PAPERS AND SHORT REPORTS

Micrometastases in bone marrow in patients with primary breast cancer: evaluation as an early predictor of bone metastases

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Abstract

The bone marrow of 307 patients with primary breast cancer was examined for tumour cells by immunocytochemistry using an antiserum to epithelial membrane antigen. Micrometastases were found in 81 cases (26.4%) and their presence was related to various poor prognostic factors: spread to lymph nodes, vascular invasion, T stage, and pathological size. The median duration of follow up was 28 months. Seventy five patients relapsed, 60 at distant sites. Of these 60 patients, 26 had micrometastases detected at presentation and 34 were free of micrometastases initially. The relapse free interval was significantly shorter for patients with micrometastases, and these patients had a shorter survival. Analysis of the sites of relapse showed that the test

predicted bone metastases only. Thus 10 out of 19 patients (53%) who developed bone metastases at first relapse had micrometastases at presentation compared with only 41 out of 288 patients (14%) who remained free of bone metastases or relapsed in non-skeletal sites.

The presence of micrometastases detected at the time of initial surgery in a patient with primary breast cancer is a useful predictor of early relapse in bone and may help in selecting patients for subsequent systemic treatment.

Introduction

Ninety five per cent of patients who present with breast cancer have local disease only on staging by conventional methods.¹ Nevertheless, 40% of these patients relapse and die within five years, and this percentage has not changed appreciably for 50 years despite improvements in radiotherapy and surgical techniques. It is widely accepted that a proportion of patients have disseminated disease at presentation which cannot be detected by currently available methods. Hence many doctors use indirect prognostic criteria such as spread to lymph nodes, tumour size, and the presence of tumour emboli in vascular spaces associated with the primary tumour (vascular invasion) in an attempt to identify a group of patients who have apparently local disease but are at high risk of developing metastases and would perhaps benefit from more aggressive adjuvant systemic treatment.

The skeleton is the commonest site of distant metastases from breast cancer^{2,3} and is frequently the first organ in which metastases are detected. By using conventional haematological techniques the likelihood of isolating tumour cells in the bone marrow at the time of presentation is small,^{1,4,5} but with the advent of immunocytochemistry the ability to detect not only small foci of tumour cells but also single cells has been greatly enhanced.^{2,6,7} Furthermore, by increasing the number of marrow sites examined a larger number of tumour cells can be detected.²

Patients entered into this study had multiple bone marrow aspirates taken under general anaesthesia immediately preceding initial surgery and were followed up for a median of 28 months.

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Patients and methods

From July 1981 to August 1985, 307 consecutive patients (mean age 59, range 26-85 years) were entered into the study as they presented with primary breast cancer at the Royal Marsden Hospital, Sutton and London; St George's Hospital, London; and St Helier Hospital, Carshalton. Each patient was screened for metastatic disease by clinical examination, measurements of plasma alkaline phosphatase and γ -glutamyltransferase activity, skeletal survey, and bone scan. An ultrasound scan of the liver was also done if either hepatomegaly or abnormal liver function was noted. The type of operation performed (mastectomy, wide local excision, or excision biopsy) was dependent on the size and site of the tumour and the patient's age. A specimen of the original tumour was taken for determination of oestrogen receptors, measured by the dextran coated charcoal method and considered to be positive if it contained 15 fmol or more/mg cytosol protein. Pathological size of the tumour, histological findings, and the presence or absence of vascular invasion were assessed by standard methods.⁸ One hundred and sixteen patients received adjuvant treatment beginning within one month of the primary treatment according to various trial protocols: 53 patients received tamoxifen, 33 aminoglutethimide, and 30 a combination of cyclophosphamide, methotrexate, and fluorouracil.⁹ The remaining 191 received no systemic treatment until proved relapse.

PREPARATION, STAINING, AND ANALYSIS

Eight marrow aspirates were taken from the following sites under general anaesthesia immediately before breast surgery: upper and lower sternum and sacrum and right and left anterior and posterior iliac crests. Roughly 2-4 ml was taken from each site and pooled for further analysis and 15 ml peripheral blood taken simultaneously from the antecubital vein.

Preparation and staining were done as described by Dearnaley *et al.*⁶ All smears were stained by using an antiserum to epithelial membrane antigen, the antiserum being raised in rabbits against human milk fat globule membranes.^{10,11} Epithelial membrane antigen is widely distributed in epithelial tissues and tumours derived from them; though expression of the antigen is generally absent from normal and neoplastic haemopoietic, lymphoid, osseous, and other connective tissues, a weak non-specific staining of normal bone marrow cells, in particular plasma cells, has been noted.^{7,12,13} Affinity purified goat antirabbit antiserum conjugated to alkaline phosphatase was used as the second antibody, and cancer cells were subsequently visualised by using a Brentamine fast red substrate. Peripheral blood was prepared for examination in an identical manner.

The red stained cells were denoted "antiserum positive" provided that they conformed to the identification of tumour cells on morphological grounds, as detailed elsewhere (fig 1).^{6,7} All slides were examined by a pathologist (UB). As far as possible the number of tumour cells seen for each patient was counted and expressed as few (<20) or many (≥ 20). In the first 200 patients bone marrow smears were prepared for conventional staining with Giemsa and examined by a haematologist; all smears were negative for tumour cells, however, and this procedure was discontinued.

FOLLOW UP

Follow up comprised a clinical examination, chest x ray film, and estimations of γ -glutamyltransferase and alkaline phosphatase activity every

three months together with isotope bone scans every six months. If the markers were abnormal (γ -glutamyltransferase activity >50 IU/l or alkaline phosphatase activity >105 IU/l) or hepatomegaly was noted the liver was scanned by ultrasonography. A skeletal survey was done if the patient had an abnormal bone scan or complained of bone pain. Relapse was defined according to criteria of the International Union Against Cancer.¹⁴ All patients were fully staged¹⁵ at relapse to detect disease at other sites.

STATISTICAL ANALYSIS

Relations between the presence of micrometastases and other known prognostic factors were assessed by χ^2 tests and χ^2 trend tests, as appropriate. To examine whether the presence of micrometastases was related to either relapse free interval or survival standard survival analyses were used.¹⁶ Cox regression analysis was carried out to assess the effect of the presence of micrometastases on relapse free interval independently of other prognostic factors. These more detailed analyses are not presented for overall survival because the total number of deaths (36) was too small. Separate survival and Cox regression analyses are presented for the bone relapse free interval. In all analyses the relapse free interval is taken to imply the distant metastasis free interval.

ETHICS

The study was approved by the ethical review boards of the Royal Marsden and St George's Hospitals. All patients signed a form consenting to the procedure after being informed of the purpose and risks of the study.

Results

Tumour cells were detected in the bone marrow of 81 (26.4%) of the 307 patients.

RELATION TO PROGNOSTIC FACTORS

Table I shows the relation between finding micrometastases and the various prognostic factors considered (T stage, pathological size of tumour, nodal state, presence or absence of vascular invasion, oestrogen receptor state, and menopausal state). There was a significant association between the presence of tumour cells in the bone marrow and T stage ($p < 0.001$), pathological size ($p = 0.006$), presence of positive nodes ($p = 0.013$), and vascular invasion ($p < 0.001$) but not oestrogen receptor or menopausal state. The actual number of cells stained showed very little relation to any of these prognostic factors—for example, the proportions of patients with tumours of 2 cm or more or vascular invasion were no greater in the group with 20 or more cells stained than in those with fewer than 20 cells stained.

With regard to histological type of tumour most patients (294/307) had infiltrating ductal carcinomas. Fifteen had lobular carcinomas and five tubular carcinomas; six had carcinomas of colloid type. The numbers of rare histological subtypes were therefore too small to determine their relation to the presence of micrometastases.

FIG 1—(a) and (b): Appearances of micrometastases in bone marrow of patient with primary breast cancer.

RELATION TO RELAPSE

A total of 75 patients relapsed after a median follow up period of 28 months; 60 of the relapses were at distant sites. Of these 60 relapses, 26 occurred among the 81 patients (32%) with micrometastases detected at presentation and 34 among the 226 patients (15%) free of micrometastases initially. The relapse free interval among patients with micrometastases at presentation was worse than in the other group (fig 2; $p < 0.005$). This difference in relapse free interval was still present when local as well as distant relapses were taken into account ($p < 0.005$). The number of cells

SITES OF RELAPSE

Of 19 patients in whom bone was the site of first relapse, 10 (53%) had micrometastases at presentation compared with only 41 of 288 patients (14%) who remained free of disease or had relapse in distant sites other than bone. When time to first relapse in bone was considered the predictive effect of the presence of micrometastases on relapse was more pronounced than in the analysis of all relapses (fig 3; $p < 0.005$). The Cox regression analysis (table II) for relapse in bone alone was qualitatively similar to that for all distant relapses. There was some suggestion that the relapse free interval at

TABLE I—Clinical and pathological characteristics of patients in relation to presence or absence of tumour cells in bone marrow at time of primary surgery

	No of patients	Micrometastases present		Micrometastases absent		Significance
		No	%	No	%	
T Stage:						
T ₀ -T ₂	222	44	20	178	80	$\chi^2 = 15.6$, df=1 $p < 0.001$
T ₃ -T ₄	84	36	43	48	57	
Missing value	1	1		0		
Pathological size (cm):						
<2	100	15	15	85	85	χ^2 (trend)=7.54 df=1 $p = 0.006$
2-5	173	57	33	116	67	
>5	12	3	25	9	75	
Missing value	22	6		16		
Nodal state:						
Negative	152	29	19	123	81	$\chi^2 = 6.19$, df=1 $p = 0.013$
Positive	135	44	33	91	67	
Not tested	20	8	40	12	60	
Vascular invasion:						
Not seen	191	32	17	159	83	$\chi^2 = 22.8$, df=1 $p < 0.001$
Present	116	49	42	67	58	
Oestrogen receptor:						
Negative	92	31	34	61	66	$\chi^2 = 2.45$, df=1 $p = 0.12$
Positive	199	48	24	151	76	
Not tested	16	2	13	14	88	
Menopausal state:						
Premenopausal	77	20	26	57	74	$\chi^2 = 0.000$, df=1 $p = 0.96$
Postmenopausal	230	61	27	169	73	

TABLE II—Cox regression analysis of time to first metastasis

Factor	Baseline level	Coefficient	Standard error	Hazard ratio	95% Confidence interval
<i>All distant relapses</i>					
Pathological size	<2 cm 2-5 cm	1.00	0.48	2.71	1.06 to 6.96
	<2 cm >5 cm	1.45	0.46	4.26	1.73 to 10.50
Lymph nodes	Negative Positive	0.66	0.32	1.92	1.03 to 3.62
Vascular invasion	Absent Present	0.50	0.32	1.65	0.88 to 3.09
Micrometastases	Absent Present	0.31	0.30	1.36	0.76 to 2.45
<i>Bone relapse only</i>					
Pathological size	<2 cm 2-5 cm	0.36	0.74	1.43	0.34 to 6.11
	<2 cm >5 cm	1.15	0.66	3.17	0.87 to 11.50
Lymph nodes	Negative Positive	0.35	0.53	1.42	0.50 to 4.00
Vascular invasion	Absent Present	0.90	0.55	2.46	0.84 to 7.23
Micrometastases	Absent Present	0.81	0.50	2.24	0.84 to 5.99

Independent effect of each factor on relapse free survival assessed by modelling effect at different levels of factor compared with baseline. Coefficients obtained and hazard ratio are given in table. Hazard ratio estimates ratio of relapse rate in patients with given level of factor to relapse in patients with baseline level.

stained did not appear to be useful in predicting relapse; patients with 20 or more tumour cells in the bone marrow relapsed at a similar rate to those with fewer than 20. Neither adjuvant endocrine treatment nor adjuvant chemotherapy affected the disease free interval.

RELEVANCE TO PROGNOSIS

To investigate whether the presence of micrometastases was a significant predictor of relapse free interval independent of other prognostic factors (vascular invasion, disease in lymph nodes, pathological size) a Cox multiple regression analysis was performed (table II). T Stage was omitted from this analysis because it was so strongly associated with pathological size. Pathological size was clearly shown to be an important prognostic factor, and lymph node disease was also significant. Though there was a small positive effect of vascular invasion and micrometastases on relapse, once pathological size and lymph node state were taken into account neither was independently significant (hazard ratios 1.65 and 1.36, respectively).

FIG 2—Left: Disease free survival according to presence or absence of micrometastases at time of initial surgery. Right: Overall survival. Relapse at all sites (that is, local and distant) represented by: — antiserum negative; --- antiserum positive. Relapse at distant sites only represented by: .... antiserum negative; — antiserum positive. Figures along x axis are numbers of patients at risk.

sites other than bone was also shorter in patients with micrometastases (fig 3; $p > 0.1$), but this difference was not significant.

We also examined the different sites of relapse in bone for each patient at the time bony metastases were first noted. Pelvic deposits were found in a higher proportion of patients with tumour cells in the marrow than in those without (7/15 v 3/13), but the difference was not significant ($p > 0.1$).

FIG 3—Left: Probability of first relapse in bone. Right: Probability of first relapse at other distant site according to presence (antiserum positive —) or absence (antiserum negative ....) of micrometastases at time of initial surgery. Figures along x axis are numbers of patients at risk.

OVERALL SURVIVAL

A total of 36 (11.7%) patients died, 15 (18.5%) of the 81 patients with micrometastases detected at presentation and 21 (9.3%) of the 226 patients free of metastases initially; in 14 and 17 patients, respectively, death was due to metastases. The difference between the two groups was significant ($p < 0.05$; fig 2), but there were too few deaths to assess whether the effect was independent of other prognostic factors.

MORBIDITY OF PROCEDURE

Morbidity associated with multiple marrow aspiration under general anaesthesia was negligible; no patient complained of pain or bruising at the sites of aspiration and there were no short term or long term sequelae or deaths related to the anaesthetic.

Discussion

This study shows that the presence of micrometastases in the bone marrow of patients with primary breast cancer is a predictor of the subsequent development of overt metastases. We have also shown that they are related to certain prognostic factors, such as size of tumour, spread to lymph nodes, and vascular invasion.^{17,18} As expected, this relation was clearest with bone metastases; there was, however, some suggestion that micrometastases may predict the development of metastases at other sites, but the association was not significant.

The relation between tumour cells in bone marrow and the invasion by tumour cells of lymphatic ducts and blood vessels associated with the primary tumour ($p < 0.001$) is of particular interest. Vascular invasion is known to be an independent prognostic variable depicting a poor outlook for those patients in whom it is detectable.¹⁹⁻²¹

The presence of micrometastases does not appear to be an independent prognostic factor; when measurement of pathological size and vascular invasion was available the hazard ratio predicting relapse between a patient who was negative and one who was positive for micrometastases was only 1.36. Recent studies by our group suggest that this may be improved by detecting more cells. This in turn may be achieved by aspirating more sites and also by using a "cocktail" of antisera; we now find that adding an antikeratin monoclonal antibody to antiserum to epithelial membrane antigen can increase the number of tumour cells

visualised, and this combination may well detect more patients with micrometastatic disease.

The clinical relevance of our findings may be considered under two separate headings. Firstly, the technique may provide a method for developing a more rational therapeutic strategy for preventing the evolution of symptomatic bone metastases; and regular sampling of bone marrow may be carried out to evaluate the effectiveness and optimum duration of adjuvant treatment in addition to first and second line measures. Secondly, intensive treatment may be warranted for patients who have micrometastases. The optimum form of intensive treatment is being explored in several centres, but a problem common to all is the danger of reinfusing micrometastases in autologous marrow rescue procedures. Our findings underscore the need for satisfactory methods to rid bone marrow of micrometastases, such as the method recently described by our group in which bone marrow was taken from patients with advanced breast cancer, treated with a monoclonal antibody-toxin conjugate (LICR-LON-Fib/abrin A-chain), and returned to the patient after high dose chemotherapy.²²

We have shown that the detection of micrometastases in primary breast cancer is a useful, albeit not independent, prognostic factor; possibly intensive therapy may benefit these patients.

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