Breast Cancer Research and Treatment **36**: 83–91, 1995.

Report

Improved prognostication in small (pT1) breast cancers by image cytometry

Michaela Aubele,[1] Gert Auer,[2] Ursula Falkmer,[2] Andreas Voss,[1] Karsten Rodenacker,[1] Lars-Erik Rutquist,[3] and Heinz Höfler[1,4]

[1] *GSF, Forschungszentrum für Umwelt und Gesundheit GmbH, Institut für Pathololologie, Ingolstädter Landstraße 1, 85764 Oberschleißheim, Germany;* [2] *Institute of Tumorpathology, Karolinska Institute, 10401 Stockholm, Sweden;* [3] *Oncologic Centre, Radiumhemmet, Karolinska Hospital, 17176 Stockholm, Sweden;* [4] *Institut für Pathologie, Technische Universität München, Germany*

Key words: pT1 breast cancer, prognosis, image cytometry, DNA, morphometry, texture

Summary

Feulgen-stained samples from 460 small (pT1) primary breast cancers were investigated by means of an image analysis system. Several DNA, morphometrical and textural parameters were evaluated for each patient, and the prognostic meaning of these parameters was then investigated by the Cox regression analysis. As prognostic criterion a distant recurrence-free survival of five years was considered.

All investigated DNA- and morphometrical parameters as well as several textural parameters showed a significant univariate correlation with the clinical course. In a multivariate approach the axillary nodal status was the most important prognostic parameter, followed by a morphometric parameter (anisokaryosis) and two textural parameters (runlength and co-occurrence). None of the DNA histogram derived parameters could add prognostic information in this multivariate approach. By the linear combination of the four selected variables, an individual prognostic factor was calculated. Using this factor the patients could be split into several groups according to their risk for distant metastases. Thus a low risk group of pT1 patients could be identified with a distant recurrence rate of only 2% after 5 years, and also a group of patients with a considerably worse prognosis and a 5-year distant recurrence rate of 53%. In contrast, using the nodal status as single parameter allows the identification of a low risk group of patients (pN0pT1) with a disant recurrence rate of 10.6%. Therefore, morphometrical and textural parameters can provide powerful prognostic information in small breast carcinomas and may allow a better selection of patients for adjuvant therapy.

Introduction

Knowledge of prognostic factors in breast cancer is a prerequisite for the selection of women for adjuvant hormone- or chemotherapy. The more precise the diagnostic (prognostic) methods in detecting groups with a higher risk (or lower risk) for aggressive disease and recurrence, the better the possibilities for individual treatment. Several prognostic indicators are available today. Of these, the presence of axillary lymph node metastases is regarded as the most reliable one [1–3] and, to a lesser degree, also tumor size [1, 3, 4], histologic grade [5, 6], and hormone receptor status [7–9]. Due to mammography screening programs and other factors an increasing number of breast cancers are small in size at their

Address for offprints: M. Aubele, GSF-Forschungszentrum für Umwelt und Gesundheit GmbH, Institut für Pathologie, Ingolstädter Landstraße 1, 85764 Oberschleißheim, Germany

detection. Such small primary breast cancer tumors are usually associated with favorable outcome [4, 10]. However, some of these patients will have recurrence and therefore could benefit from more aggresive therapy, whereas patients with a low risk for distant metastases do not need adjuvant treatment.

A large number of potential prognostic factors has been investigated in the last years. One of the most frequently assessed markers is the DNA content of Feulgen stained cells and a variety of parameters which are calculated on the DNA histograms. Several studies have demonstrated an independent prognostic value of DNA ploidy [11–15], while others could not confirm or only partly confirmed these findings [16–19]. The visual classification of DNA histograms, developed by Auer and coworkers [20], was shown to have an independent prognostic value in breast cancers [13, 21–24]. Aneuploid DNA histograms have been found more frequently in rapidly metastasizing tumors, whereas patients with diploid tumors in general show a better clinical course [21–23]. Lymph node negative patients with aneuploid DNA distribution were reported to have a higher rate of relapse than those with a diploid distribution [19, 25]. In the last years also some more parameters, which are calculated on the DNA histograms, were shown to yield prognostic information. The most important ones may be the entropy [26], the ploidy balance [27], the 2c deviation index [28], the exceeding rates, as well as the S-phase fraction as an expression of proliferative activity [10, 29, 30].

In addition to these DNA parameters, some morphometric features have also been reported to be of prognostic value in breast cancer patients. The nuclear shape was demonstrated to deliver prognostic information [1, 2, 30–33]. Also the variability of nuclear size as a morphometric equivalent of anisokaryosis was found to be of prognostic value [1, 35]. Finally, a few studies have noted the prognostic relevance of specific chromatin features in breast cancer [36–38]. However, these cytometrical studies were usually performed on consecutive cases of breast cancers, consisting of various portions of small (pT1) and larger primary tumors. In the present study the prognostic significance of cytometrically assessed DNA, morphometrical and chromatin parameters was investigated exclusively on small (pT1) breast cancers. Prognosis was related to a five year distant recurrence-free survival of patients.

Material and method

Tumor samples

Cellular measurements were performed on 460 fine needle aspirates and imprints from primary invasive breast carcinomas, sampled at the Karolinska Hospital in Stockholm from 1971 to 1986. Based on the pathology reports all tumors were ≤ 2 cm in diameter (pT1). Histologically they were classified as ductal invasive (nos, n = 348), lobular (n = 29), medullary (n = 6), and others (n = 28). The histopathological grading according to Bloom and Richardson [5] was available from 324 cases. Twenty-seven of the tumors were classed as grade I, 155 as grade II, and 142 as grade III. As neither histological type nor grade could add independent prognostic value in our multivariate analysis, all 460 pT1-patients were kept in the study cohort. Most of the patients were lymph node negative (n = 329). Seventy-six patients had one axillary lymph node metastasis, and 55 had ≥ 2 lymph node metastases.

The patients were treated by radical mastectomy, modified radical mastectomy, or quadrantectomy. All tumors were resected without residual tumor and without distant metastases at the time of operation (R_0M_0). Only patients with lymph node metastases received subsequent adjuvant irradiation, including the chest wall and the regional lymph nodes. None of the patients received systemic adjuvant therapy. Differences in individual therapy were not taken into account in our analyses. Due to the long period of sampling (from 1971 to 1986), data on hormone receptor status were available only from a portion of the patients. Therefore this parameter could not be included in our analyses. The mean age of patients was 58 years (± 11). Patients aged 50 years or younger were considered as premenopausal (n = 97), patients older than 50 years as postmenopausal (n = 363). The mean follow-up was 84 months (± 35). After five years, 76 (= 16.5%) of

the patients had distant metastatic disease recurrence.

Cytometrical measurements

The imprints and fine needle aspirations from the primary tumors were stained according to Feulgen (5N HCl, 60 min., room temperature). From each patient one slide was investigated and in each slide 100 tumor cells and up to 30 leukocytes as an internal DNA reference were selected. The single cell measurements were performed with an Axiomat-microscope (Zeiss, Oberkochen, Germany) and a TV-camera (Bosch, Stuttgart, Germany). The nuclei were scanned in transmission at 100 magnification (oil immersion) using an optical narrow band filter of 546 nm wavelength [39]. After segmentation of the nuclei, the images were transformed pixelwise into extinction, and DNA-scaling was then performed by the mean IOD value of the leukocytes [39, 40].

DNA parameters

On the DNA histograms the following parameters were calculated: the *ploidy* (c-value of the stemline), the *S-phase fraction* (percentage of cells with DNA values between 2.5c and 3.5c), the 2.5c and 5c *exceeding rates,* the *2c deviation index* according to Böcking et al. [28], the *ploidy balance* according to Opfermann *et al.* [27], and the *entropy* of the histograms according to Stenkvist *et al.* [26]. In addition the *histograms* were *typed* visually according to the criteria described by Auer *et al.* [20]: type I (diploid or near diploid stemline), type II (tetraploid stemline or two well-defined peaks around the 2c and 4c region), type III (diploid stemline with a sizeable number of cells in the S-phase region), and type IV (irregular aneuploidy).

Morphometrical and textural parameters

As morphometric parameters the *area of the nuclei* and the radius of the *largest inscribable circle* were determined [41]. The calculation of *textural* parameters was performed on the extinction image as well as on the flat texture image [41]. Thirteen co-occurrence features as described by Haralick *et al.* [42] and five runlength features as described by Galloway [43] were calculated on the extinction image (*CO1–CO13,* and *RL1–RL5*) as well as on the flat texture image (*NC1–NC13*, and *NR1–NR5*) [41]. The formulas for the features relevant for our results are given in the Appendix.

All morphometrical and textural parameters were calculated for each single nucleus. For survival analyses these single cell features (*xx*) were transformed into slide features by calculating the mean value (*xx*-M), the standard deviation (*xx*-SD), and the coefficient of variation (*xx*-CV) per slide.

Statistical methods

The statistical analyses were done using the SAS (SAS Inst. Inc., USA) and the BMDP software (Statistical Software Inc., USA). Univariate and multivariate analyses were performed by the Cox regression analysis [44]. In multivariate analyses, the features were selected in a stepwise manner. With this method the first selected variable is the one with the highest univariate chi^2-value. All following variables which are added improve the result significantly. Their chi^2-value is multivariate. A prognostic factor for each individual patient was calculated by the linear combination of the selected variables, as described by Haybittle *et al.* [45].

For demonstration of univariate significance of single parameters the Kaplan-Meier analysis was performed [46]. Therefore continuous variables were grouped according to various quantitation models. The significance of differences between groups was determined by the log-rank test.

In all analyses a statistical significance was indicated when $p \leq 0.05$. All analyses were performed for a distant recurrence-free survival of patients of five years.

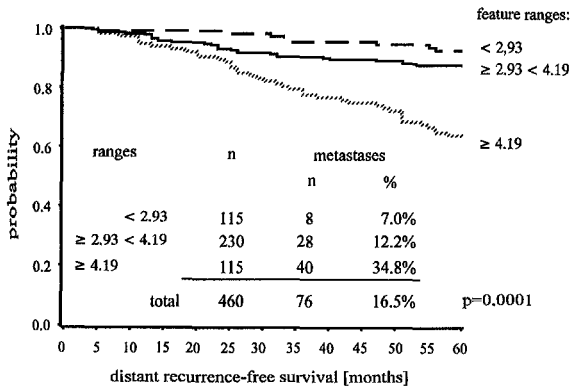

Fig. 1. Kaplan-Meier curves for pT1-breast cancer patients, stratified according to their value in anisokaryosis (Rad-SD), with about 25%, 50%, and 25% of the patients in the groups. The feature ranges, the number of patients, as well as the number of distant recurrences within groups are also given.

Results

Univariate significance of parameters

The average five year distant recurrence rate of the investigated patients with pT1 cancer was 16.5% (n = 76). In univariate analyses the age of patients as well as the histological tumor type failed to deliver significant prognostic information ($p > 0.05$). The three malignancy grades showed significant differences in the clinical course. The 5-year distant recurrence rate was 7.4% for grade I tumors, 10.3% for grade II, and 22.5% for grade III tumors ($p = 0.005$).

According to nodal status most of the patients (n = 329) were lymph node-negative. Forty-seven of the patients had one positive node, and 84 patients had ≥ 2 positive nodes. These three groups showed also a significantly different clinical course in Kaplan-Meier analysis ($p \leq 0.0001$). The average distant recurrence rate of node negative patients was 10.6%, whereas patients with ≥ 2 positive nodes showed a distant recurrence rate of 39.3% ($p \leq 0.0001$).

From the 460 patients, 184 showed a diploid or near diploid DNA distribution (type I), 55 showed a type II distribution, 56 a type III, and 165 showed an irregular/aneuploid histogram pattern (type IV). In the Kaplan-Meier analysis the distant recurrencerate after five years was 7.6% for patients with type I distributions, 20.0% for type II, 14.3% for type III, and 26.1% for patients with type IV distributions. In comparison to the average distant recurrence rate of all pT1-patients (16.5%), the histogram types enable a maximum deviation of 26%. However, type III histograms with a distant recurrence rate of 14% show a better clinical course than type II with a recurrence rate of 20%. According to the log-rank test the four histogram types are significantly different ($p \leq 0.0001$). Also significant in univariate Cox analysis were all other DNA parameters ($p \leq 0.05$): the ploidy (c-value of stemline), the S-phase fraction and the 2.5c and the 5c exceeding rates as expression of proliferative activity, the 2c deviation index, the entropy of the histograms, and the ploidy balance, which shows inverse correlation with the clinical course of pT1 patients.

The standard deviation of the nuclear radius (Rad-SD) and the nuclear area (A-SD) as well as the coefficient of variation of radius (Rad-CV) and area (A-CV) all showed a significant univariate correlation with the occurrence of distant metastases ($p \leq 0.05$). As an example the result of the Kaplan-Meier analysis for pT1-patients, stratified according to their Rad-SD value, is shown in Fig. 1. According to this, pT1 patients with a low variation in nuclear radius (Rad-SD < 2.9) show an average distant recurrence rate of 7% after 5 years, whereas pT1-patients with a large variation (Rad-SD ≥ 4.2) show a distant recurrence rate of 35%. The three classes of nuclei radius variation are significantly different ($p = 0.0001$). In Fig. 1 the Kaplan-Meier curves for pT1 patients are shown, stratified according to their radius variation. Also given are the number of patients as well as the number of distant recurrences within the groups.

Of the textural parameters, several co-occurrence and runlength features were significant in univariate analyses. The most important ones were the runlength feature NR2-M ($p = 0.0086$), and the co-occurrence features NC13-M ($p \leq 0.0001$) and CO12-M ($p = 0.0003$) (for feature description see also Appendix).

Multivariate analyses

In multivariate analysis all parameters with a univariate significance of $p \leq 0.05$ were entered. The most important prognostic parameter in this approach was the axillary nodal status (Table 1). Its prognostic information, however, could significantly be improved by a morphometric parameter (standard deviation of nuclear radius, Rad-SD), and the textural parameters runlength (NR2-M) and co-occurrence (CO12-M). In this multivariate approach the histopathological grading was not selected as an independent prognostic factor. In Table 1 the selected features are shown with the chi^2-values, the p-values, and the coefficients obtained by the Cox analysis. The latter indicate how much each selected variable contributes to the patients' hazard. According to Haybittle and coworkers [45] a multivariate prognostic factor (PF) was then calculated for each individual patient by the following linear combination of the variables:

PF = 0.72*pN + 0.54*Rad-SD + 1.21*NR2-M + 40.96*CO12-M.

As all coefficients are positive, the risk of pT1 patients for a distant recurrence is larger as the values of these variables increase. Consequently, the best prognosis show node negative pT1-patients with a low variation in nuclear size (Rad-SD) and low values in the textural features runlength (NR2-M) and co-occurrence (CO12-M). The calculation of this multivariate factor leads to a continuous distribution of patients' values according to their risk. This is shown in Fig. 2 as histogram with the cases with distant recurrence plotted in black. It demonstrates, that cases with distant metastases within five years (black) are clearly shifted to the right side of the histogram (high prognostic factor), whereas the left side of the histogram consists mainly of distant recurrence-free patients (low prognostic factor). For splitting of the patients into several classes according to their risk, several cut-off points were examined to determine the most prognostically informative point. The final quantitation model was about 25% of the patients in each group, except the group with highest factor, which could be split again. The arrows in Fig. 2 indicate these final cut-off points. Performing Kaplan-Meier analysis for these groups resulted in five classes of pT1 patients with significantly different risk. Figure 3 shows the Kaplan-Meier curves for these groups. The corresponding result of this analysis is given in Table 2 with the numbers of patients in the groups and the numbers of distant recurrences. By this classification a group of patients with a considerable worse prognosis and a 5-year distant recurrence rate of 53% (highest factors) could be identified, and also a group of pT1-patients with the lowest risk and a recurrence-rate of only 2% (lowest factor). In contrast to the latter, the group of patients with the lowest risk according to nodal status (pN0pT1) showed a five year distant recurrence rate of 10.6%.

Discussion

The good prognosis of breast cancers with a small primary tumor has been confirmed by several studies [4, 10, 47]. Carter *et al.* [4] found breast cancer with tumor size ≤ 2 cm to be associated with a 91% 5-year survival rate. In the report of von Rosen *et al.*

Table 1. Result of the multivariate Cox regression analysis for 460 pT1 patients (distant recurrence within five years). Given are the stepwise selected variables, representing independent prognostic factors, their contribution to the regression improvement, and the regression coefficients

Stepwise selected variables	Improvement		
	Chi^2	p-value	Coefficient
Nodal status	32.8	≤ 0.0001	0.72
Anisokaryosis (Rad-SD)	25.0	≤ 0.0001	0.54
Runlength (NR2-M)	11.6	0.001	1.21
Co-occurrence (CO12-M)	5.3	0.021	40.96

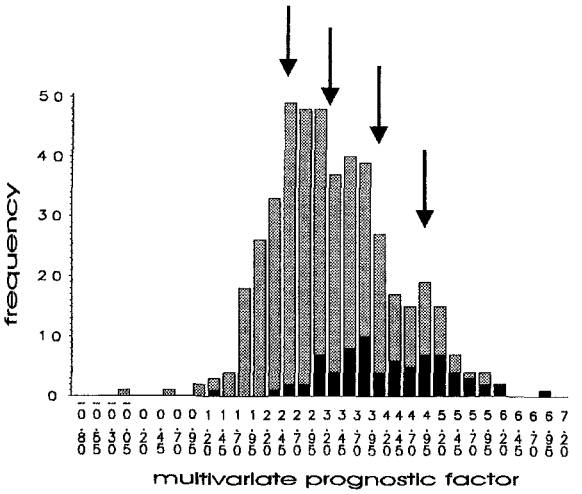

Fig. 2. Distribution of the multivariate prognostic factor for 460 pT1-patients. This factor was calculated by the linear combination of the following parameters: lymph node status (pN), anisokaryosis (Rad-SD), runlength (NR2-M), and co-occurrence feature (CO12-M). Patients with a distant recurrence within 5 years are plotted in black. These patients are clearly shifted to the right side of the histogram (high factors), whereas the left side consists mainly of distant recurrence free patients (low factors). The arrows indicate the cut-off points for subsequent grouping of the patients for Kaplan-Meier analysis.

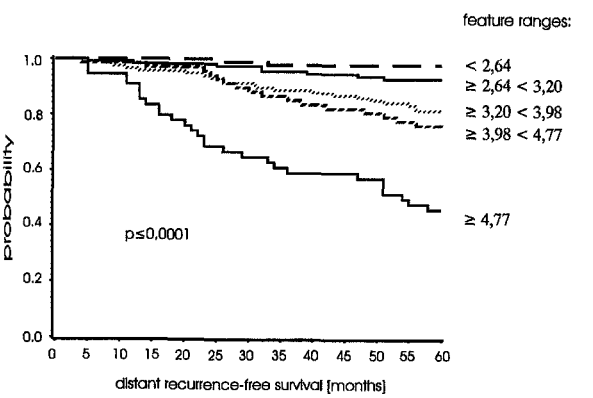

Fig. 3. Kaplan-Meier curves for pT1 breast cancer patients, stratified according to their multivariate prognostic factor. The feature ranges for the five groups and the significance of differences (Log-Rank-test) are also given. The numbers of patients in the groups and the numbers of distant recurrences are shown in Table 2.

[47] women with pT1pN0 cancer had a 91% 10-year survival rate. In our study the average 5-year distant recurrence rate of pT1 patients was about 17%. 72 per cent (= 329) of these pT1 patients were node-negative (pT1pN0) with a 5-year distant recurrence-rate of 11%.

Several potentially prognostic factors in breast cancer have been investigated in the last years. The most frequently investigated one may be the DNA value of tumor cells. Several studies confirm the independent prognostic value of DNA parameters [11–15], while others could not or could only partly confirm these findings [16–19]. One possible reason for this contradictory results may be, that most of the prognostic studies are performed on consecutive cases, consisting of a heterogeneous group of breast cancers at least according to lymph node status and tumor size. Only a minority of these studies were performed exclusively on small (pT1) breast cancers [10]. In our study on 460 pT1 breast cancers, all DNA content derived parameters correlated significantly with the clinical course in univariate analysis. In multivariate analysis, however, none of these parameters could add prognostic information. In contrast, beside the lymph node status, ani-

Table 2. Result of the Kaplan-Meier analysis for pT1 patients. The patients were classed into five groups according to their multivariate prognostic factor. Also given are the feature ranges, the numbers of patients in the groups, and the numbers of distant recurrences

Multivariate factor		Number of patients		Occurrence of distant metastases	
		absolute	relative	absolute	relative
	< 2.64	101	22%	2	2.0%
≥ 2.64	< 3.20	117	25%	8	6.8%
≥ 3.20	< 3.98	117	25%	21	17.9%
≥ 3.98	< 4.77	70	15%	16	22.9%
≥ 4.77		55	12%	29	52.7%
Total		460	100%	76	16.5% $p \leq 0.0001$

sokaryosis and two textural parameters were proven to be of independent prognostic value.

A strong prognostic value of morphometric parameters has been described in some studies. A significant correlation was found between the clinical course and nuclear size and shape parameters [1, 2, 30–34]. These findings are confirmed by our study, where the anisokaryosis (Rad-SD) was proven to be an independent prognostic factor in multivariate analysis. Using the Rad-SD as a single parameter leads already to a classification of a low risk group with a 5-year distant recurrence-rate of about 7%, whereas the group of node-negative pT1 patients show a distant recurrence rate of 11%. Therefore, for identification of a low risk group of pT1 patients, the anysokaryosis can also be used as a single prognosticator.

It was suggested that high resolution image cytometry allows the extraction of a series of chromatin parameters, which define properties of individual chromatin regions [36]. Those parameters would allow the recognition of subtle deviations in the highly ordered chromatin organization. Coarseness of chromatin network and patterns of chromatin clumping were shown to be characteristic of aggressive breast cancers [37]. A correlation between chromatin features and the metastatic potential of tumor cells as well as a correlation between chromatin condensation and the survival of patients was shown [36, 38]. However, cytometric studies about the prognostic meaning of chromatin features in breast cancers are very few. In our investigation, co-occurrence and runlength features, as described by Haralick *et al.* [42] and Galloway [43], were of importance for the prognosis of pT1 patients. Those features are statistical second order parameters, derived from frequency matrices [41–43]. The biologic explanation of those textural parameters could be, that patterns of chromatin structure relate to gene activity and that an increase in malignancy may be reflected by changes in this structure [38].

The aim in searching for prognosticators in breast cancer is to get more precise information about the aggressiveness and the metastatic potential of the tumor. The better this prognostic diagnosis, the more precise and appropriate the selection of patients for adjuvant therapy. In our study, exclusively small (pT1) breast cancers were investigated, which show in general a quite good prognosis. In multivariate analysis the nodal status was the strongest prognostic factor. Its prognostic information, however, could be significantly improved by a morphometric (anisokaryosis) and two textural (co-occurrence and runlength) parameters. By this feature combination, pT1 patients could be split into several groups according to their risk for distant recurrence. Thus a low risk group comprising 25% of the patients with a distant recurrence rate of only 2%, and also a group with a considerably higher risk and a recurrence rate of 53% could be identified. Therefore, a classification of small breast cancers by a combination of nodal status and cytometrical parameters could benefit at least those patients, and, therefore, can guide treatment.

Acknowledgements

The authors would like to thank their colleague U. Jütting for evaluation of the DNA parameters.

This work was supported by grants from the 'Wilhelm-Vaillant-Foundation', München, Germany, the Swedish Cancer Society, and the Cancer Society in Stockholm, Sweden.

Appendix

Formulas of co-occurrence and runlength features, relevant for our results:

Co-occurrences (41, 42), with: H_x, directed entropy (x); H_y, directed entropy (y); H_{xy}, entropy; H_{xyl}, mixed entropy 1; H_{xy2}, mixed entropy 2;

$$CO12/NC12 = \frac{H_{xy} - H_{xyl}}{\max(H_x, H_y)}$$ measure of correlation 1;

$$CO13/NC13 = \sqrt{1-e^{-2(H_{xy2}-H_{xy})}}$$ measure of correlation 2;

Runlength (41, 43) with (i,j) = coordinates of the runlength matrix; N_r = maximum run (128); p(i,j) = probability of run (j) with grey value (i)

$$RL2/NR2 = \sum_{i=0}^{N_g-1} \sum_{j=1}^{N_r} j^2 p(i,j)$$ long runs emphasized;

References

1. Baak JPA, van Dop H, Kurver PHJ, Hermans J: The value of morphometry to classic prognosticators in breast cancer. Cancer 56: 374–382, 1985
2. Eskelinen M, Lipponen P, Papinaho S, Aaltomaa S, Kosma VM, Klemi P, Syrjänen K: DNA flow cytometry, nuclear morphometry, mitotic indices and steroid receptors as independent prognostic factors in female breast cancer. Int J Cancer 51: 555–561, 1992
3. Nemoto T, Vana J, Bedwani RN, Baker HW, McGregor GH, Murphy GP: Management and survival of female breast cancer: Results of a national survey by the American College of Surgeons. Cancer 45: 2917–2924, 1980
4. Carter CL, Allen C, Henson DE: Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. Cancer 63: 181–187, 1989
5. Bloom HJG, Richardson WW: Histological grading and prognosis in breast cancer. Br J Cancer 11: 359–377, 1957
6. Freedman LS, Edwards DN, McConnell EM, Downham DY: Histological grade and other prognostic factors in relation to survival of patients with breast cancer. Br J Cancer 40: 44–55, 1979
7. Lozowski M, Greene GL, Sadri D, Stanick D, Pai P, Harris MA, Lundy J: The use of fine needle aspirates in the evaluation of progesterone receptor content in breast cancer. Acta Cytol 34: 27–30, 1990
8. Pestana CB, Donozo N, Pinto AJ, de Almeida PC, Machado-Santelli GM: Sequential determination of immunocytochemical estrogen receptor and nuclear DNA content in fine needle biopsies from breast carcinoma. Breast Cancer Res Treat 19,1: 34–46, 1991
9. Thorpe SM, Rose C, Rasmussen BB, Mouridsen HT, Bayer T, Keiding N: Prognostic value of steroid hormone receptors: multivariate analysis of systemically untreated patients with node negative primary breast cancer. Cancer Res 47: 6126–6133, 1987
10. Joensuu H, Toikkanen S: Prognosis of breast cancer with small primary tumor (pT1). Acta Oncol 30: 793–796, 1991
11. Balslev I, Christensen IJ, Bruun Rasmussen B, Larsen JK, Lykkesfeldt AE, Thorpe SM, Rose C, Briand P, Mouridsen HT: Flow cytometric DNA ploidy defines patients with poor prognosis in node-negative breast cancer. Int J Cancer 56: 16–25, 1994
12. Beerman H, Kluin PM, Hermans J, Velde CJH, Cornelisse CJ: Prognostic significance of DNA-ploidy in a series of 690 primary breast cancer patients. Int J Cancer 45: 34–39, 1990
13. Guzman J, Rückmann A, Glaser A, Wittekind C, Schönfeld B, Kiefer G: DNA cytophotometric analysis of breast cancer. Follow-up for 10 years. Anal Quant Cytol Histol 14: 427–432, 1992
14. Norden T, Lindgren A, Bergström R, Holmberg L: Defining a high mortality risk group among women with primary breast cancer. Br J Cancer 69: 520–524, 1994
15. Sigurdsson H, Baldetorp B, Borg A, Dalberg M, Fernö M, Killander D, Olsson H: Indicators of prognosis in node-negative breast cancer. New Engl J Medicine 322: 1045–1053, 1990
16. Baldetorp B, Fernö M, Fallenius A, Fallenius-Vecchi G, Idvall I, Olsson H, Sigurdsson H, Akerman M, Killander D: Image cytometric DNA analysis in human breast cancer analysis may add prognostic information in diploid cases with low S-phase fraction by flow cytometry. Cytometry 13: 557–585, 1992
17. Batsakis JG, Sneige N, el-Naggar AK: Flow cytometric (DNA content and S-phase fraction) analysis of breast cancer. Cancer 71: 2151–2153, 1993
18. Longin A, Fontaniere B, Pinzini V, Catimel G, Souchier C, Clavel M, Chauvin F: An image cytometric DNA-analysis in breast neoplasms. Parameters of DNA-aneuploidy and their relationship with conventional prognostic factors. Path Res Pract 188: 466–472, 1992
19. Merkel E, Winchester DJ, Goldschmidt RA, August CZ, Wruck DM, Rademaker AW: DNA flow cytometry and pathologic grading as prognostic guides in axillary lymph node-negative breast cancer. Cancer 72: 1926–1932, 1993
20. Auer GU, Caspersson T, Wallgren A: DNA content and survival in mammary carcinoma. Anal Quant Cytol Histol 3: 161–165, 1980
21. Auer G, Eriksson E, Azavedo E, Caspersson T, Wallgren A: Prognostic significance of nuclear DNA content in mammary adenocarcinomas in humans. Cancer Res 44: 394–396, 1984
22. Fallenius AG, Auer GU, Carstensen JM: Prognostic significance of DNA measurements in 409 consecutive breast cancer patients. Cancer 62: 331–341, 1988
23. Fallenius AG, Franzen SA, Auer GU: Predictive value of nuclear DNA content in breast cancer in relation to clinical and morphologic factors. Cancer 62: 521–530, 1988
24. Mir R, Johnson H, Margolis M, Teplitz S, Wise L: Prognostic significance of DNA measurement determined by image analysis in human breast carcinoma. J Surg Oncol 50: 168–172, 1992
25. Yuan J, Hennessy C, Givan AL, Corbett IP, Henry JA, Sherbet GV, Lennard TW: Predicting outcome for patients with node negative breast cancer: a comparative study of the value of flow cytometry and cell image analysis for determination of DNA ploidy. Br J Cancer 65: 461–465, 1992
26. Stenkvist B, Strande G: Entropy as an algorithm for the statistical description of DNA cytometric data obtained by image analysis microscopy. Anal Cell Pathol 2: 159–165, 1990
27. Opfermann M, Brugal G, Vassilakos P: Cytometry of breast carcinoma: Significance of ploidy balance and proliferation index. Cytometry 8: 217–224, 1987
28. Böcking A, Adler CP, Common HH, Granzen B, Auffermann W: Algorithm for a DNA-cytophotometric diagnosis and grading of malignancy. Anal Quant Cytol 6: 1–8, 1984
29. Siitonen SM, Kallioniemi O-P, Helin HJ, Isola JJ: Prognostic value of cells with more than 5c DNA content in node-negative breast cancer as determined by image cytometry from tissue sections. Hum Pathol 24: 1348–1353, 1993
30. Tamura G, Masuda T, Satoh T, Satodate R, Ishida M, Saitoh

K: Karyometric and DNA content analysis of cancer cells in Stage III breast cancer with reference to prognosis. Jpn J Clin Oncol 20: 78–82, 1990
31. Baak JPA, Chin D, van Diest PJ, Ortiz R, Matze-Cok P, Bacus SS: Comparative long-term prognostic value of quantitative HER-2/neu protein expression, DNA ploidy, and morphometric and clinical features in paraffin-embedded invasive breast cancer. Lab Invest 64: 215–223, 1991
32. Mariuzzi GM, Mambelli V, Criante P, Sisti S: Quantitative evaluation of morphological parameters for infiltrating breast cancer prognosis. Path Res Pract 185: 698–700, 1989
33. Umbricht C, Oberholzer M, Gschwind R, Christen H, Torhorst J: Prognostic significance (relapse, non-relapse) of nuclear shape factors in lymph node negative breast cancer. Anal Cell Path 1: 11–23, 1989
34. Uyterlinde AM, Schipper NW, Baak JPA, Peterse H, Matze E: Limited prognostic value of cellular DNA content to classical and morphometrical parameters in invasive ductal breast cancer. Am J Clin Pathol 89: 301–307, 1989
35. van der Linden JC, Lindeman J, Baak JPA, Meijer CJLM, Herman CJ: The multivariate prognostic index and nuclear DNA content are independent prognostic factors in primary breast cancer patients. Cytometry 10: 56–61, 1989
36. Komitowski D, Kett P, Janson C, Jarasch E-D: Quantitative aspects in defining prognostic factors of breast cancer. Path Res Pract 185: 621–624, 1989
37. Komitowski D, Janson C: Quantitative features of chromatin structure in the prognosis of breast cancer. Cancer 65: 2725–2730, 1990
38. Komitowski D, Hart MM, Janson CP: Chromatin organization and breast cancer prognosis: Two-dimensional and three-dimensional image analysis. Cancer 72: 1239–1246, 1993
39. Rodenacker K, Aubele M, Burger G, Gais P, Jütting U, Gössner W, Oberholzer M: Cytometry in histological sections of colon carcinoma. Path Res Pract 188: 556–560, 1992
40. Aubele M, Burger G, Rodenacker K: Problems concerning the quality of DNA measurements on Feulgen-stained imprints. A study of five fixation techniques. Anal Quant Cytol Histol 16: 226–232, 1994
41. Rodenacker K: Invariance of textural features in image cytometry under variation of size and pixel magnitude. Anal Cell Pathol, accepted, 1994
42. Haralick RM, Shanmugam K, Dinstein IH: Textural features for image classification. IEEE Transactions on Systems, Man, and Cybernetics 3(6): 610–621, 1973
43. Galloway MM: Texture analysis using gray level run lengths. Computer Graphics and Image Processing 4: 172–179, 1975
44. Cox DR: Regression models and life tables. J Royal Stat Soc Series B, 34: 187–220, 1972
45. Haybittle JL, Blamey RW, Elston CW, Johnson J, Doyle PJ, Campbell FC, Nicholson RI, Griffiths K: A prognostic index in primary breast cancer. Br J Cancer 45: 361–366, 1982
46. Kaplan EL, Meier P: Nonparametric estimation for incomplete observations. J Am Stat Assoc 53: 457–481, 1959
47. von Rosen A, Rutquist LE, Carstensen J, Fallenius A, Skoog L, Auer G: Prognostic value of nuclear DNA content in breast cancer in relation to tumor size, nodal status, and estrogen receptor content. Breast Cancer Res Treat 13: 23–32, 1989