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DNA Ploidy and Survival in Breast Cancer Patients[1]

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Flow cytometric DNA ploidy measurements using frozen or deparaffinized tumor specimens were performed on 565 primary breast cancers from patients treated in the period 1975–1984. Twenty-nine percent of the cases were diploid, 61% had a single aneuploid stemline, and 10% were multiploid. Aneuploid tumors more often had negative estrogen receptor values than diploid tumors, but no significant correlation was found between ploidy class and TNM stage. Patients with more than ten positive axillary lymph nodes had predominantly aneuploid tumors. Overall and distant relapse-free survival were higher for patients with diploid tumors and low-aneuploid tumors. Stratification of the patients according to degree of lymph node involvement, TNM stage, and menopausal stage showed that the prognostic effect of aneuploidy was apparent predominantly in patients with locally advanced disease. Postmenopausal node-positive patients with diploid tumors had a significantly better prognosis than those with aneuploid tumors, but this difference was not found for the comparable premenopausal group. Multivariate analysis with the Cox proportional hazards model indicated that ploidy is an additional, independent prognostic factor in postmenopausal patients.

Key terms: Breast cancer, aneuploidy, flow cytometry, prognosis

Several prognostic factors have been described in breast cancer such as the degree of axillary lymph node involvement, tumor size, histological grade, and estrogen receptor content. Since some of these factors may reflect the age of the tumor rather than its biological potential, there is a great need for additional biological factors for refinement of prognosis assessment at the level of the individual patient (28). Aneuploidy is a primary tumor characteristic that has been found to be of prognostic value for an increasing number of solid tumor types (11,12,34,36). In breast cancer, a very long follow-up period and a large number of patients are needed to evaluate the prognostic effect of aneuploidy, and the majority of reported prospective studies do not fulfill these requirements yet (21). The technique developed by Hedley et al. (14), enabling flow cytometric (FCM) DNA measurements on deparaffinized tissue sections, has now made it possible to use archival tumor material from breast cancer patients with longer clinical follow-up (15). By combining FCM DNA measurements from paraffin embedded tissue as well as from prospectively sampled frozen tissue, we have studied the prognostic value of aneuploidy in a total number of 565 patients with primary breast cancer with follow-up periods ranging from 1 to 10 years. Multivariate analysis with Cox's proportional hazards model has been used to evaluate the independence of aneuploidy as a prognostic variable. The results indicate the necessity of studying the prognostic effect of aneuploidy for subsets of patients matched for clinicopathological prognostic factors.

MATERIALS AND METHODS

Selection of Patients

Prospectively, tumor tissue was collected from 233 out of the 476 patients with histologically proved primary breast cancer treated in the period 1980–1984 at the Department of Surgery. In order to assure an unbiased composition of the series, paraffin-embedded tissue blocks were included from those cases from which no fresh tumor tissue had been sampled. To include patients with longer clinical follow-up, paraffin-embedded tissue blocks were retrieved from a consecutive series of 209 patients treated in the period 1975–1977 from the archives of the Department of Pathology, State University of Leiden. A complete chronology of each patient's disease history including initial treatment, postsurgical TNM classification, and follow-up data was retrieved from the Oncological Documentation Service of the Academic Hospital, Leiden. Patients who developed a contralateral breast cancer or another type of malignancy after the diagnosis of the first breast cancer were consid-

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ered to be censored from the time of diagnosis of the second malignancy. A total of 120 patients were excluded from analysis because of reasons listed in Table 1. Patients who died with distant metastases were counted as cancer deaths, whereas patients who died without known metastases were counted as noncancer deaths. The interval to recurrence or death was calculated from the onset of primary therapy. Information on tumor size and degree of axillary lymph node involvement was retrieved from the histopathology reports. There was no significant difference in age distribution and degree of lymph node involvement between the cohorts of patients treated in the period 1975–1977 and 1980–1984 (Table 2).

Table 1
Reasons Patients Were Excluded From Analysis

Total number of patients	685
Not eligible	
No follow-up	13
Bilateral disease or previous history of other malignancy	78
No material available for ploidy measurements	29
Evaluable patients	565

Table 2
Age Distribution, Degree of Lymph Node Involvement and Follow-up Period of Patients Treated in the Periods 1975–1977 and 1980–1984

	1975–1977	1980–1984	All patients
No. of patients	170	395	565
Mean age ± SD (yr)	57.1 ± 14.0	57.7 ± 14.8	57.5 ± 14.5
Mean No. of positive nodes ± SD	2.0 ± 3.5	2.3 ± 4.4	2.2 ± 4.2
Median follow-up (mo)	85.5	25.0	33

Table 3
Treatments Received by Patients

	No. of treatments
Surgical	
Incision biopsy only	16
Lumpectomy plus axillary dissection	91
Modified radical mastectomy	458
Postsurgical	
Radiotherapy	355
Adjuvant chemotherapy	58
Adjuvant hormonal therapy	6

Table 4
UICC Stage of Patients

Stage	TNM	No. of patients
I	T1NOMO	118
II	T1N1MO	49
II	T2NOMO	149
II	T2N1MO	103
III	T1-3N2-3MO	61
III	T3NO-1MO	43
III	T4N1-3MO	15
IV	T1-4NO-3NXM1	12
	Incomplete data	15
Total		565

Therapy

The different treatments received by the patients are listed in Table 3. The majority of patients was treated by a modified radical mastectomy followed by postsurgical irradiation. Adjuvant chemotherapy was given to premenopausal node-positive patients. Patients who presented with distant metastases at diagnosis received hormonal or chemotherapy or a combination of these. There was no significant change in treatment policy over the period 1975-1984 except for the introduction of breast-saving therapy for tumors less than 3 cm in 1980. As this mode of therapy essentially is not less aggressive and results so far are not different from radical mastectomy series, treatment period was not considered to bias the results of the survival analysis by pooling of the patients.

Stage

The clinical stages of the patients are presented in Table 4. The staging was based on the postsurgical TNM classification according to the recommendations of the International Union against Cancer (UICC, 1978).

Estrogen Receptor (ER) Determination

The presence of estrogen receptors was measured according to the dextran-coated charcoal method at the Department of Chemical Pathology, using calf uterus as a positive control. For the ER determinations performed before March 15, 1983, the cut-off value for a positive receptor content was $\geqslant$20 fmol/mg protein. After this date, the sensitivity of the assay was increased by decreasing the dilution of the ER in the incubation medium, resulting in a cut-off value of $\geqslant$ 10 fmol/mg protein. Progesteron receptors were not determined during the period 1975–1984.

Statistical Analysis

The statistical analysis of the data was done with the BMDP and SPSS statistical packages. Actuarial survival curves were calculated according to the Kaplan-Meier method. Overall survival was calculated by counting as cancer deaths only patients who died with known metastases and censoring for noncancer deaths. Survival without censoring for noncancer deaths is referred to as "crude survival." Multivariate analysis of the prognostic variables was done according to the Cox proportional hazards model. A difference was considered to be statistically significant for P values $< .05$.

Tumor Sampling

Samples from fresh tissue consisted either of pieces of tumor tissue stored at −70°C or scrapings made from lamellated biopsy or mastectomy specimens suspended in about 3 ml 40 mM citrate buffer, pH 7.6, containing 250 mM sucrose and 5% (v/v) dimethylsulfoxide (DMSO) and stored at −70°C (33). From 20 tumors, scrapings were fixed in 70% ethanol. To avoid sampling errors, imprint preparations were made from scraped tissue blocks for Papanicolaou staining and cytologic screening for the presence of a sizeable proportion (> 10%) of tumor cells. The presence of tumor cells in frozen tissue specimens or in archival paraffin blocks was confirmed by histologic examination of H&E stained sections.

DNA Cytometry

Cell suspensions were prepared from paraffin-embedded tissue blocks by treating deparaffinized 35 μm sections with a solution of 0.5% pepsin (Sigma No. P-7000, Sigma Chemical Company St. Louis, MO. in saline, pH 1.5, for 30 min, according to the procedure of Hedley et al. (14). From frozen tissue blocks or cell scrapings, suspensions of single nuclei were prepared with the detergent-trypsin procedure of Vindeløv et al. (33) and stained with propidium iodide (PI). Ethanol-fixed samples were stained with PI according to a previously described procedure (4). Rainbow trout red blood cells (TRBC) were added to the suspensions of isolated nuclei prepared from the frozen samples as an internal ploidy standard. Stained samples were measured on an ICP 22 flow cytometer (Ortho, Westwood, MA) with the appropriate filter combinations for the excitation and measurement of PI and DAPI fluorescence. Filtered demineralized water was used as a sheath fluid. PI-stained ethanol-fixed samples were measured on a FACS IV (Becton Dickinson, Mountain View, CA), as described previously (4). At least 20,000 cells per sample were measured.

Classification of Ploidy Abnormalities

The ploidy of the tumor was expressed by the DNA index which is the ratio between the model channel numbers of the $G_{0,1}$ peak for the tumor cell population and of the $G_{0,1}$ peak representing non-neoplastic cell types such as leucocytes, fibroblasts, etc. that usuallly are present in tumor samples. Tumors with a distinct $G_{0,1}$ population with DI ≠ 1.0 were classified as aneuploid. When more than one aneuploid $G_{0,1}$ population was present, the tumor was classified as multiploid. For samples from fresh or frozen tumor tissue, it was possible to identify the diploid population on the basis of the $G_{0,1}$/TRBC ratio between 1.20 and 1.30 (32). For samples prepared from paraffin-embedded tissue this was not possible (27), but the presence of a sufficient proportion (> 10%) of non-neoplastic cells that could serve as a diploid reference was verified by histologic examination.

RESULTS

Ploidy Distribution

Figure 1 shows DNA profiles from a frozen tissue sample and a deparaffinized tissue section from the same tumor specimen. In general, a lower resolution was obtained for the DNA profiles from deparaffinized samples resulting in a higher incidence (34%) of diploid cases than for the unfixed samples (22% diploid cases). The overall incidence of diploid, single-aneuploid, and multiploid cases is listed in Table 5. The frequency distribution of DI shows a typical bimodal pattern with clustering of stemlines in the (near)-diploid and (hypo)-tetraploid region with a minimum around DI = 1.40 (Fig. 2).

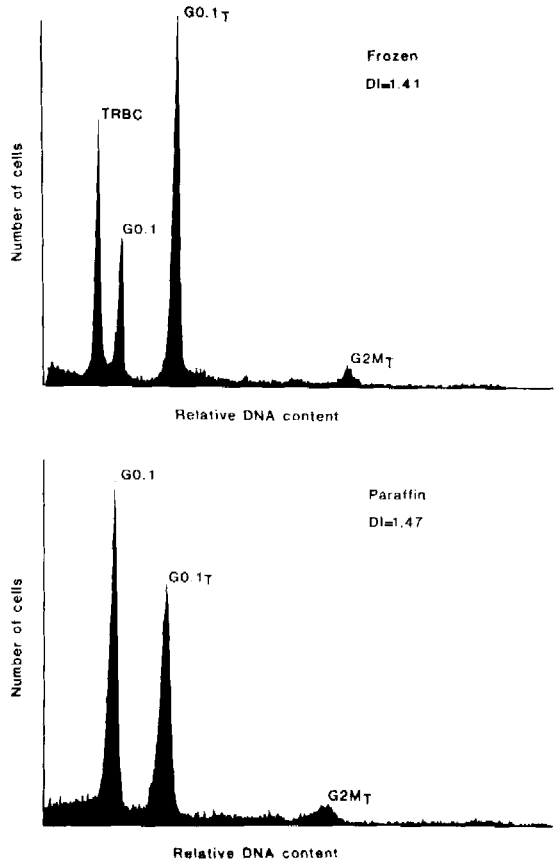


FIG. 1. DNA profiles from two different samples from the same tumor. Upper panel: PI-stained suspension prepared from frozen tissue with trout red blood cells (TRBC) as internal ploidy standard. Lower panel: DAPI-stained suspension prepared from deparaffinized tissue. The lower profile shows broader peaks, but DI are similar (1.41 and 1.47, respectively).

Ploidy Class and Clinicopathological Variables

Table 6 shows the correlation between ploidy class and various clinicopathological variables. Patients with a high degree of axiallary lymph node involvement (≥ 10 positive nodes) showed a markedly higher incidence of aneuploid tumors than patients with a lower or zero number of involved nodes. There was also a trend toward a higher aneuploidy incidence in patients with involvement of the infraclavicular lymph nodes and patients with "grave signs" like lymph or blood vessel involvement or breakthrough of the lymph node capsule. A significantly higher incidence of aneuploidy was found in patients with T2 tumors (2.1–5 cm in diameter)

compared to those with T1 tumors (diameter ⩽ 2 cm) but the incidence of aneuploidy for T3 (diameter > 5 cm) and T4 tumors (tumors of any size with direct extension to the chest wall or skin) was similar to that of T1 tumors. For the ER data obtained with the more sensitive assay introduced after March 15, 1984, an inverse correlation between aneuploidy and ER status was found as expressed by the higher aneuploidy incidence in the ER− group compared with that in the ER+ group. This difference appeared to be more pronounced for patients with negative lymph nodes (N0) than for patients with positive lymph nodes (N+).

Survival Analysis

Ploidy was not associated with a difference in local-regional recurrence rate, but overall survival and distant relapse-free survival were lower for the group of patients with aneuploid tumors (Fig. 3). However, the differences were rather small and statistically significant for overall survival only. Moreover, the overall survival curves show a tendency to merge after longer follow-up periods. Patients with multiploid tumors behaved similarly to those with tumors having a single aneuploid stem line, and, therefore, these two groups were combined. Combination of low-aneuploid tumors (DI < 1.40) into one group with the diploid tumors led to an increased difference in overall survival and distant relapse-free survival with the remaining aneuploid group (P = 0.006 and P = 0.003, respectively). Survival of patients with diploid tumors who developed distant metastases was only slightly better and not statistically significant (Fig. 4).

Table 5
Ploidy Distribution in 565 Breast Carcinomas

Ploidy class	No. of patients	Percentage
Diploid	162	28.8
Aneuploid	348	61.6
Multiploid	55	9.7
Total	566	100

Ploidy and Survival in Different Subsets of Patients

The relationship between ploidy and survival for different subpopulations of patients is shown in Table 7. For comparison, crude survival (no censoring for noncancer deaths) and overall survival rates are also given without stratification for ploidy. On the whole, crude survival rates were slightly lower than overall survival rates. Both showed a highly significant inverse correlation with nodal stage, tumor size, and UICC stage (P <

Table 6
Relationship Between Ploidy and Various Clinicopathological Variables

Variable	No. of patients	Percentage aneuploid
Nodal status		
N0	263	71.9
1–3 N+	138	74.6[a]
4–9 N+	73	64.4 P < .05
10 N+	30	93.3[a]
Infraclavicular nodes neg.	495	70.5
Infraclavicular nodes pos.	71	76.1
Tumor size		
2.0 cm (T1)	180	67.2[a] P < .05
2.1–5.0 cm (T2)	291	75.6[a]
5.0 cm (T3)	62	69.4
UICC stage		
Stage I	118	66.1
Stage II	301	73.1
Stage III	118	72.9
Stage IV	16	62.5
Without "grave signs"	489	70.1
With "grave signs"	77	77.9
Hormonal status		
All patients		
ER−	125	72.0
ER+	262	69.8
Patients treated after March 15, 1983		
All		
ER−	29	93.1[a] P < .05
ER+	98	70.4[a]
Stratified for nodal status		
N0, ER−	15	100[a] P < .05
N0, ER+	49	69
N+, ER−	12	83
N+, ER−	41	73

[a] 2 × 2 Contingency table.

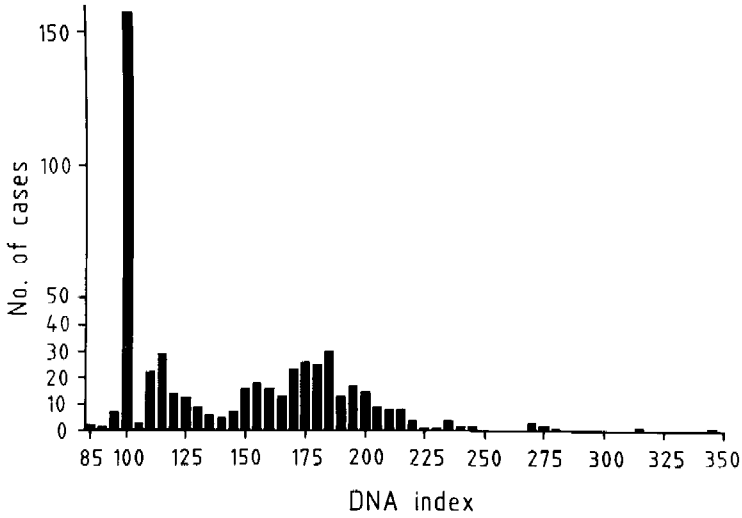


FIG. 2. Frequency distribution of DI (× 100) showing a clustering of stemlines in the (near)-diploid and (hypo)-tetraploid range.

Table 7
Survival Rates for Patients Stratified According to Clinicopathological Prognostic Variables and Tumor Ploidy

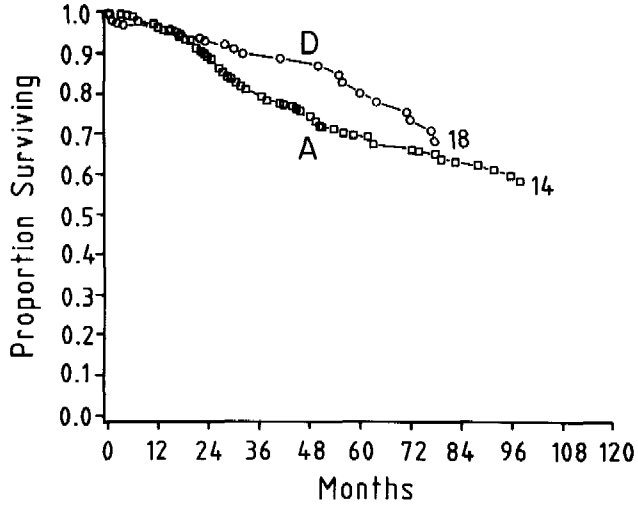
	Cumulative proportion surviving (%)							
	Crude (5 yr) Diploid + Aneuploid	Overall (5 yr)				Distant relapse-n free (3 yr)		
		Diploid + Aneuploid	Diploid	Aneuploid	P*	Diploid	Aneuploid	P*
All patients	70	73	80	69	.04	78	67	.06
Nodal status								
N0	83	88	93	85	.3	88	82	.04
1–3 N+	69	73	80	70	.4	75	64	.6
>3 N+	57	58	72	50	.03	66	43	.03
Tumor size (cm)								
≤2.0	85	90	97	85	.07	87	85	.2
2.1–5.0	68	70	78	67	.06	78	67	.09
>5.0	62	63	65	60	.07	70	45	.2
Stage I	86	92	(92)[a]	(92)[a]	.3	87	92	.9
Stage II	72	86	80	72	.1	83	72	.1
Stage III	63	63	86	64	.04	72	47	.01
ER−	65	69	87	59	.2	78	65	.3
ER+	72	88	80	75	.4	72	70	.3
Premenopausal	75	75	81	76	.6	75	67	.5
Postmenopausal	67	73	82	70	.04	78	66	.05

*Mantel-Cox test, significance threshold $P < .05$.
[a]Only 3-year survival rate available.

0.0001). ER status had a significant effect on overall survival but not on crude survival (P = .04), whereas the reverse trend was seen for menopausal status (P = .05).

There was no statistically significant effect of ploidy on overall survival and distant relapse-free survival in N0 patients, although the survival rates for the diploid group tended to be somewhat higher (Table 7). The same ws true for patients with 1–3 positive lymph nodes (1–3 N+) However, for the group with more than three positive nodes (> 3 N+), both distant relapse-free as well as overall survival were significantly better for patients with diploid tumors (Fig. 5). Stratification according to tumor size again showed a trend toward higher survival rates for patients with diploid tumors (Table 7). No effect of aneuploidy was seen for stage I patients, but a progressive effect was found for stage II and stage III patients which, for the latter group, reached the level of statistical significance both for overall as well as for distant relapse-free survival (Table 7, Fig. 5).

No prognostic effect of ploidy was found in the ER+ group of patients. In the ER− group, those with aneu-



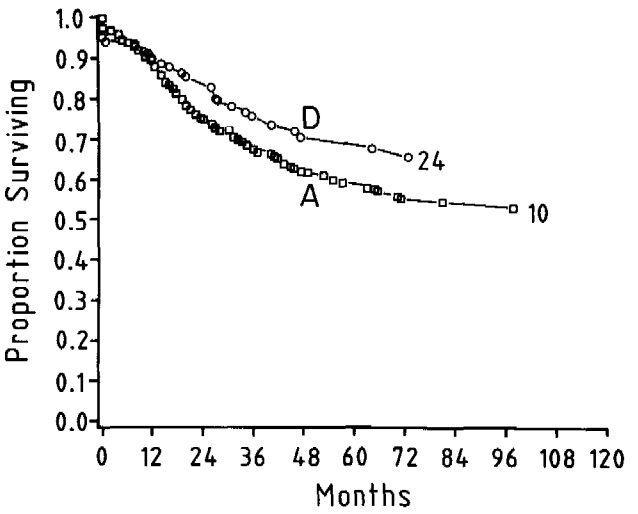


FIG. 3. Kaplan-Meier survival curves for 565 patients with breast cancer stratified according to ploidy class (162 diploid, 403 aneuploid). Upper panel: overall survival, P = .04 (Mantel-Cox). Lower panel: distant relapse-free survival, P = .06). D, diploid; A, aneuploid. Numbers at the end of the curves indicate the number of patients still at risk.

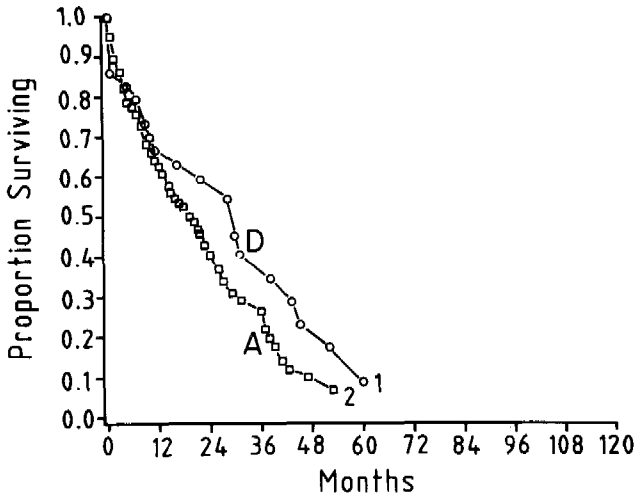
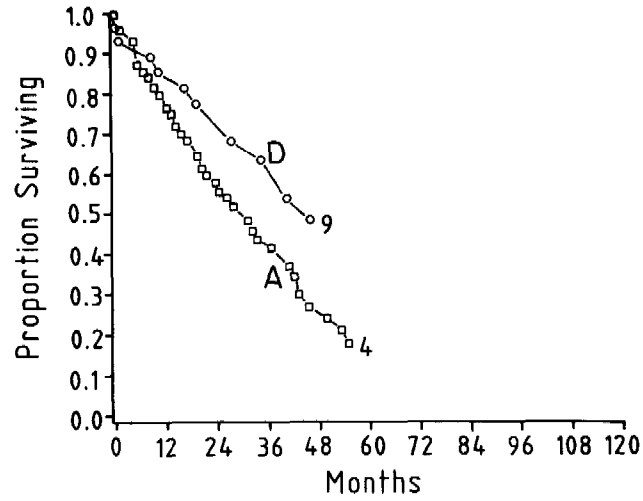


FIG. 4. Survival of patients with diploid (D) (n = 37) and aneuploid (A) (n = 121) tumors after distant relapse. No significant difference (P = 0.1). Numbers at the end of the curves indicate the number of patients still at risk.



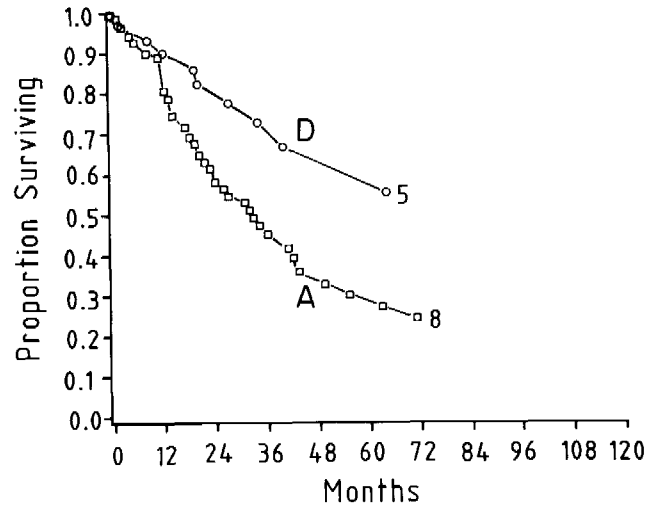


FIG. 5. Upper panel: effect of ploidy on distant relapse-free survival for patients with four or more positive lymph nodes. Note significant difference between the diploid (D) (n = 29) and aneuploid (A) (n = 74) groups (P = .03). Lower panel: patients with stage III tumors (diploid, n = 32; aneuploid, n = 86; P = .01). Numbers at the end of the curves indicate the number of patients still at risk.

ploid tumors did somewhat worse, but the difference did not reach the level of statistical significance (Table 7). Aneuploidy was associated with a significantly lower overall survival in postmenopausal but not in premenopausal patients (Table 7). A similar difference was found for distant relapse-free survival, but here it was on the border of statistical significance. Further stratification of the patients for nodal status showed a better overall and distant relapse-free survival for the postmenopausal

N+ group with diploid tumors but not for the aneuploid N+ group, which behaved in a manner similar to the corresponding premenopausal group (Fig. 6). The different behavior of the postmenopausal diploid N+ group was not associated with significant differences in ER content compared with the aneuploid N+ group (data not shown).

Multivariate Analysis

The stepwise Cox regression model was used to investigate the independence of ploidy as a prognostic variable in relationship to other prognostic variables. The following variables were included in the analysis: ploidy (diploid vs. aneuploid) nodal status (0, vs. 1–3, vs. > 3 positive axillary nodes), ER content (positive vs. negative), T1 vs. T2, 3, 4, and menopausal status. The nodal status was included as two dummy variables as is usual in this type of statistical model. The analysis requires complete data for all included variables which were available for 353 out of 565 patients. The main cause of incompleteness was the lack of ER data for 177 patients, mostly from the 1975–1977 cohort. Table 8 shows the selection of ploidy as an independent prognostic parameter for overall survival as well as distant relapse-free survival, although the contribution to the latter is only marginal. Stratification for menopausal status shows the prognostic effect to be limited to postmenopausal patients with a stronger impact on distant relapse-free survival than on overall survival (Table 9).

DISCUSSION

In this study we have investigated the prognostic value of aneuploidy in a series of 565 unselected breast cancer patients with 1 to 10 years clinical followup. The overall percentage of 71.2 aneuploid tumors was within the 60–90% range reported in other studies (6,8,10,15,17,23–25). Like Hedley et al. (13), we found that the lower resolution of DNA profiles from deparaffinized samples leads to a slight underestimation of the incidence of low-aneuploid tumors. However, the disturbing effect on the survival analysis is probably small since, overall, patients with low-aneuploid tumors appeared to have similar prognosis as those with diploid tumors. The typical bimodal distribution of DI has been reported by a number of other investigators (8,13,17,23–25) and corresponds to the distribution of modal chromosome numbers found by karyotype analysis of breast carcinomas (26). As pointed out by Ewers et al. (8), the evolution of hypotetraploid stemlines may have occurred via a polyploidization of (near-)diploid precursors followed by chromosome segregation up to the point where cells start to lose vital genes. This may explain the paucity of stemlines in the (near-)triploid range. A similar mechanism has been proposed recently by Devonec et al. (7) for (hypo-)tetraploid stemlines in bladder cancer.

We found a highly increased aneuploidy incidence (93.3%) in patients with ten or more positive axillary lymph nodes, whereas no significant differences were found for the groups with 0, 1–3 or 4–9 positive nodes.

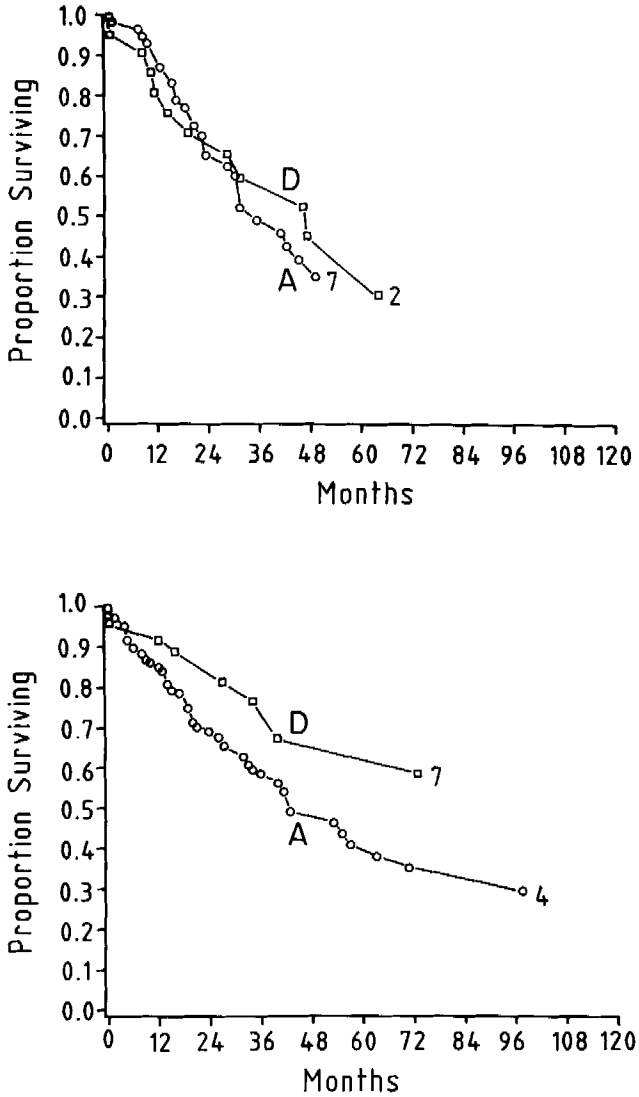


FIG. 6. Effect of ploidy on distant relapse-free survival for patients with positive axillary lymph nodes stratified according to menopausal status. Upper panel: premenopausal patients showing no difference between the diploid (D) (n = 22) and aneuploid (A) (n = 62) group (P = .8). Lower panel: postmenopausal patients showing a significant difference between the diploid (n = 42) and aneuploid (n = 115) group (P = .03). Numbers at the end of the curves indicate the number of patients still at risk.

Table 8
Results of Stepwise Cox Regression Model[a]

Step No.	Variable entered	Improvement (P value)
Overall survival		
1	>3 N+	.0000
2	ER+	.005
3	Aneuploidy	.02
4	1–3 N+	.07
Distant relapse-free survival		
1	>3 N+	.0000
2	Tumor size (T2–4)	.02
3	1–3 N+	.06
4	Aneuploidy	.08

[a]No stratification (353 patients with complete data).

Table 9
Results of Stepwise Cox Regression Model

Step No.	Variable entered	Improvement (P value)
Premenopausal patients (n = 112; complete data)		
Overall survival		
1	>3 N+	0.03
2	1–3 N+	0.07
Distant relapse-free survival		
1	Tumor size (T2–4)	0.003
Postmenopausal patients (n = 241; complete data)		
Overall survival		
1	>3 N+	0.001
2	ER+	0.006
3	Aneuploidy	0.05
Distant relapse-free survival		
1	>3 N+	0.000
2	Aneuploidy	0.04

These results are similar to those of Hedley et al. who found 85% aneuploid cases in the 10+ group (personal communication). Associations betwen the degree of nodal involvement and aneuploidy incidence have been reported by Fosså et al. (10) and Jakobsen et al. (17) but not by other investigators (8,20,23). Therefore, it seems reasonable to conclude that, overall, the association between nodal involvement and ploidy is rather weak.

Ewers et al. (8) found a significantly lower aneuploidy incidence in T1 tumors (43%) than in T2 and T3 tumors (62% and 64%, respectively). We found a higher incidence in T2 tumors only (75.6– aneuploid cases) compared to 67.2% in T1 and 69.4% in T3 tumors, whereas Hedley et al. (personal communication) obtained similar percentages (71%) for T1 and T2 tumors but a higher percentage (82%) for T3 tumors. Thus, as for lymph node involvement, there again is no unanimity on the correlation between ploidy and tumor size. The overall lower aneuploidy incidence found by Ewers et al. (8) could be related to the exclusion of tetraploid tumors from the aneuploid group. Clinical stage (UICC) was also positively correlated with aneuploidy incidence in their study, but in our series of patients no statistically significant trend was found.

Several authors (3,17,20,24) have reported an inverse correlation between ER status and ploidy where others have not found such a relationship (6,10,16,25). In the present study a significant correlation was found only with more recently determined ER values after increasing the sensitivity of the assay. It is not unlikely that similar differences in sensitivity of ER determinations may have contributed to the discrepancies in the published data. The proportion of 67.9% aneuploid Er+ tumors found with the improved assay was similar to that found by Hedley et al. (67%, personal communication) and Jakobsen et al. (68%) (17). The proportion of 93% aneuploid ER– tumors was higher than the 78% found by Hedley et al. (personal communciation), but differences in size and composition of the patient series (117 cases, present study vs., 326 cases, Hedley et al.) may be responsible for this. A similar high proportion of aneuploid ER– tumors (92%) has been reported by Jakobsen et al. (17) for postmenopausal patients only. Our results thus confirm the inverse correlation between ploidy and ER status in breast cancer tumors reported by several other groups.

Evidence for an association between aneuploidy and an impaired prognosis in breast cancer has been obtained in various studies using Feulgen densitometry (1,2). These results are not directly comparable to those obtained with FCM because, apart from differences in resolution and statistical representation of the DNA profiles, deviating definitions of aneuploidy have been used. The results from the present study indicate that ploidy is an additonal prognostic factor for overall and distant relapse-free survival. Although in most of the subgroups of patients, some favorable, although not statistically significant, effect of diploidy was observed, the effects were more pronounced in patients with locally advanced disease and predominantly limited to the postmenopausal group. This seems to be in contrast with the preliminary results obtained in a prospective FCM study by Ewers et al. (8) showing a lower recurrence rate for patients with low stage euploid tumors. However, apart from the short mean followup period (16 months), these data are not completely comparable with those derived from Kaplan-Meier survival curves.

The majority of patients with clinical stage III tumors or more than three positive axillary lymph nodes is adversely likely to have systemic disease. Therefore, ploidy apparently must have some modulating effect on the growth of occult metastases in these patients, rather than be associated with a difference in metastatic potential. This effect could involve a higher growth rate of metastases from aneuploid tumors. This would be in agreement with the higher S-phase fractions of aneuploid tumors reported by other investigators (9,13,20), although the S-phase fractions of diploid tumors are likely to be underestimated by FCM due to the contamination with non-neoplastic cells. The unfavorable prognostic effect of a high growth rate of the primary tumor in breast cancer has been established in several [^{3}H]thymidine labeling studies (22,29,31). Survival after distant relapse appears not to be influenced by the labeling index, however (31). Similarly, no significant prognostic effect of ploidy on survival after distant relape was found by Hedley's group (11,15) or in the present study. An alternative explanation is that although the proportion of diploid and aneuploid tumors that ultimately metastasize may be similar, the threshold volume at which aneuploid tumors metastasize may be lower than for diploid tumors. This too can lead to an earlier appearance of clinically detectable metastases, as shown by Koscielny et al. (19).

It is remarkable that in the present study the prognostic effect of aneuploidy appears to be limited predominantly to the postmenopausal group. This seems to contrast with the results reported by Hedley et al. (15) for a series of 169 patients showing a significantly better survival for the premenopausal patients with diploid tumors. However, this difference has disappeared in the results from a larger series of 473 patients now studied

by these investigators which in turn show a trend toward a better prognosis for the postmenopausal diploid group (personal communciation). The more favorable prognosis of postmenopausal patients with diploid tumors could be associated with their tendency to be more frequently ER+ and, therefore, more likely to have lower [^{3}H]thymidine labeling indices and S-phase fractions (31). As a result, occult metastases in postmenopausal patients with diploid primary tumors would progress more slowly than in patients with aneuploid tumors.

It is difficult to envisage how a global phenomenon like aneuploidy, covering a great variety of possibly accompanying structural chromosome aberrations could exert a specific growth-promoting effect. Current insights in the role of oncogenes suggest a function in the signalling pathway that determines the cell's response to growth-stimulating factors (35) leading to more autonomous growth. It is conceivable that apart from gene-dosage effects, aneuploidy could be associated with higher genetic instablility leading to a higher probability of oncogene activation by gene rearrangements, (onco-)gene amplification, or loss of dominant repressor genes (18). Each of these mechanisms could lead to a growth promotion speeding up tumor progression, which is in line with the reported correlation between aneuploidy and high S-phase fractions.

The results of the Cox regression analysis indicate that tumor ploidy is an additional, independent prognostic factor in breast cancer patients that may help to identify a subgroup of postmenopausal node-positive patients with a relatively better prognosis. However, longer follow-up is needed to evaluate whether this is a permanent difference or a temporary effect, if it remains limited to postmenopausal patients, and whether it will become expressed in the N0 group too. The latter case would be of higher clinical relevance.

It can be concluded that aneuploidy is associated with aggressive biological behavior of postmenopausal breast cancers, probably involving an enhanced growth rate or an earlier dissemination to other organs. Finally, the results from this study illustrate the necessity to analyze the prognostic value of ploidy in breast cancers in relationship to other prognostic factors (21). Such a stratification probably would require about 1,000–2,000 patients with at least 10 years followup.

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