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AUTOMATED GRADING IN BREAST CANCER BY IMAGE ANALYSIS OF HISTOLOGICAL SECTIONS

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ABSTRACT

The aim of the study was to test the solidity of the conventional grading of breast cancer and to divide otherwise non-specified invasive ductal carcinomas into different groups by means of automated microscopic image analysis. Therefore, a pilot study was started in which biopsies were examined from 350 patients who had undergone standardized operations between 1984 and 1987. A wide range of methods was used for characterization of tumor differentiation by image analysis of Feulgen-stained histological sections, including karyometry, chromatin structure analysis, and histometry. For measurements, the Robotron A 6471 image analysis system with AMBA/R software was used. Based on previous studies into preneoplasia of the mammary gland, the prevalence of karyometric features could be demonstrated. Moreover, some new parameters of the nuclear chromatin structure seem to be also correlated with histological grading and, therefore, might be of prognostic relevance, although this will have to be proved by statistical analysis of morphometric and clinical data when the follow-up study will be finished.

Keywords: Breast cancer, histology, grading, image analysis, karyometry, histometry

INTRODUCTION

A clinico-morphological pilot study in brest cancer was undertaken to establish a histological tumor gradig with high reproducibility by means of image analysis of histological sections. The aim of the study was a splitting of non-otherwise specified invasive ductal carcinoms (NOS), to separate morphometrically defined tumor groups and to find out prognostically significant morphometric features. Based on previous studies into fibrocystic breast disease and preneoplasia of the mammary gland in which the prevalence of karyometric features has been demonstreted (Guski et al. 1988), chromatin structure analysis of tumor cell nuclei was suggested to be useful for prognostic validity.

MATERIAL AND METHODS

Clinical material

Material collected in the Institute of Pathology from 1984 to 1987 from 439 female breast cancer patients was investigated. All patients were operated in the Department of Surgery, Humboldt University of Berlin, applying a standard therapy protocol (Winzer et al. 1989).

Case selection

Twenty cases of primary invasive ductal breast cancer, histologically non-otherwise specified (NOS) according to UICC criteria, were selected for image analysis from 350 morphologically examined tumor cases. All patients of the study group were premenopausal and in the TNM stage pT1, with a tumor size from 5 to 20 mm of diameter. Nineteen of these patients had no axillary lymph node metastases (pN0) and one patient was staged as pN1. All patients were treated by lumpectomy, axillary lymphadenectomy, and axillary radiation. In 50 % of the cases a fibrocystic disease was noticed. The follow-up data were available from 35 to 60 months (Table 1).

Preparation of histological sections

From specimens fixed in 10 % buffered formalin for 24 hours and embedded in paraffin, four micrometer hematoxylin-eosinstained sections were taken for histological typing and grading according to Bloom and Richardson (1957), modified by Schnürch et al. (1986). For image analysis, Feulgen staining was performed using the method of Kiefer (1978) as described

previously (Simon et al. 1984). For each measured microscopic field (test field), a grading was performed in Feulgen-stained sections using the same criteria as for the histological grading of hematoxylin-eosin-stained sections. The results of both tumor case grading and test field grading were included in the statistical analysis of morphometric data.

Table 1

Clinical data of patients with breast cancer and biopsy material examined by automated image analysis. Staging of fibrocystic breast disease after Prechtel (1972).

INVASIVE DUCTAL CARCINOMAS (NOS)
pT1 pNO

CASE No	AGE (years)	INTERVALL AFTER OPERATION (months)	TUMOR SIZE (mm)	FIBROCYSTIC BREAST DISEASE (degree)
1	44	60	20	III
2	48	57	15	
3	49	57	10	
4	47	55	20	
5	42	55	15	III
6	44	50	15	
7	45	50	20	I
8	44	48	20	II
9	31	47	10	III
10	42	47	15	III
11	50	46	20	II
12	47	46	20	III
13	49	46	15	III
14	50	44	20	II
15	45	43	17	
16	47	41	12	
17	33	40	20	
18	47	38	13	
19+	34	36	20	
20	46	35	5	

+ pT1 pN1

Microscopic image analysis

The Robotron A6471 image analysis system with AMBA/R software (Roth 1989) combined with an automated scanning microscope, was used for karyometric and histometric measurements. A userfriendly algorithm for interactive corrections of imperfect results in cell nuclei isolation after automated image analysis was implemented, especially for cell nuclei conglomerates. Voronoi diagram and Johnson-Mehl tesselation have been applied as models for histometric characterization (Hufnagl 1990). For every tumor case, 10 microscopic fields were selected in the invasive zone of the tumor. This selection was performed randomly at low magnification (objective 12.5) and the coordinates of each field were stored in the memory. The test fields were then measured by using an objective 100 (oil immersion) with a magnification of 800 x. As a result of the automatic and interactive seperation of touching nuclei three types of nuclei appeared: 1. Nuclei without any artificial changes of contour and texture, 2. Nuclei with original contour, but texture influencd by manipulations, 3. Nuclei with modified contour and texture. For nuclei of the first type it is sensible to measure their size and to analyse their texture. For the second type of nuclei only the measurement of the contour is possible. The nuclei of the third kind may only be counted (Table 2).

Table 2

Criteria for classification of cell nuclei (Nc) in breast cancer and number of measured and counted nuclei, respectively (for details see material and methods).

CLASS OF NUCLEI	OBJECT OF MEASUREMENTS	TEXTURE OF NUCLEI	CONTOUR OF NUCLEI	NUCLEI MEASURED	NUCLEI COUNTED	MEAN NUMBER PER TUMOR
1	Nc of tumor cells	correct	correct	yes	yes	180
2	Nc of tumor cells	non correct	correct	yes	yes	100
3	Nc of tumor cells	non correct	non correct	no	yes	470
4	Nc of stromal cells	correct	correct	yes	yes	50
5	Nc of lymphocytes	any texture	correct	yes	yes	20
6	Nc of blood cells	any texture	any contour	no	yes	60
7	Nc of stromal cells	non correct	non correct	no	yes	90

Seven cell classes were distinguished and measured or only counted, using the above criteria. The classification of the objects was performed as an interactive operation in all cases. In each case, at least 280 tumor cell nuclei were measured, from which 180 nuclei were analysed for features of the chromatin structure (Figs. 1,2).

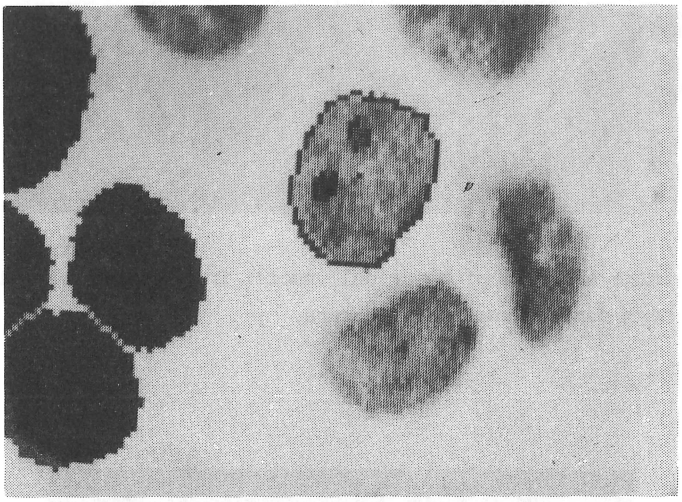

Figure 1.: Image analysis of tumor cell nuclei in breast cancer with low dense chromatin structure.

For this nuclear texture analysis, a sophisticated computer program was developed which will be described separately in detail (Wolf et al., in press). Karyometric and densitometric measurements were also performed for nuclei of stromal cells (fibroblasts, fibrocytes, histiocytes etc.) and lymphocytes. Voronoi and Delauney analysis was included for histometric features, such as artificial tumor cell boundary reconstruction and analysis of touching and overlapping tumor cell nuclei. For the separation of such conglomerates of nuclei, automated operations (Wolf and Voss 1987) and interactive procedures were used (Figs. 3,4).

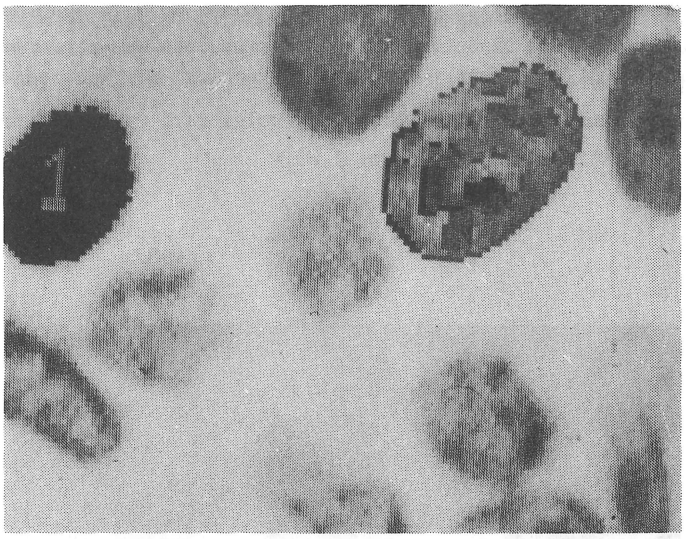

Figure 2.: Image analysis of tumor cell nuclei in breast cancer with high dense chromatin structure.

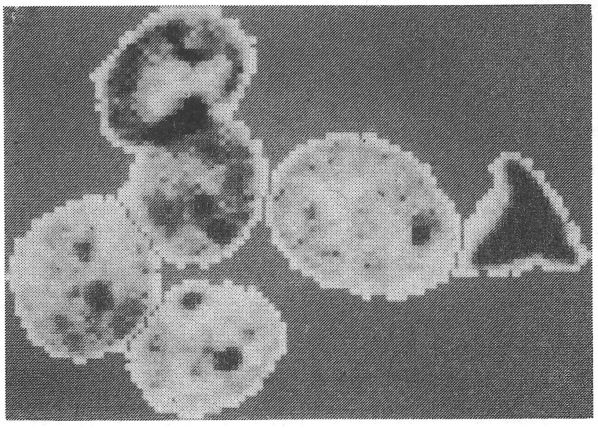

Figure 3.: Separation of touching tumor cell nuclei in breast cancer by automated or interactive procedures for isolation and measurement of nuclei.

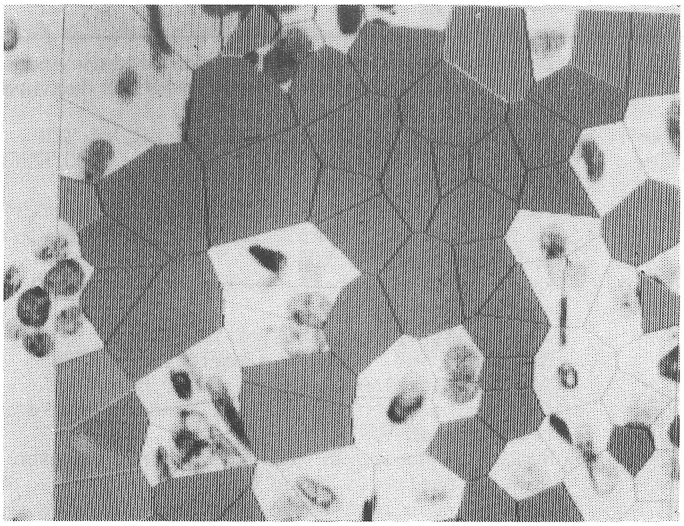

Figure 4.: Reconstruction of tumor cell boundaries by application of Voronoi diagram tesselation.

For the characterization of cellular differentiation and for the comparison of tumor case grading with tumor test field grading, 28 karyometric and histometric features were selected (Table 3).

Table 3

Morphometric features selected for tumor grading by automated image analysis.

SELECTED FEATURES (n = 28)

No	Feature	Description
12	ACO(M)+	Area of tumor cell nuclei (TCN)
15	SEX1(R)	Sum of grey values of TCN
22	HEX1(M)+	Relative hole area of TCN
25	REX1(R)	Range of grey values of TCN
27	MEX1(R)+	Mean grey value of TCN
28	GANZ(M)+	Total number of texture line points of TCN
30	HANZ(M)+	Next neighbor texture line points to clear areas of TCN
32	DANZ(M)+	Next neighbor texture line points to dark areas of TCN
34	HDAN(M)+	Next neighbor texture line points to clear and/or dark areas of TCN
36	HLNG(M)+	Length of sample lines to clear areas of TCN
38	DLNG(M)+	Length of sample lines to dark areas of TCN

No	Feature	Description
40	HDLN(M)	Length of sample lines to clear or dark areas ot TCN
42	ANZD(M)+	Difference of number of next neighbor texture line points of TCN
46	TXTF(M)+	Texture line points per area points of TCN
48	KNTR(M)+	Feature of contrast: (HLNG - DLNG . 1000/HLNG + DLNG) + 1000 (TCN)
58	HDST(M)+	Sample line distribution to clear areas of TCN
60	HDST9	90 % quantile of sample line distribution to clear and dark areas of TCN
61	FREQ(M)	Ratio of sign changing number of grey level to contour area of TCN
67	ZNTR(M)+	Centricity of chromatin of TCN
69	SIZE(M)	Run length of chromatin particles of TCN
72	RKPL(R)+	Nucleus/cytoplasm ratio of tumor cells
73	VAB(M)+	Distance between centre of TCN and circum-scribing rectangle of VORONOI cells
75	RNKO(M)	Number of nuclei in tumor cell conglomerates
77	KACO(M)	Area of nuclei in tumor cell conglomerates (M)
78	KACO(R)	Area of nuclei in tumor cell conglomerates (R)
81	ACO4(M)	Area of stromal cell nuclei
83	ACO5(M)	Area of lymphocytic nuclei
86	SEX5(R)	Sum of grey values of lymphocytic nuclei

\+ = Features selected in both groups, test field grading and tumor case grading, respectively
(M) = Mean value
(R) = Standard deviation

Statistical evaluation

Multivariate analysis was performed by a commercially available statistical software program NCSS (Number Cruncher Statistical System). For the feature selection discriminant analysis based on regression coefficients was used. The correlation between features and tumor differentiation was determined using robust rank correlation.

RESULTS

In total, 28 features selected for statistical analysis were found to be suitable for characterization of tumor differentiation, ranging from 3 to 9 points, when adeno-tubular formation, polymorphism of nuclei, and frequency of mitoses were determined. Statistical correlation was observed for 11 parameters in cases with moderate and poor tumor differentiation (6 to 9 points), whereas for well differentiated tumors (3 to 5 points) a correlation was proved only for 5 parameters (MEX1/R), TXTF (M), HDST(M), RKPL(R), VAB(M), see Table 3). When test field grading was applied, a strong correlation was found for the whole range of tumor

differentiation for 7 parameters (MEX1(R), HANZ(M), HLNG(M), ANZD(M), VAB(M), KACO(R)). Comparison of tumor case grading with test field grading showed a correlation for 5 features (ACO(M), HEX(M), MEX1(R), DANZ (M), HDAN(M)). For test field grading, a stronger correlation was found to exist between the feature values and tumor differentiation than for the grading of the whole tumor, but in both cases the standard deviation was considerably (Fig. 5). Tumor grading was performed in each case twice by two pathologists with intraobserver and interobserver reproducibilities of 72,7 % and 63,3 %, respectively.

DISCUSSION

Recent studies have confirmed the prognostic significance of nuclear DNA distribution pattern in breast cancer patients (Fallenius et al. 1988, Böcking et al. 1989 a, b). DNA measurements were performed in fine needle slide preparations or in monolayer smears prepared from paraffin-embedded tissues using microspectrophotometry or automated microscopic image analysis, respectively. However, DNA measurements in histological sections are not possible without limiting assumptions (Rigaut et al. 1987; Rigaut and Persoz 1989). Moreover, the correlation between histological and cytological values is low (Fallenius 1986). Image analysis of nuclear chromatin texture, therefore, could be more useful with respect to the prognostic relevance of karyometric features when using sections. In the pilot study, some parameters which describe nuclear texture are clearly correlated with the histological tumor grading, containing prognostic information as demonstrated by different studies (for a review, see Guski et al. 1989). The wide range of standard deviation of values obtained by image analysis (see Fig. 5) is probably caused by errors in subjective histological grading and other factos, too, mainly by the presence of well and poorly differentiated areas within the same tumor.

In contrast to the morphometric study published recently by Umbricht et al. (1989), nuclear shape parameters did not show a correlation with histological tumor grading. Umbricht et al. demonstrated that the correlation between tumor pleomorphism (described by several form factors) and prognosis was stronger than the correlation between tumor grading and prognosis. It is also our conclusion that nuclear pleomorphism could be one of the prognostically most relevant components of tumor grading in breast cancer. Therefore, it should be necessary to complete our

pilot study by establishing additional form descriptors which are adequate to characterize the shape of tumor cell nuclei.

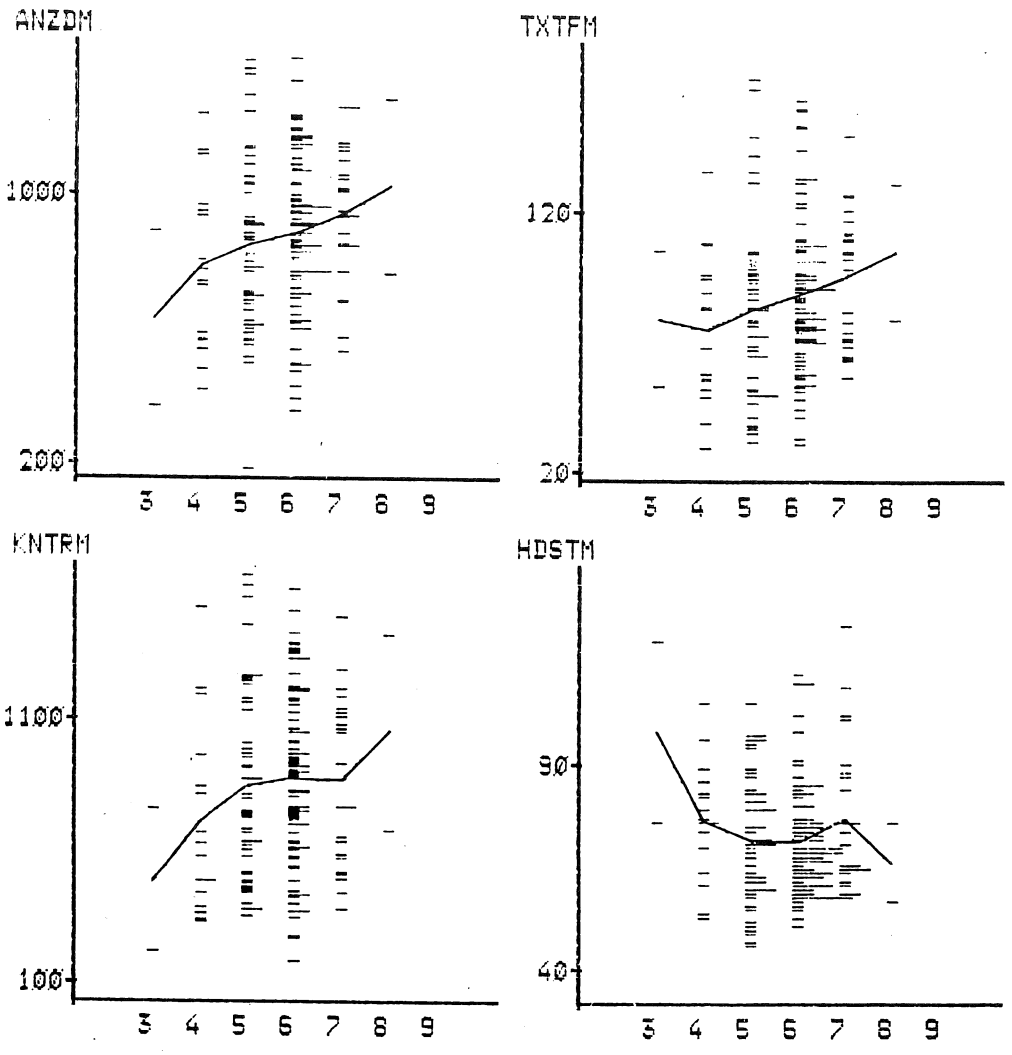

Figure 5.: Results of chromatin structure analysis of tumor cell nuclei (description of features see Table 3) for histological grading (3 to 9 points after Bloom and Richardson) in breast cancer.

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