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DNA DISTRIBUTION, CYTOSOL ESTROGEN RECEPTORS AND AXILLARY NODES AS PROGNOSTIC PREDICTORS IN BREAST CARCINOMA

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Abstract

One hundred and fifty patients with breast carcinoma were examined to compare axillary node status, estrogen receptor level and cellular DNA content as prognostic indicators. Seventy-four per cent of the patients were postmenopausal and forty per cent had axillary node metastases. Estrogen receptor was measured by isoelectric focusing in polyacrylamide gel. DNA was measured in individual cell nuclei by means of Feulgen-acriflavine-sulphate stained imprints. Fifty-two per cent of the tumors had diploid and/or tetraploid DNA pattern, and the rest aneuploid pattern. Axillary node metastases, aneuploid DNA pattern and low level of estrogen receptor were related to recurrence. When introduced into Cox's proportional hazards procedure, axillary nodes and estrogen receptor level but not DNA pattern remained as significant predictors of recurrence.

There is an urgent need for reliable predictors of recurrence in breast carcinoma, to make possible individualized treatment. Traditionally, both staging and degree of differentiation of the tumour have been used. The most important staging predictor is the presence and number of involved axillary lymph nodes (14). Numerous attempts have been made to correlate the morphologic grading of the tumour with the clinical outcome, but they have been only partially successful (7, 8, 17). Objectively measurable features in the tumours which can be correlated to the prognosis are accordingly of great practical importance. Two such are the DNA pattern of the tumour and the content of estrogen receptor. The amount of DNA in individual tumour cells is readily measurable (3). Most patients in whom the breast carcinoma recurred within 2 years the tumour had an irregular DNA pattern, whereas patients who remained disease-free at 5 or 10 years often had a near-normal diploid or tetraploid pattern (3). There are several reports on the prognostic value of estrogen receptor content. Tumours with high cell estrogen receptor content carry a better prognosis than tumours containing little or no estrogen receptor (19).

In the present investigation the prognostic information to be obtained from DNA pattern and tumour cell content of estrogen receptors was explored in patients with breast carcinoma with a follow-up of approximately 5 years.

Material and Methods

Patients were included in the study if they had been treated for primary breast carcinoma in 1978, if they were in clinical stage I or II at diagnosis, if the initial surgical treatment included axillary dissection, and if a frozen tumour specimen was available for DNA analysis.

The series comprised 150 women (median age 61 years, range 26–86). The patients were considered as postmenopausal if more than a year had elapsed

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since the last menstrual period. Owing to previous hysterectomy, the menopausal status was unknown for 2 patients. One of them, aged 41 years, was considered as premenopausal, and the other, who was 56 years of age, as postmenopausal.

The mean follow-up was 60 months. In this cohort of patients, 40 had had local or distant recurrence of the disease. There were 9 deaths unrelated to recurrence of breast carcinoma.

The axillary nodal status of the patients was based on rescrutiny of the original microscopic slides. The distribution of patients according to absence of axillary node metastases, the presence of one to 3 or 4 or more metastatically involved nodes, and the number of examined nodes in the axillary specimen is given in Table 1.

Cytosol estrogen receptor assays were made by means of isoelectric focusing on slabs of polyacrylamide gel (27). The assays were performed on frozen tumours that had been stored at −70°C for not more than 7 days. An estradiol binding of more than 0.10 fmol/μg DNA characterized estrogen receptor rich tumours; estrogen receptor poor tumours bound 0.10 fmol/μg DNA or less. In the present series 36 per cent of the premenopausal and 18 per cent of the postmenopausal women had estrogen receptor poor tumours (Table 2).

Single cell DNA measurements were performed on imprint specimens from thawed tumour material. The specimens were fixed, hydrolyzed and stained with acriflavine-SO_2, and the DNA quantitation was done in a Leitz MPV3 cytophotometer. The technical details of preparation and analysis are previously described (6). In each sample more than 150 tumour cell nuclei were selected and their DNA content measured. Histograms showing the distribution of DNA content in the cells were constructed for each patient. The DNA histograms were classified according to the principles suggested by AUER et coll. (3). AUER et coll. (3, 4) found minor differences between types 1 and 2 and between types 3 and 4 with respect to tumour aggressiveness. Therefore only 2 groups were distinguished in the present investigation: Group A, consisting of patients in whom the DNA histograms showed a distinct peak at the normal diploid or at the tetraploid DNA value, or both, with only a few cells outside the range of these peaks (corresponding to AUER's types 1 and 2 'distinct modal'); and Group B, patients with a considerable number of cells outside the diploid-tetraploid peaks, or with irregular or aneuploid distribution of DNA in the nuclei, (corresponding to AUER's types 3 and 4 'aneuploid').

Table 1

Distribution of patients according to evaluated variables

Variable and characteristics	No. of patients	Per cent
Menopausal status		
Premenopausal	39	29
Postmenopausal	111	74
Number of examined axillary nodes		
<5	43	32
>5	93	68
Axillary nodal status		
Node negative	90	60
Node positive	60	40
1–3	42	28
4+	18	12
Estrogen receptors		
Poor	34	23
Rich	116	77
DNA pattern		
Distinct modal	78	52
Aneuploidy	72	48

Table 2

Relation between estrogen receptor and DNA pattern

Menopausal status, DNA pattern	Estrogen receptor (No. of patients)		Total
	Poor	Rich	
Premenopausal			
Distinct modal	6	16	22
Aneuploidy	8	9	17
Subtotal	14 (36 %)	25 (64 %)	39
Postmenopausal			
Distinct modal	5	51	56
Aneuploidy	15	40	55
Subtotal	20 (18 %)	91 (82 %)	111
All patients			
Distinct modal	11	67	78
Aneuploidy	23	49	72
Total	34 (23 %)	116 (77 %)	150

Tumours. The size ranged between 5 and 70 mm (mean 22.4 mm). Four patients had a tumour exceeding 50 mm in its greatest dimension. Histologic classification showed 79.5 per cent ductal, 13.4 per cent lobular, 2.8 per cent papillary and 4.3 per cent

colloid cancers. The histologic classification proposed by McDIVITT et coll. (20) was used.

Statistical methods. Correlations between the variables were analysed by means of ordinary contingency table analyses. The time to any recurrence was the endpoint of the study. For confirmation, the analyses were repeated using death or recurrence as endpoint.

For the multifactorial analyses of the prognostic information of the variables the proportional hazards linear model procedure according to COX (13) with BRESLOW's (10) modification for tied data was used. The computer program was the PHGLM of the SAS institute (16). Differences between the recurrence free survival rates of groups of patients were tested for their statistical significance by means of the log rank test (23).

Results

DNA pattern in comparison with other evaluated variables. In 78 of the 150 women the DNA pattern corresponded to the distinct diploid or tetraploid values (group A), and in 72 the nuclei showed a much wider variation of the DNA content (group B).

The DNA pattern was not significantly correlated to age (p=0.54), or menopausal status (p=0.25), and there was no significant correlation to axillary lymph node status (Table 3, p=0.18).

The DNA pattern was correlated to the cytosol estrogen receptor content (Table 2, p=0.01) even when menopausal status was taken into account. Of the women with receptor poor tumours 11 of 34 (32%) showed a DNA pattern belonging to group A, whereas this DNA pattern was found in 67 of 116 (58%) of the estrogen receptor rich tumours.

Recurrence-free survival. The 5-year recurrence-free survival rate was 83 per cent in patients with no axillary nodal involvement. Patients with positive nodes had a significantly lower recurrence-free survival rate (57%, p=0.0002) (Fig. 1). There was no significant correlation between estrogen receptor contents and nodal status (p=0.98). The cellular estrogen receptor content was a predictor of recurrence in patients with axillary node metastases, but not in those without. The recurrence-free survival rate at 5 years in node positive patients dropped from 64 per cent if the tumour was receptor rich to 31 per cent if the tumour was receptor poor (p=0.002) (Fig. 2). The predictive value of the DNA pattern on the recurrence-free survival in patients

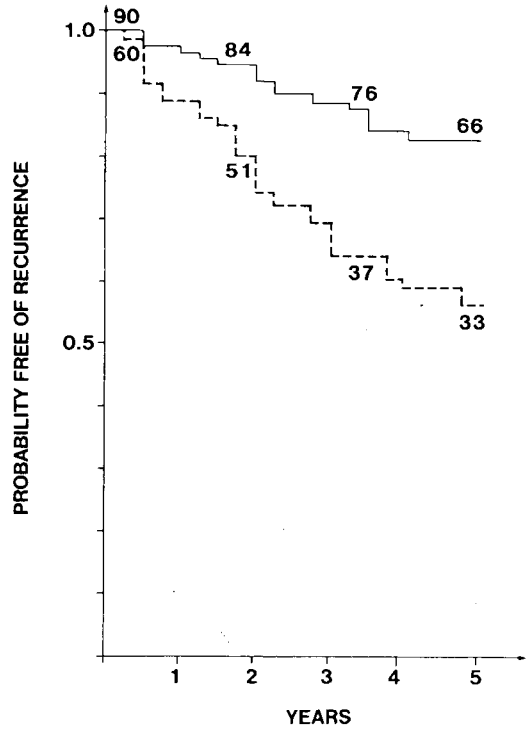


Fig. 1. Recurrence-free survival in patients without (——) and with (– – –) axillary lymph node involvement.

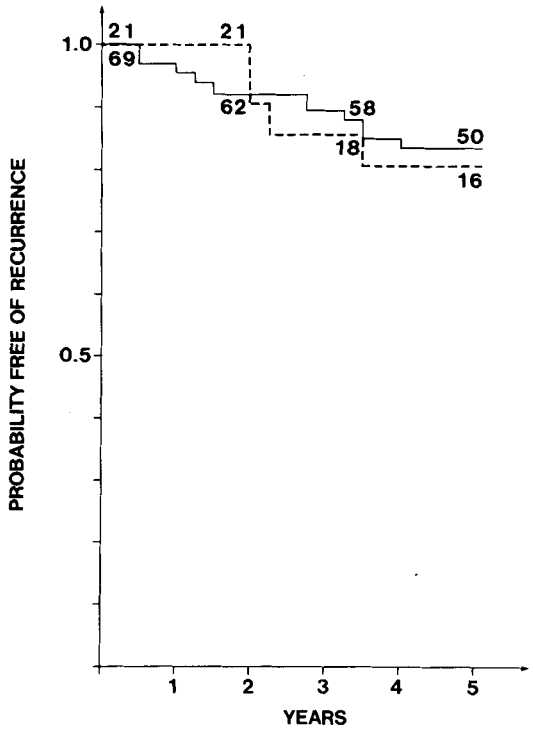
Table 3

Correlation between axillary nodal status and DNA pattern

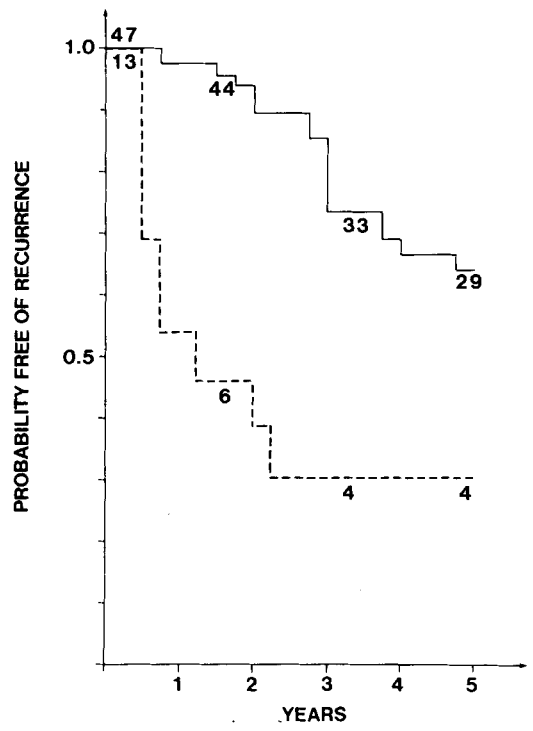
DNA pattern	Axillary nodal status (No. of patients)			Total
	0	1–3	4+	
Distinct modal	47	25	6	78
Aneuploidy	43	17	12	72
Total	90	42	18	150

with and without lymph node involvement is shown in Fig. 3. The DNA pattern improved the prediction of recurrence-free survival only among patients with nodal involvement. In patients with positive axillary nodes and a DNA pattern belonging to group A the 5-year recurrence-free survival rate was 70 per cent; the figure was 42 per cent when the DNA pattern was irregular (group B). This difference is statistically significant (p=0.02).

Result of the Cox proportional hazards model procedure. Age and menopausal status failed to contribute to the prediction of recurrence, whether introduced alone or together with other predictors.

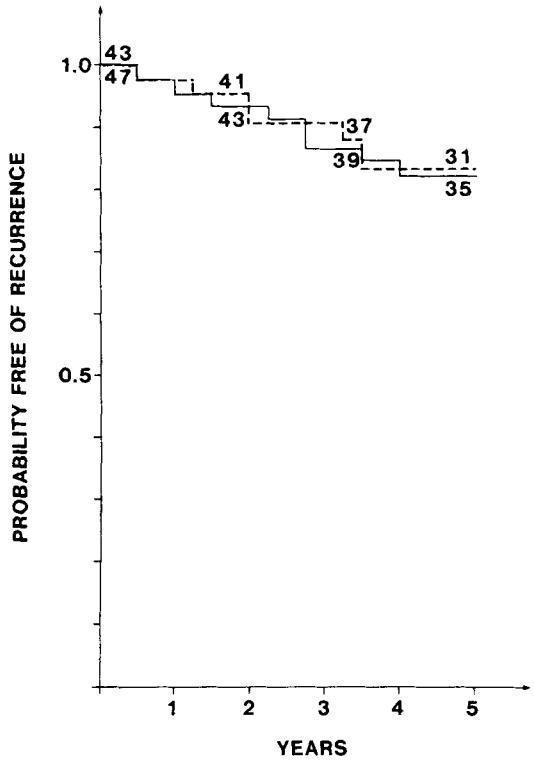


a

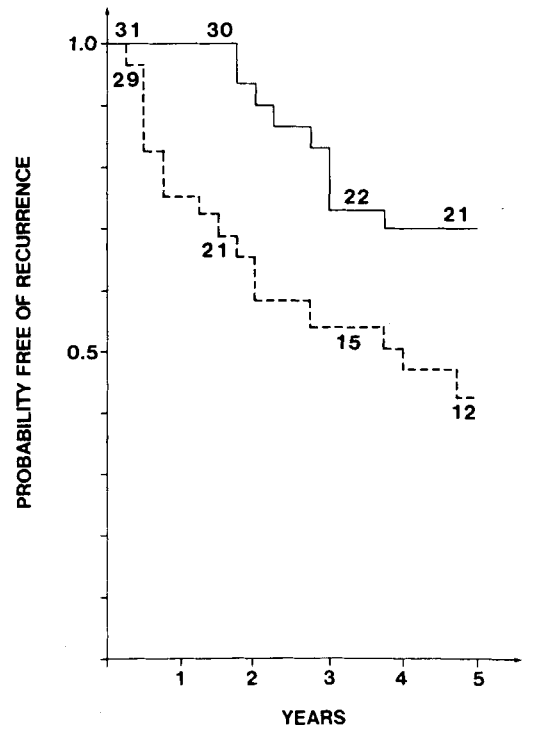


b

Fig. 2. Recurrence-free survival in patients without (a) and with (b) axillary lymph node involvement in relation to estrogen receptor content. ER>0.10 (——), ER<0.10 (– – –).



a



b

Fig. 3. Recurrence-free survival in patients without (a) and with (b) axillary lymph node involvement in relation to DNA pattern. Group A (——), group B (– – –).

When introduced as a single predictor, nodal status (no metastases versus metastases) significantly contributed to the prediction of recurrence ($p=0.0008$), as did estrogen receptor content ($p=0.05$). DNA pattern however failed to be a significant predictor ($p=0.13$). When combined with nodal status, estrogen receptor content ($p=0.01$) but not DNA pattern ($p=0.08$) was a significant predictor. When all predictors were simultaneously introduced into the model only nodal status ($p=0.0001$) and estrogen receptor content ($p=0.01$) remained significant predictors.

The information contained in the number of involved nodes (1–3, versus 4+) was then added to the model. In combination with this variable, the DNA pattern had no significant influence on recurrence ($p=0.20$). In combination with the number of involved lymph nodes estrogen receptor content retained its predictory value ($p=0.004$).

The Cox model analyses were repeated using time to death or recurrence as endpoint, but this did not change the results.

Discussion

Breast carcinoma has DNA modal values that differ from the normal 2c value in 50 to 90 per cent of the cases. The modal values tend to fall into either an approximately diploid group, or an aneuploid group commonly centred near the tetraploid region (9). Tumours with diploid-type DNA pattern are more often rich in estrogen receptor (4, 5, 24) and tend to be more differentiated (22). No correlation factors such as menopausal status, age, tumour size or node status were found. In earlier studies a diploid DNA pattern has been correlated to improved prognosis (1–3, 12, 18, 25). In the present series the DNA pattern was related to prognosis among patients with axillary node metastases, but when it was tested in the Cox model, alone or together with axillary node status or estrogen receptor content, it failed to be a predictor of recurrence.

The cellular content of estrogen receptor was a predictor of recurrence that was unrelated to the degree of lymph node involvement. There was a significant correlation between estrogen receptor content and DNA pattern which is in good agreement with previous reports. Tumours rich in estrogen receptors often showed a regular diploid or tetraploid DNA pattern. Further, tumours with little estrogen receptors were often associated with aneuploid DNA distribution. This probably means that both factors are separate markers of tumour cell differentiation. As a predictor of recurrence the estrogen receptor content replaced DNA pattern when introduced into the proportional hazards model in combination with lymph node status. Estrogen receptor content also remained a significant predictor of recurrence even when the number of involved lymph nodes was introduced into the model. This is in accordance with previous findings (26). The cellular content of estrogen receptors thus apparently gives information with respect to prognosis over and above that contained in the DNA distribution pattern. Unfortunately, neither DNA pattern nor estrogen receptor content could predict recurrence in patients with no lymph node involvement. This may be explained by the low number of recurrences, which makes it almost impossible to find a significant predictor. Recurrences among patients without lymph node metastases occur during prolonged time periods. A repeat analysis of this group at a later time point is therefore planned.

The DNA measurements were made on individual cells by means of static cytophotometry, a method which has the advantage that it enables the selection of morphologically identifiable cells for analysis. The main drawback, over and above the risk that such selection may introduce bias, is slowness, so that readings are based on only a few hundred cells with limited resolution of the DNA distribution patterns. The variation coefficient for the stem line peaks ranged from 5 to 15 per cent. The analysis is consequently based on subjective grouping of the patients into a few groups in which DNA modal values up to 2.5c are considered 'diploid'. Flow cytometry gives better resolution and stemlines with DNA values exceeding 2.1c can be distinguished as aneuploid (22). This may explain why a greater proportion of aneuploid tumours are found with flow cytometry (21, 22, 24). Static cytophotometry and flow cytometry appear to be complementary (11, 15), and improved prognostic information could perhaps be obtained by combining both methods.

The present analysis stresses the importance of accurate axillary staging, because none of the laboratory tests can yet replace the prognostic information available from the presence and number of involved axillary lymph nodes. This strong correlation between prognosis and nodal status is surprising because the number of examined nodes varied markedly. It is tempting to speculate whether care-

ful standardized dissection of the axillary tissue might further improve the predictive value of node status. The finding that, in contrast to DNA pattern as here measured and defined, estrogen receptor status added significantly to the prediction of recurrence to the axillary status, is interesting and will be further investigated.

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