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PROGNOSTIC FACTORS IN LOCALLY ADVANCED BREAST CANCER (T3, T4) WITH SPECIAL REFERENCE TO TUMOR CELL DNA CONTENT

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Abstract

The prognostic value of DNA analysis was studied retrospectively in 91 patients with locally advanced breast cancer (T3, T4) and a follow-up time of 3–7 years. Tumor cell DNA analysis was performed by static cytometry on aspiration biopsy specimens in 42 cases and on tissue sections in 49 cases. The tumors were classified as euploid or aneuploid. Sixty-four percent of the tumors were aneuploid. DNA pattern correlated significantly to histologic grade and axillary perinodal growth and also to survival. DNA pattern, histologic grade and axillary node metastases correlated significantly to disease-free survival (DFS). In this series of patients with locally advanced breast cancer DNA determinations gave important prognostic information.

Key words: Breast, neoplasms; T3 and T4 carcinoma, DNA pattern, prognosis.

The prognosis for women with breast cancer is difficult to anticipate. The most valuable prognostic indicator so far has been clinical staging according to the TNM system regarding tumor size, involvement of regional lymph nodes and the presence or absence of distant metastases (16). Histological examination reveals not only differentiation grade, but also tumor type and extent of metastatic growth. Such information may be of prognostic value (26, 32). Estrogen receptor content has been shown to correlate to the prognosis of breast cancer (1). The receptor content appears to be independent of tumor size and lymph node involvement. Patients with estrogen receptor-rich tumors seem to have a favorable course concerning survival, relapse-free time and post-relapse survival. Estrogen receptor content gives additional prognostic information to the morphological prognostic parameters (1, 2, 22, 29).

With the advent of new and modified treatment modalities, or combinations thereof, there is a growing need for independent and reliable prognostic factors. Lately a great deal of interest has been focused on tumor cell DNA pattern. Many authors have found a prognostic value of DNA determinations, while other studies have given more ambiguous results (Table 1). Although the DNA distribution pattern seems to be a prognostic tool in breast cancer disease, its precise clinical and biological importance remains to be elucidated. The aim of our study was to investigate the correlation between DNA pattern and clinical course in a cohort of patients with locally advanced breast cancer (T3 and T4) but without generalized metastases, with special emphasis on survival and disease-free survival (DFS). Patients with locally advanced breast cancer generally have a poor prognosis, and we expected that the prognostic information given by DNA pattern should be observable already after a relatively short observation time.

Material and Methods

Patient material. All breast cancer patients in the 3 most northern counties of Sweden have been reported to a regional Oncology Centre and Cancer Registry according to a special protocol since 1980. Tumor size has been judged by palpation, and the TNM system used for classification (31). The records from all 121 patients reported during 1980–82 for T3 and T4 breast cancer were studied. Thirty patients were excluded. (Three patients had bilateral breast cancer, one had cytosarcoma phyllodes and 12 had distant metastases at the time of diagnosis. In 14 patients morphological material was unavailable or unsuitable for DNA analysis.) In the remaining 91 patients (53 T3, 38 T4) DNA analysis was performed and follow-up data were available.

DNA analysis. Aspiration biopsy specimens from 42

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Table 1

Prognostic value of DNA analysis according to different authors. (FCM = flow cytometry, SCM = static cytometry)

Authors (ref)	No. of patients	Patient material	DNA analysis	Prognostic value	Follow-up (years)
Atkin 1972 (3)	67	Stage not given	SCM on smears	+	8
Auer et al. 1980 (4)	112	Retrospective, selected Mainly stage I–II	SCM on smears	+	5–10
Cornelisse et al. 1987 (9)	565	Prospective and retrospective. All stages	FCM on fresh or paraffin embedded tissue	+	1–10
Coulson et al. 1984 (10)	74	Retrospective, selected. All stages	FCM on frozen tissue	+	3
Deshpande et al. 1982 (11)	705	Prospective. Stage I–II	DNA weight measured on frozen tissue	–	Median 2 1/2 (2–84 months)
Dowle et al. 1987 (12)	354	Retrospective. Stage not given	FCM on paraffin embedded material	(+) only short term prognosis	7
Ewers et al. 1984 (14)	638	Prospective, consecutive. All stages	FCM on frozen tissue	(+) on T1 and T2NO tumours only	Mean 1 1/2
Fallenius 1986 (15)	409	Retrospective. Stage I–III	SCM on smears	+	8–13
Hedley et al. 1984 (18)	165	Retrospective. Stage II	FCM on paraffin embedded material	+	5
Klintenberg et al. 1985 (21)	150	Retrospective. Stage I–II	SCM on smears from thawed tumour tissue	(+) only node positive patients	5
Klintenberg et al. 1986 (22)	210	Prospective. Stage I–III	SCM and FCM on frozen tissue	(+)	5–6
Thorud et al. 1986 (30)	59	Prospective. Stage I–II	FCM on frozen tissue	–	≥4
Current study 1987	91	Retrospective. Stage III	SCM on smears or tissue sections	+	3–7

patients were examined after staining with acriflavine, and in the remaining 49 patients tissue sections were examined after Feulgen staining. DNA analysis was performed by static cytometry using a Leitz MPV 3 cytophotometer. The FLUORA software program was used for the acriflavine-stained smears and the HISTOSCAN program for the Feulgen-stained histologic sections, according to Bjelkenkrantz et al. (6, 7). About 100 tumor cells and 20 control cells (lymphocytes) were measured in each specimen. The DNA histograms were classified into 4 different groups (I–IV) according to the criteria used by Auer et al. (4). Our classification was mainly based on the morphology of the DNA histograms, but in some cases we also paid regard to the DNA index. A tumor with a DNA index >1.25 was considered aneuploid. Type I and II histograms were pooled in one group named 'euploid' and type III and IV histograms in another group named 'aneuploid'. Thus type III tumors with a high fraction of S-phase cells were classified as aneuploid. All histologic specimens were classified and graded (33, 34) by two of us (LB and SOE) until consensus was reached.

Treatment. The surgical prodcedures are shown in Table 2. Fifteen patients were inoperable because of old age, fixed axillary nodes, supraclavicular node metastases, tumor fixation to the thoracic wall or extensive involvement of the skin. Most patients operated on with mastectomy and axillary dissection or sampling and less than 75 years of age received postoperative radiation treatment and they were also included in a study on adjuvant hormonal treatment. This consisted of tamoxifen in patients older than 55 years and tamoxifen plus ovarian irradiation in patients younger than 55 years. Women less than 70 years of age were also cross-randomized to a study on adjuvant cytotoxic treatment, including 8 cycles of doxorubicin and cyclophosphamide. When progressive disease was diagnosed the adjuvant treatment was stopped, and individualised treatment was started. The adjuvant hormonal and cytotoxic treatment is shown in Table 3. Inoperable patients were given individual treatment.

Statistic analysis. Chi-square test was used for contingency tables (17). Curves for survival and DFS were calculated by the method of Kaplan-Meier and a log rank test was used for comparison between the curves (20). No corrections were made for deaths unrelated to cancer. A p-value less than 0.05 was considered statistically significant.

Table 2

Surgical treatment vs. DNA pattern

Treatment	Euploid		Aneuploid	
	No.	%	No.	%
Mastectomy and axillary dissection	18	55	27	46
Mastectomy and axillary sampling	7	21	14	24
Local excision and axillary sampling	0	0	1	2
Simple mastectomy	4	12	4	7
Local excision without axillary sampling	0	0	1	2
Inoperable	4	12	11	19
Total	33		58	

Table 3

Adjuvant hormonal and cytotoxic treatment vs. DNA pattern

Treatment	Euploid		Aneuploid	
	No.	%	No.	%
Tamoxifen	1	6	6	14
Tamoxifen + ovarian irradiation	3	18	2	5
Cytotoxic treatment	0	0	6	14
Tamoxifen + cytotoxic treatment	1	6	3	7
Tamoxifen + ovar. irrad. + cytotoxic treatment	2	12	1	2
No randomized adjuvant treatment	10	59	24	57
Total	17		42	

Table 4

Cytologic grading vs. DNA pattern. The figures within parenthesis state the number of disease-free patients

	Diploid	Aneuploid
Well differentiated ductal carcinoma	1 (1)	1 (1)
Intermediately diff. ductal carcinoma	4 (2)	17 (5)
Poorly differentiated ductal carcinoma	3 (1)	12 (2)
Total	8 (4)	30 (8)

Table 5

Histologic classification vs. DNA pattern. The figures within parenthesis state the number of disease-free patients

	Euploid	Aneuploid
Well differentiated ductal carcinoma	7 (5)	4 (2)
Intermediately diff. ductal carcinoma	9 (5)	24 (6)
Poorly differentiated ductal carcinoma	4 (0)	11 (4)
Lobular carcinoma	9 (4)	5 (0)
Papillary carcinoma	1 (1)	0 (0)
Medullary carcinoma	0 (0)	5 (2)
Mucinous carcinoma	1 (1)	1 (0)
Total	31 (16)	50 (14)

Table 6

Histologic axillary lymph node metastases vs. DNA pattern

	Diploid		Aneuploid	
	No.	%	No.	%
Positive nodes	16	64	33	79
Negative nodes	9	36	9	21
Total	25		42	

Table 7

Axillary perinodal metastatic growth vs. DNA pattern

	Diploid		Aneuploid	
	No.	%	No.	%
Perinodal growth	9	36	26	63
No perinodal growth	16	64	15	37
Total	25		41	

Results

The follow-up time was 3–7 years. DNA analysis revealed an euploid DNA pattern in 36% and an aneuploid pattern in 64% of the tumors (histogram type I 36%, type II 0%, type III 8% and type IV 56%). Age distribution and mean tumor size did not differ between the euploid and aneuploid groups.

In the 38 tumors graded on smears we found a weak tendency for more aneuploid tumors in the combined group of intermediately and poorly differentiated cancers than among the well differentiated cancers. However, this difference was not statistically significant ($p=0.38$, Table 4). In 81 tumors graded on histologic specimens, the combined group of intermediately and poorly differentiated ductal cancers showed a statistically significant higher fraction of aneuploid tumors than the well differentiated cancers ($p=0.027$, Table 5). Concerning the more unusual histologic types of breast cancer, 36% of the lobular and all medullary cancers were aneuploid.

No statistically significant correlation between the occurrence of microscopically verified axillary lymph node metastases and DNA pattern could be found ($p=0.15$, Table 6). However, occurrence of perinodal growth in the axillary nodes was closely related to aneuploidy ($p=0.027$, Table 7).

Tables 2 and 3 show that treatment was similar in the euploid and aneuploid groups. The number of deaths from other causes than breast cancer was similar in both groups (8.1% and 7.6% respectively). Pre- and perimenopausal age ($\leq$55 years) compared to postmenopausal age (>55 years) showed no strong relation to survival rate ($p=0.096$). Cytologic grade (well, intermediately and poorly differentiated) in ductal cancer did not significantly

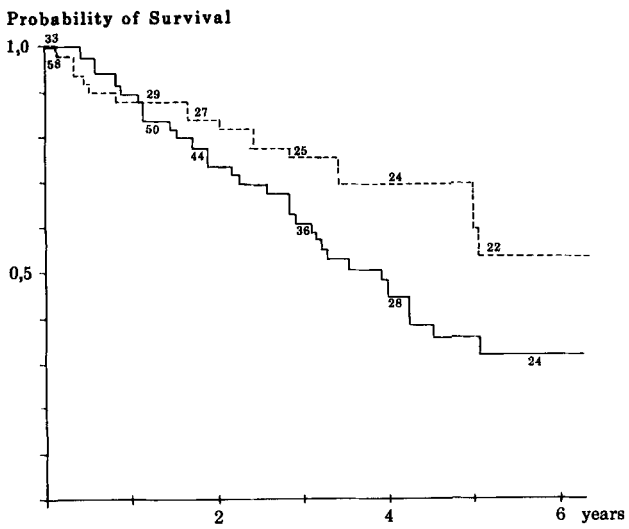

Fig. 1. Survival in patients with euploid (n=33, – – –) and aneuploid (n=58, ——) DNA pattern (p=0.045).

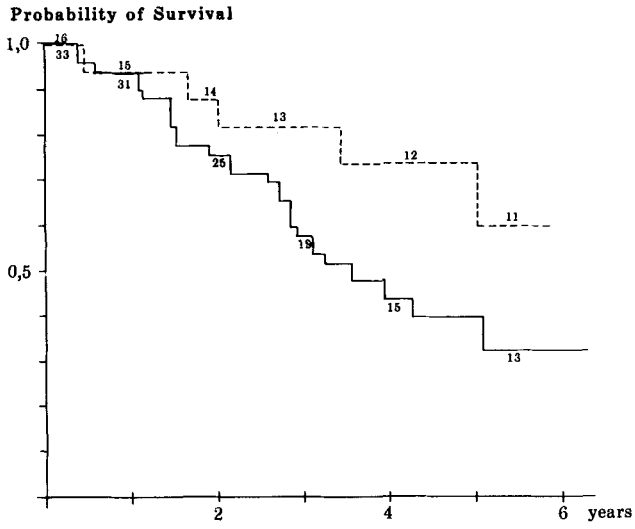

Fig. 2. Survival in patients with axillary lymph node metastases in relation to euploid (n=16, – – –) and aneuploid (n=33, ——) DNA pattern (p=0.064).

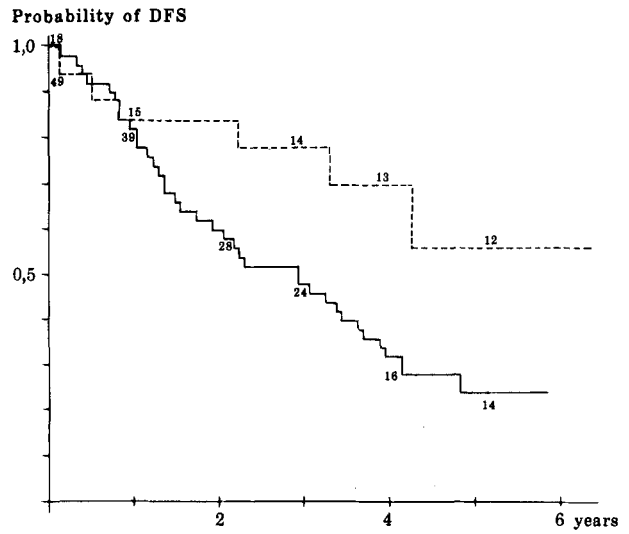

Fig. 3. DFS in patients without (n=18, – – –) and with (n=49, ——) axillary lymph node metastases (p=0.028).

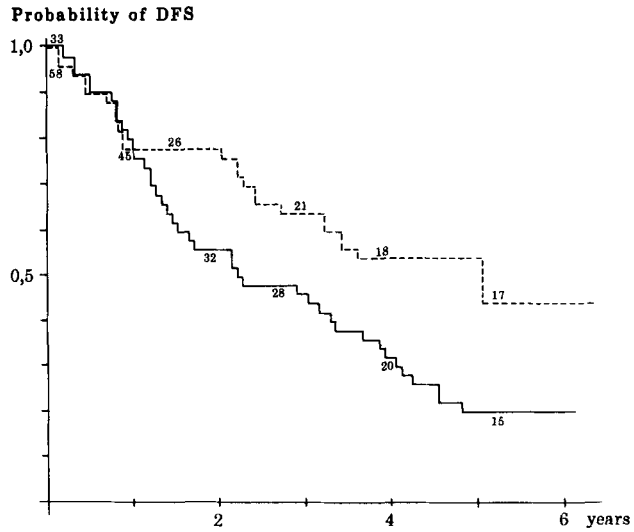

Fig. 4. DFS in patients with euploid (n=33, – – –) and aneuploid (n=58, ——) DNA pattern (p=0.035).

correlate to survival rate (p=0.25) and histologic grade (well, intermediately and poorly differentiated) showed no better correlation (p=0.22). However, if the histologically examined well differentiated ductal cancers were compared to the combined group of intermediately and poorly differentiated cancers there was a difference in survival rate between the 2 groups, but the difference was not statistically significant (p=0.10). In the 67 patients who were operated on with axillary dissection or sampling, the 5-year survival rate was 64% for the node-negative and 52% for the node-positive patients. This difference was not statistically significant (p=0.21).

DNA pattern, however, carried a statistically significant correlation to survival. Euploid breast cancer patients had a 5-year survival rate of 64% compared to 36% for the aneuploid breast cancer patients (p=0.045, Fig. 1). When the subgroups of node-positive and node-negative patients were analyzed, there was still a considerable difference between the euploid and aneuploid groups, which, however, was not statistically significant. Fig. 2 demonstrates survival among the node-positive patients (p=0.064).

DFS rate was significantly higher in the pre- and perimenopausal than in the postmenopausal patient group (p=0.019). Cytologic grade (well vs. less well differentiated) did not correlate significantly with DFS (p=0.18), but

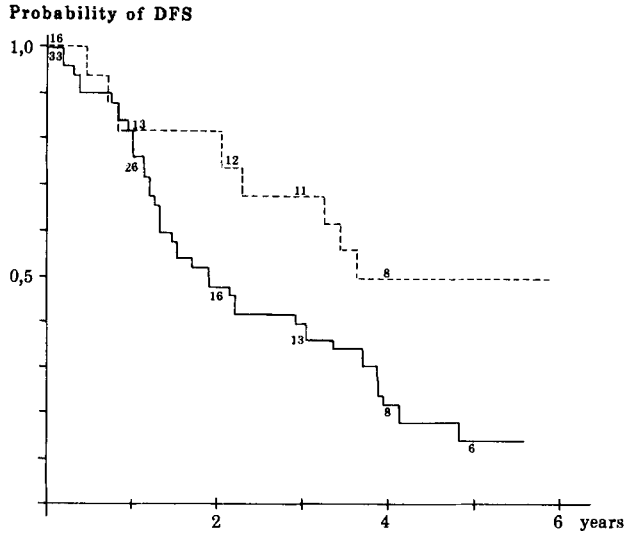

Fig. 5. DFS in patients with axillary lymph node metastases in relation to euploid (n=16, - - -) and aneuploid (n=33, ——) DNA pattern (p=0.034).

histologic grading showed that patients with well differentiated ductal cancer had a significantly higher DFS rate than patients with less differentiated ductal cancer (p=0.036). Only 36% of the lobular cancers were grouped as aneuploid, but the DFS rate (29%) was similar to that for poorly differentiated ductal cancers (27%). All medullary cancers were aneuploid, but the DFS rate (40%) was still higher than that for the poorly differentiated ductal cancers (27%). In the 67 patients with microscopically examined axillary lymph nodes the 5-year DFS rate was 56% for the node-negative and 24% for the node-positive patients. This difference was statistically significant (p=0.028, Fig. 3). DNA pattern showed a statistically significant correlation to DFS, i.e. patients with euploid tumors had a 5-year DFS rate of 54% compared to 20% for the patients with aneuploid tumors (p=0.035, Fig. 4). In the subgroups the statistical significance of this difference became restricted to patients with histologically verified axillary lymph node metastases (p=0.034, Fig. 5).

Discussion

The fraction of aneuploid breast cancer (64%) was higher in the present series than reported by most other authors using static cytometry and DNA classification according to Auer et al. (4, 5). Cornelisse et al. (8, 9) reported 60% aneuploid tumors, but they also included tetraploid tumor in the aneuploid group. Klintenberg et al. (21) found 48% aneuploid tumors in 150 tumor samples from patients with breast cancer stage I–II, and Fallenius (15) found 47% aneuploid tumors in 409 patients with breast cancer stage I–III. The high aneuploidy rate in our material can probably be explained by the fact that the series only included patients with T3 and T4 tumors (stage III). Ewers et al. has shown that aneuploidy rate increases with tumor size and stage (14).

In agreement with some other authors we found a relationship between histologic differentiation grade and DNA pattern (11, 19, 25, 27, 28, 30). Our results also indicate that the DNA pattern does not give good prognostic information in lobular and medullary carcinomas and this is in agreement with the results of Erhardt et al. (13). Hedley et al. (18) found more extensive axillary lymph node involvement in patients with aneuploid tumors, but in accordance with most authors (11, 14, 23–25) we did not find any correlation between DNA pattern and the existence of lymph node metastases. However, a correlation was found between aneuploidy and perinodal growth (p=0.027, Table 6). It will be interesting to examine whether this relationship also exists in less advanced breast cancers.

Atkin (3) examined 67 primary breast cancer specimens for modal DNA values and reported that the 8-year survival rate was better in patients with a near-diploid DNA pattern than in patients with a triploid-tetraploid DNA pattern. Auer et al. (4) studied a selected retrospective material of 112 breast cancers. They found that patients with a survival of less than 2 years had abnormal DNA distribution patterns in 81%, whereas on the contrary, women who survived more than 10 years had normal (euploid) DNA histograms in 80%. Hedley et al. (18) found that aneuploidy correlated to a worse prognosis in women with stage II breast cancer. Seventy-two percent of the patients with diploid tumors were disease-free after 4 years compared to 43% of the patients with aneuploid tumors. Ewers et al. (14) found that the recurrence rate was twice as high in patients with aneuploid tumors than in patients with euploid tumors. However, this difference was noted only in patients with less advanced stages of breast cancer disease (T1 and T2) whereas Deshpande et al. (11) on the contrary did not find any significant differences in tumor DNA content between short- and long-term survivors. The literature is summarized in Table 1.

The DNA pattern had a statistically significant correlation to both survival and DFS in this series of patients. This correlation was statistically significant when all patients were included. When subgroups were analyzed the difference was significant only among the node-positive patients, which may be explained by the fact that only 18 patients were node-negative.

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