mitotic activity index; M/V, volume corrected mitotic index) and nine nuclear factors with special emphasis on disclosing prognostic factors during a follow-up of 12 years. The M/V index (P = 0.0018), tumour size (P = 0.0052), MAI (P = 0.0115) and histological grade (P = 0.0565) predicted the recurrence-free survival. MAI (P = 0.0007), M/V index (P = 0.0046), tumour size (P = 0.0133), histological grade (P = 0.0528) and S.D. of the nuclear perimetry (P = 0.07) predicted the disease-related survival. In Cox's analysis, MAI (P = 0.004), adjuvant therapy (P = 0.03) and tumour size (P = 0.09) predicted survival independently. Recurrence-free survival was related independently to nuclear perimetry (P < 0.001), SD of nuclear area (P = 0.01) and MAI (P = 0.019) in Cox's analysis. In small (diameter ≤20 mm) tumours, S.D. of nuclear perimetry predicted recurrence-free survival (P = 0.03) in Cox's analysis. The results advocate the use of mitotic indexes and nuclear factors in place or in combination with conventional histological grading in predicting the survival and tumour recurrence in axillary lymph node-negative breast carcinomas.
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INTRODUCTION

QUANTITATIVE VARIABLES have significant predictive value in several epithelial tumours [1–20]. S-phase fraction [5, 12, 13], DNA ploidy [3, 5, 11, 12, 14], mitotic activity [1, 2, 12, 16, 17] and some nuclear factors [1, 2, 16, 18, 19] have proved to be significant prognostic factors in breast cancer as well.

Recently, the role of flow cytometry [3, 5] in prognostication of breast cancer has been studied more intensely than that of quantitative morphometry [1, 2]. For axillary lymph node-negative breast carcinomas, the majority of the prognostic data is derived from the studies using flow cytometry [11, 13, 14, 16]. However, in other epithelial tumours, multivariate analyses have shown that flow cytometric variables are inferior or at best equal to the mitotic indexes and morphometrically measured nuclear factors in predicting the long-term survival [8]. Thus, there is ample evidence to indicate that relatively simple morphometric methods are accurate in prediction of even the localised (T1) breast carcinomas [4, 8, 10]. It seems to be established that, compared with, e.g. flow cytometry, there are major advantages confined to morphometric methods, including a high reproducibility [6, 20] and relative simplicity.

From the clinical point of view, an accurate prognostic prediction of axillary lymph node-negative breast carcinomas is one of the key issues in breast oncology [22–24]. The currently used histological grading systems are subject to considerable variation [25], and consequently, the estimates of prognosis in individual cases are inaccurate [25].

On the basis of the above, the present study was prompted, in which the predictive value of the morphometric factors was assessed in 281 patients with axillary lymph node-negative (pN−) breast carcinomas followed-up for over 12 years at Kuopio University Hospital during 1968–1990.

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PATIENTS AND METHODS

Patients

A total of 281 consecutive women with axillary lymph node-negative breast carcinomas were treated and followed up at Kuopio University Hospital of Kuopio during 1968–1990. From these patients, complete follow-up histories and archival paraffin