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Prognostic Scoring using Cytomorphometry and Lymph Node Status of Patients with Breast Carcinoma

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Abstract—*Applying morphometry on FNA smears may lead to meaningful prognostic subgrouping of breast carcinomas. A statistical analysis of the cytomorphometric and clinical data of 73 patients with breast carcinoma was performed. By multivariate analysis, taking into account various prognostic factors, it was shown that variations in nuclear area and the presence of axillary metastases were the most important prognosticators. By weighing these factors a prognostic score was obtained. According to that score the patients were classified into 4 score groups: the decreasing survival with increasing score was evident. The prognostic score was also related to the metastasis-free period. This prognostic scoring is relatively easy to perform, and can be done in routine pathology laboratories.*

INTRODUCTION

THE CLINICAL course of patients operated on for carcinoma of the breast depends to a great extent on the potential of the tumor to form distant metastases in the pre-operative period. It is known that this varies greatly between different tumors and is only partly expressed in the finding of metastatic tumor in axillary lymph nodes [1]. Not only the presence of axillary metastases but also the histologic grade of the tumor [2–4] and the tumor size are related to the prognosis [5–8]. The fact that well-differentiated tumors are seldom found to have positive axillary lymph nodes re-emphasizes the low metastatic potentials of these well-differentiated tumors [3]. The expected prognosis of a patient can be expressed by a prognostic index, in which the histologic tumor grade is incorporated [5]. However, the histological grading is based on subjective analysis of the tumor and is, in consequence, less successful when the grading is performed by different pathologists. There is therefore a need for objective grading systems.

Baak *et al.* [9] have shown that objective quantitative assessment of the tumor is highly consistent. It has predictive value and the applied quantitative features, the cellularity index and the mitotic index, are better prognosticators than the presence of positive axillary lymph nodes and tumor grade alone.

The quantitative features of the tumor cells in the cytological preparations of fine-needle aspirations (FNA) of breast tumors can also be measured and analysed. The mean nuclear area of the tumor cells has been shown to be related to the development of metastases and to the prognosis [10–12]. A correlation between the variance of nuclear area, mitotic frequency and recurrence rate has also been found [13].

In this paper we report on various morphometric nuclear parameters of tumor cells in FNA smears. Using these measurements in conjunction with the lymph node status, we performed a multivariate analysis to discover the best prognostic factors. It was then possible to make a weighted formula using the two most important prognosticators that allowed the patients to be classified into 4 separate groups that matched the biological patterns of the disease.

MATERIALS AND METHODS

Patients

Fine-needle aspiration (FNA) smears of breast carcinomas of 73 patients were used for this study.

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The criteria for the selection of patients were that the tumor had been confirmed histologically and that the patients had no evidence of distant metastases at the time of operation. Only patients with a complete follow-up were included. The patients were followed up for a minimum of 5 yr or until death. All patients were operated on between 1973 and 1976. Seventy-one were treated by modified radical mastectomy and two underwent simple mastectomy only. In cases with medial tumors and in those lateral tumors with positive axillary lymph nodes, post-operative radiotherapy was given. The patients were clinically re-examined at regular intervals. Local recurrences and distant metastases were treated with conventional methods.

The following data were recorded: age, menopausal status at diagnosis, T-category (UICC, 1978), axillary lymph node metastasis in the surgical specimen, time interval between operation and demonstration of local recurrence (tumor in the mastectomized region) or distant metastasis, and survival time.

Morphometric studies

The morphometric analyses were performed on air-dried Giemsa-stained aspiration smears. The smears were prepared as follows: aspiration smear air-dried for +/- 5 min. May–Grünwald–Giemsa (MGG) stain (3 min in May–Grünwald stain, rinsed with buffer, 9 min in diluted Giemsa, air-dried and mounted in Permount; pH buffer 6.5). This technique is well suited to morphometric studies due to the constancy of nuclear and cellular sizes [14]. The cytological diagnosis was positive for malignancy in 63 cases and suspicious in 10. The cytological suspicious cases were included in the study as well because we selected the patients on the basis of histological proven tumors. Different smears were examined in all cases. The smears had to be of good quality, meaning they had to contain enough (at least 100) intact tumor cells. The best smear in each case was selected according to these criteria. All aspirates were made by the same experienced cytologist (D. I. Blonk).

The morphometric analyses were all performed by one person, using a graphic tablet equipped with a camera lucida. In each smear 100 carcinoma cells were selected at random and the nuclei were outlined using the digital cursor [15]. No measurements were made of cytoplasmic area. In each case the mean and standard deviation of the 100 measured nuclei was obtained by means of the following parameters: perimeter, area and diameter $[2\sqrt{(\text{area}/\pi)}]$. A histogram of measured nuclear areas for each case was also obtained. A typical example of such a histogram is shown in Fig. 1.

The clinical data were not known to the person who did the measurements. To test whether results were reproducible, 20 cases were measured after an interval of 1 month. The agreement between the two measurements of the various parameters was satisfactory, although the standard deviation of the nuclear area appeared less reproducible in a few cases (Fig. 2).

Statistical analysis

Intercurrent-death (i.e. death not caused by breast cancer)-corrected survival curves were calculated on an actuarial basis [16]. Statistical comparison of such curves of groups of patients was done by the log-rank test [16]. To investigate simultaneously the prognostic importance of the various parameters, use was made of multivariate survival analysis methods [17]. In this way a prognostic score was obtained which weighed the various prognostic factors [18].

RESULTS

The clinical data of the 78 patients with breast carcinoma who met the selection criteria are presented in Table 1. Only 9 patients had tumors smaller than 2 cm. To investigate the prognostic

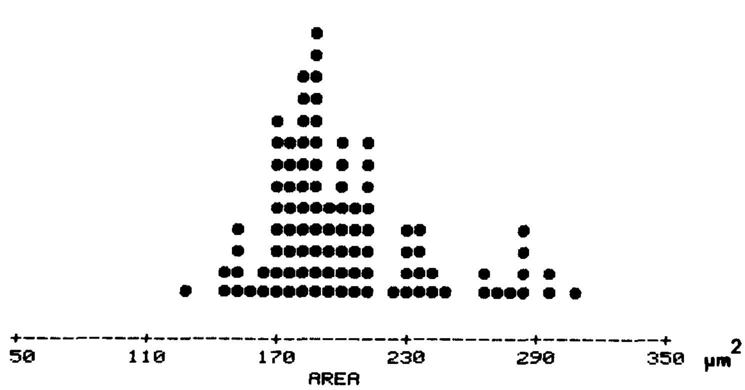

Fig. 1. Histogram of area measurements of 100 nuclei in one representative case.

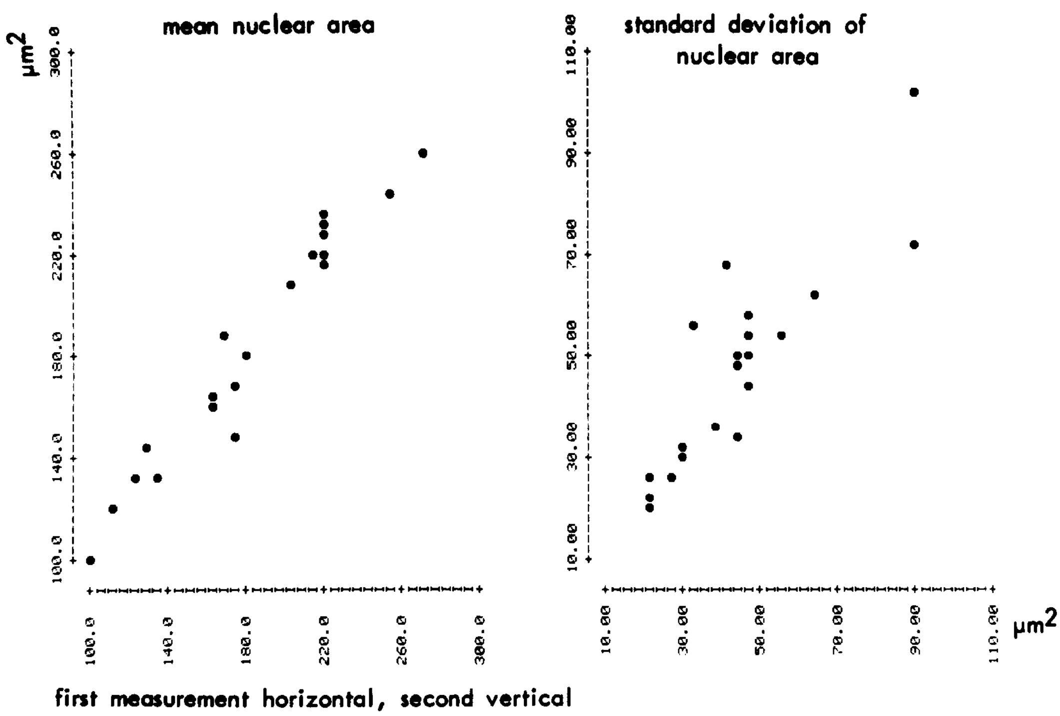

Fig. 2. Diagram of measured values of the mean (left) and the standard deviation (right) of the nuclear area of 20 cases on two different occasions.

value of each morphometric nuclear parameter separately, patients were grouped according to measured values. In Table 2 the intercurrent-death-corrected survival percentages at 5 yr are given for groups of patients with increasing values of the mean and standard deviation of the three measured parameters. These percentages according to clinical information (T-category of the tumor, presence of axillary metastases, age and menopausal status of the patient) are also given. All measured morphometric parameters showed a significant correlation with survival. The presence of axillary metastases at operation appeared to be an ominous sign, but no relation could be demonstrated between these metastases and any morphometric parameter. Although survival was slightly reduced with increasing T-category (survival percentages at 3 yr for categories T1, T2 and T3 were 89, 77 and 69 respectively), these differences were not statistically significant. Survival was related to neither the age nor the menopausal status of the patients.

By multivariate survival analysis, taking account of all investigated factors listed in Table 2, it appeared that the standard deviation of the nuclear area and the presence of axillary metastases were the most important prognosticators.

Another measure of variation in area between nuclei which was available, namely the 80%-interpercentile range, also correlated with survival (the 80%-interpercentile range is defined as the difference between the 90th and 10th percentiles, i.e. it is the range which covers 80% of measured nuclear areas). In the multivariate analysis this factor was a better prognosticator than the standard deviation of the nuclear area.

Survival of patients in relation to the 80%-percentile range of nuclear area and axillary metastases respectively is given in Fig. 3. The

Table 1. Clinical data of 73 patients with breast carcinoma

		T?	T1	T2	T3	Positive lymph nodes	Negative lymph nodes	lymph nodes?
Premenopausal	18	1	4	9	4	8	10	
Postmenopausal	54	1	5	39	9	28	24	2
Menopause unknown	1			1		1		
Total	73							

Table 2. Intercurrent-death-corrected 5-yr survival in relation to six nuclear morphometric parameters, T-category of tumor, presence of axillary metastases and age of patients

Characteristic	No. of patients	Survival (%)	Statistical significance
Mean area (μm^2)			
<160	22	86	
160–200	26	78	$P = 0.003$
>200	25	53	
Mean diameter (μm)			
<14	20	84	
14–16	29	77	$P = 0.009$
>16	24	56	
Mean perimeter (μm)			
<55	22	86	
55–62	25	77	$P = 0.008$
>62	26	55	
S.D.* area (μm^2)			
<35	29	93	
35–50	22	76	$P < 0.001$
>50	22	43	
S.D. diameter (μm)			
<1.5	30	90	
1.5–2.0	23	72	$P < 0.001$
>2.0	20	48	
S.D. perimeter (μm)			
<6.5	23	86	
6.5–8.5	29	81	$P < 0.001$
>8.5	21	45	
T-category			
T1	9	76	
T2	49	75	$P = 0.43$
T3	13	69	
Axillary metastases			
absent	34	87	
present	37	59	$P = 0.008$
Age (yr)			
<55	28	75	
55–65	20	57	$P = 0.42$
>65	25	82	
Menopausal status			
pre-	18	78	
post-	54	69	$P = 0.66$

Missing data: T-category (2), axillary metastases (2), menopause (1).
*S.D. = standard deviation.

decreasing survival with increasing range of the nuclear area was apparent for patients with, as well as for those without, axillary metastases (Fig. 4). Based on the multivariate analysis, a prognostic score was obtained which weighs the two most important prognosticators.

By combining the two variables that had statistically significant coefficients in the Cox multivariate analysis, the following prognostic score was derived: $0.03 \times \text{range} + 1.9$ (if positive lymph nodes are present). In this formula 'range' denotes the 80%-interpercentile range of nuclear area, expressed in μm^2. The value 1.9 in the formula is only added to the preceding term when positive lymph nodes are present. For example, a patient without axillary metastases and a measured value of the 80%-interpercentile range of 105 μm^2 receives a score of: $0.03 \times 105 = 3.15$.

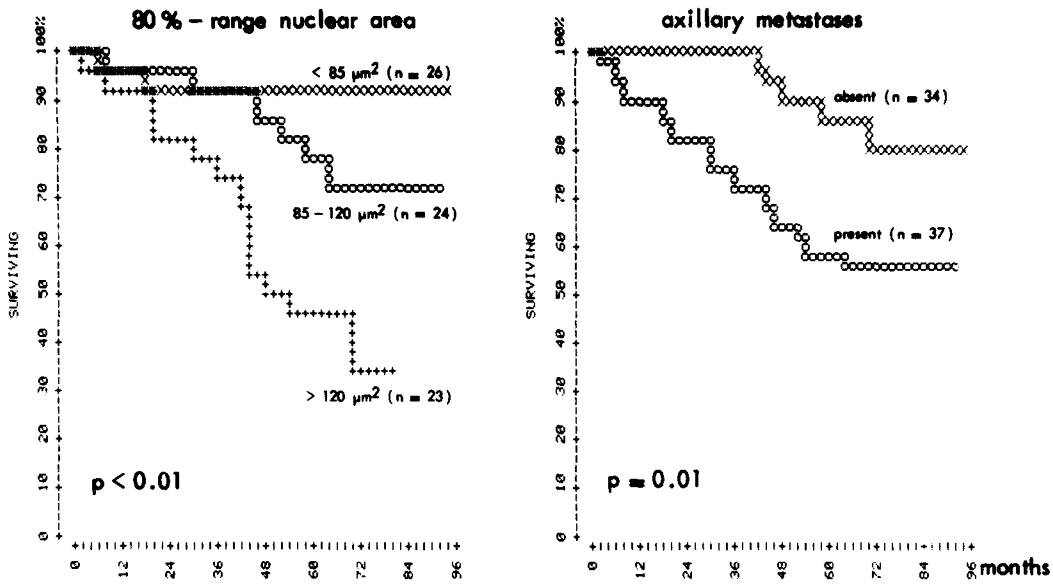

Fig. 3. Intercurrent-death-corrected survival by 80%-interpercentile range of nuclear area (left) and by presence of axillary metastases (right).

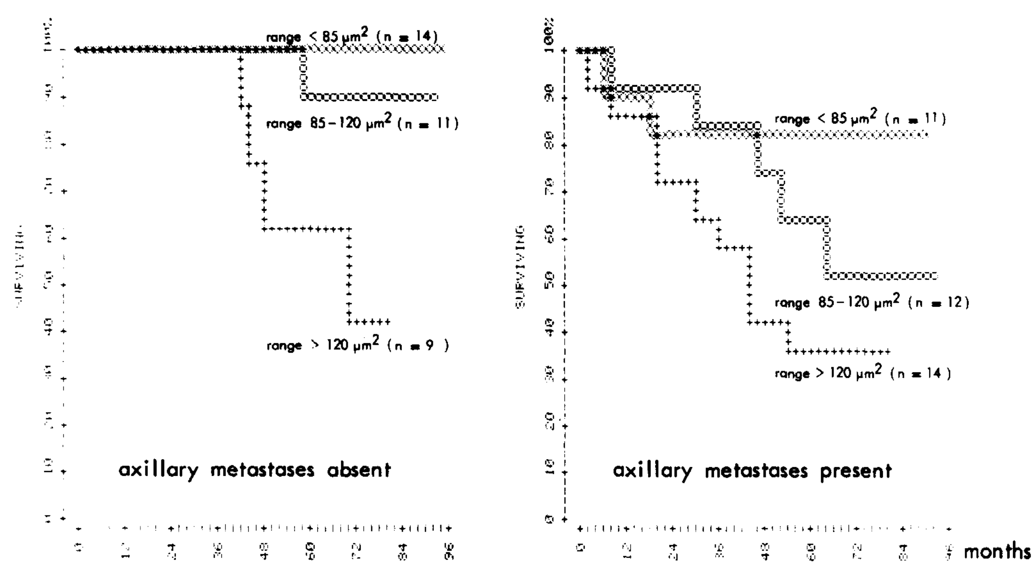

Fig. 4. Intercurrent-death-corrected survival according to 80%-interpercentile range and lymph node status.

The scoring is such that with increasing score survival decreases. After calculation of the prognostic score for each patient, the patients were grouped into 4 score groups (group I, scores <2.5; group II, scores 2.5–4.0; group III, scores 4.0–5.5; group IV, scores >5.5). The survival curves of these 4 groups of patients are plotted in Fig. 5. The decreasing survival with increasing score group is evident. Actuarial cumulative percentages of patients with distant metastases for the same 4 groups are shown in Fig. 6. There was no apparent relationship between prognostic scores and the incidence of local recurrences.

DISCUSSION

It is well documented that the presence of positive lymph nodes is a manifestation of an interrelationship between the tumor and the host that permits the outgrowth of a tumor cell population in the lymph node and, consequently, to a certain extent correlates with distant metastasis, e.g. prognosis [1]. It is logical to assume that the biological metastatic potentials of the tumor cells play a key role in this relationship. It might be expected that the morphometric characteristics of the tumor cells would reflect the biological potentials of the tumor. Indeed, we did

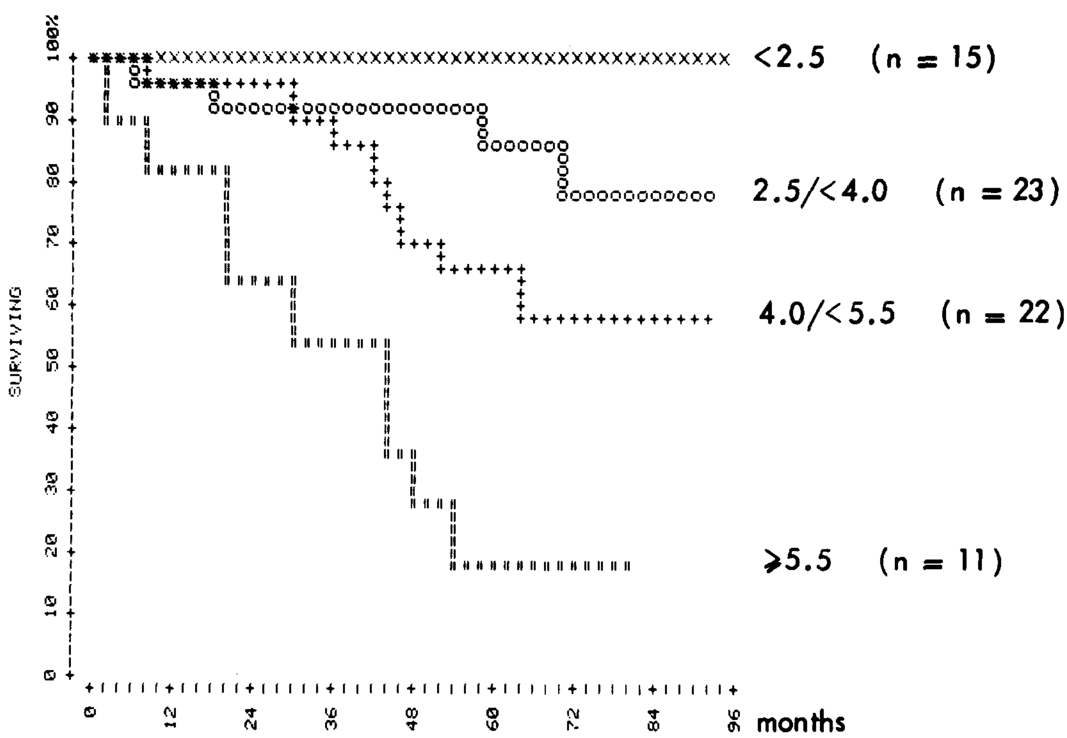

Fig. 5. Intercurrent-death-corrected survival by prognostic score [=0.03 × *range* + 1.9 (*if positive lymph nodes*)].

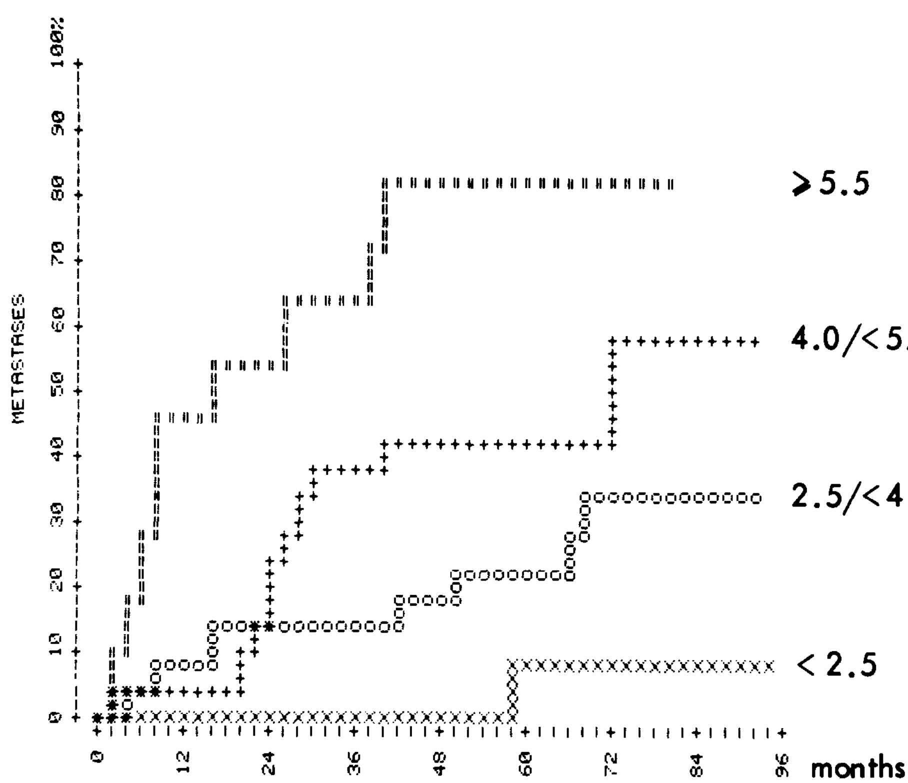

Fig. 6. Cumulative percentages of patients with distant metastases according to prognostic score.

find a relation between cytomorphometric characteristics and prognosis.

Our findings are in accordance with those that have already been published. Other authors have shown that there is a relation between the nuclear size and variance of breast cancer cells and prognosis [10–13]. Tumors with a small nuclear area and little size variation were shown to have a better prognosis. Morphometrically, there exists a significant overlap between nuclear size of benign tumors and the so-called small-type carcinomas, and these are consequently difficult to diagnose [10, 12, 15]. This phenomenon was also applicable to our material: 9 of the 10 suspicious smears in our series had relatively small nuclei, with an 80%-interpercentile range of <85.

In the scoring of the individual patients in our series, a combined use was made of morphometric data and lymph node status. This demonstrated that lymph node status and morphometric data are factors which are independently related to survival. It was therefore possible to create subgroups of patients with great differences in survival (Fig. 5). It became evident that this scoring is more closely correlated with prognosis than when lymph node status alone is used. This is in accordance with the findings of Haybittle *et al.* [8], who reported on a prognostic index based on lymph node stage, tumor size and pathological grade. They, too, found that their prognostic index gave a better discrimination than lymph node stage alone. Since lymph node stage is an important part of our index, the use of fine-needle aspiration does not avoid the necessity of carrying out subsequent surgical removal of the lymph nodes. It might be that the combination of morphometry of the breast tumor in combination with morphometry of the nodal tumor [21] could result in a more refined prognostic index.

Another aspect of the importance of the subdivision into 4 score groups is shown in Fig. 6, in which the metastasis-free periods are plotted. In groups III and IV the metastasis-free periods were generally much shorter than in groups I and II. The four score groups show the same ranking order as in Fig. 5. Table 3 shows that 36% of the patients in score group III and 81% of the patients in score group IV died of cancer. It appears that the calculated score also reveals something about the aggressiveness of the tumor as well.

The finding that none of the other morphometric parameters are represented in the prognostic formula, even though they all showed a significant correlation with survival (Table 2),

Table 3. The four score groups in relation to their follow-up

Score group	Alive and recurrence-free	Alive with recurrence	Intercurrent death	Cancer death (%)	Total
I	10	2	3	0 (0)	15
II	13	4	2	4 (17)	23
III	9	2	3	8 (36)	22
IV	1	1	0	9 (81)	11

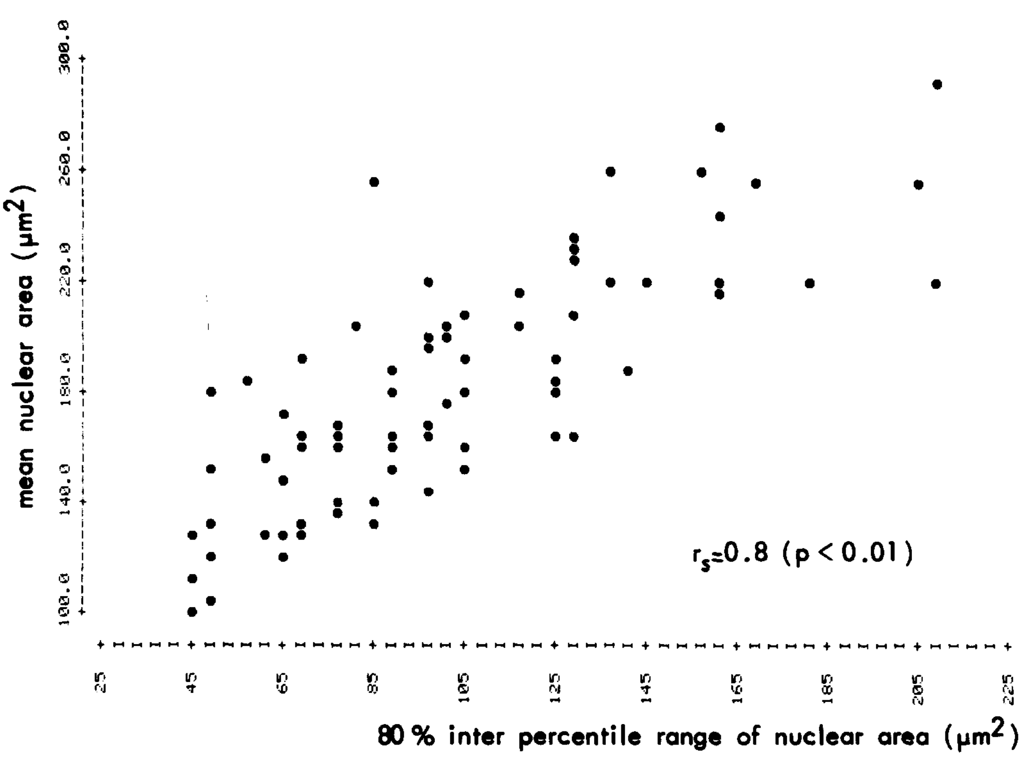

Fig. 7. Correlation between the mean and the 80%-interpercentile range of the nuclear area.

can be explained by the fact that they all correlated with the 80%-interpercentile range. For example, in Fig. 7 the correlation between the mean and the variation of the nuclear area is demonstrated. There was no additional value in the other parameters after allowing for the 80%-interpercentile range of the nuclear area. It should be stressed that our quantitative data are only valid for cytologic preparations that are fully air-dried before staining (see Materials and Methods). The sizes of cells and nuclei in cytologic preparation vary greatly, depending on the cytopreparation technique applied [14]. Even small differences in technique result in important quantitative discrepancies. For instance, in cases of follicular tumors of the thyroid, when the cells are immediately stained by a commercial variation of Wright's stain (Diff. Quick, American Scientific, McGraw Park, IL), the nuclei are only half the size of nuclei in fully air-dried MGG-stained preparations [19, 20]. In the first method the cells are not fully spread, and shrink in the first staining bath. Therefore the results of the two studies on morphometry of fine-needle aspirations of the thyroid cannot be compared.

Our findings show that morphometric assessment of the tumor cell population can give clinically important information of the prognosis of the individual patient. We are in the process of testing our prognostic index on a separate group of patients to ascertain whether or not the same predictive value applies.

Applying morphometry to FNA smears can lead to interesting and meaningful prognostic subgrouping of breast carcinomas, which may help in the selection of adjuvant therapies and in the comparison of outcomes in clinical trials if this objective method is adopted on a larger scale. The equipment needed for this assessment is relatively inexpensive and the measurement of the 100 nuclei takes approximately 40 min for a trained cytotechnician.

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