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THE PROGNOSTIC VALUE OF QUANTITATIVE HISTOPATHOLOGY IN BREAST CANCER: RESULTS OF RETROSPECTIVE STUDIES

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ABSTRACT

The results of retrospective studies of breast cancer patients, regarding the prognostic value of quantitative histopathologic estimates, are summarized. The stereologic estimates of the volume-weighted mean volume of cancer cell nuclei were of independent prognostic value, both in breast cancers of the ductal and the lobular types. This variable is easily obtained from ordinary histologic sections, highly reproducible, and suitable for routine use. In addition, in breast carcinomas of the lobular type, estimates of the mitotic frequency were strong predictors of the prognosis.

Key words: breast cancer, morphometry, nuclear volume, prognosis, stereology.

INTRODUCTION

The outcome of disease in patients with operable breast cancer can partly be predicted by a combination of tumor diameter and lymph node status. However, in about 30 per cent of the cases the course is contrary to the expected. One approach to improve the prognostic prediction is by examination of the histologic specimen, but the reproducibility of traditional *qualitative* malignancy grading of tumors has been questioned (Stenkvist *et al.*, 1979). Several *quantitative* histopathologic variables have, in contrast, been shown to be highly reproducible and of prognostic value (Aaltomaa & Lipponen 1992, and Baak *et al.*, 1985). In the current study, the prognostic significance of stereologic estimates of the volume-weighted mean nuclear volume and various two-dimensional morphometric estimates is evaluated. The relative value of these and of traditional clinicopathologic variables are assessed in multivariate survival analyses.

MATERIALS AND METHODS

Patients

Specimens from 71 consecutively treated patients with ductal breast carcinoma, diagnosed at the University Institute of Pathology, Aarhus Kommunehospital, between January 1983 and December 1985, were retrieved. All patients were included in the Danish Breast Cancer Cooperative Group 1982 protocol (Andersen & Mouridsen, 1988). According to the protocol, all patients were under 70 years of age and had operable breast cancer with known lymph node status. The median follow up was 6.1 years. The patients have been described in detail in a submitted paper. In another study (Ladekarl & Sørensen, 1993), specimens from 53 consecutively treated patients with operable lobular breast carcinoma, diagnosed from January 1980 to December 1986, were retrieved from the files at the University Institute of Pathology, Aarhus Kommunehospital and Aarhus Amtssygehus. The median follow-up of these patients was 8.7 years.

Quantitative histopathology

One or two hematoxylin-eosin stained, 4 μm thick, histologic sections from each tumor were investigated. For the estimation of the quantitative histopathologic variables, an Olympus BHS microscope equipped with a 100 W light source and a projection attachment was employed. Using a 100 X oil immersion lens, the final magnification was 1,850 X. On average, 15 fields of vision were sampled. The first field was selected at *random*, and the subsequent fields were sampled *systematically* by adjusting the distance between individual fields roughly proportional to the square root of the overall tumor sectional area. Tumor areas showing inflammation, necrosis or nuclear pyknosis were excluded. Only cancer cell nuclei in focus in the selected focal plane (of depth ~ 0.5 μm) were considered for sampling.

The volume-weighted mean nuclear volume, $\bar{v}_V(\text{nuc})$, was estimated using point-sampled intercepts (Gundersen & Jensen, 1985, and Sørensen, 1991). A test system with points and associated lines was randomly thrown on the image of the tumor (Fig. 1). If a nuclear profile of a cancer cell was hit by a test point, the length of the nuclear intercept – *i.e.* the distance from nuclear border to nuclear border through the test point along the associated test line – was measured with an l_0^3-ruler with fifteen classes. This ruler is "UFAPP" (*u*nbiased *f*or *a*ll *p*ractical *p*urposes) (Gundersen *et al.*, 1988), if more than 5 to 10 clases are used. The $\bar{v}_V(\text{nuc})$ was estimated by

$$\bar{v}_V(\text{nuc}) = \frac{\pi}{3} \cdot \bar{l}_0^3 . \qquad (1)$$

It was assumed that the nuclei were isotropically orientated in three-dimensional space. Using the area associated with each point in the test system, a(p), the nuclear volume fraction, $V_V(\text{nuc/tis})$, could be estimated by

$$V_V(\text{nuc/tis}) = P_P(\text{nuc/tis}) = \frac{N \cdot a(p)}{n_f \cdot A} . \qquad (2)$$

N is the total number of nuclei hit by points, n_f is the number of fields of vision with area A, and

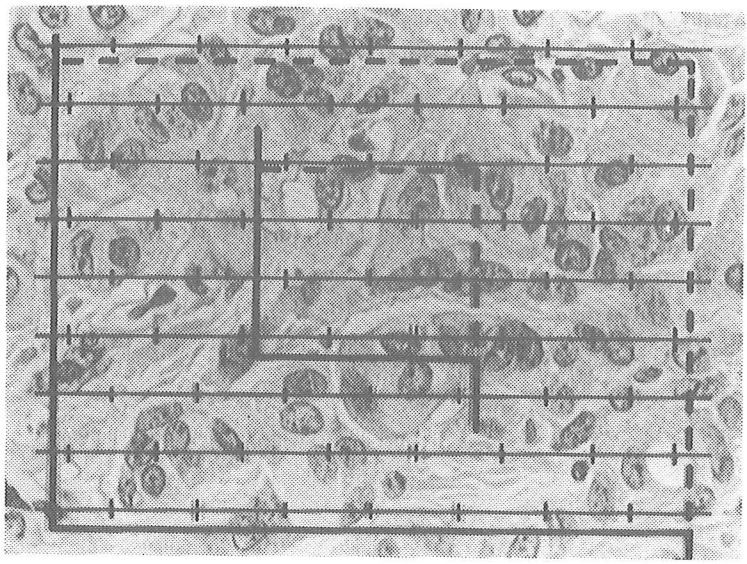

Figure 1. Field of vision from a ductal breast carcinoma projected onto a test system (original magnification X 700).

P_P(nuc/tis) is the number of points hitting nuclei as a fraction of points hitting tumor tissue. Two counting frames integrated in the test system were used for estimating profile numbers by an unbiased counting rule (Gundersen, 1977). Estimates of the nuclear profile density, ND (the number of nuclear profiles per mm^2 of tumor), were obtained by

$$ND = \frac{Q(nuc)}{n_f \cdot A_1}, \tag{3}$$

where Q(nuc) is the total number of nuclear profiles counted within the small frame of area A_1. Mitotic profiles were counted using the large counting frame (with area A). The mitotic profile frequency, MF (the number of mitotic profiles per 1000 nuclear profiles), was estimated by

$$MF = \frac{Q(mit) \cdot A}{Q(nuc) \cdot A_1} \cdot 10^3 . \tag{4}$$

Q(mit) is the total number of mitoses counted. Finally, the mean nuclear profile area, $\bar{a}_H$(nuc), was estimated by

$$\bar{a}_H(nuc) = \frac{a(p) \cdot N \cdot A_1}{Q(nuc) \cdot A} . \tag{5}$$

Statistics

Univariate survival analyses were performed by log-rank tests of Kaplan-Meier estimates, whereas the multivariate survival analyses were performed using Cox models. Median values were used as cutoff points for the quantitative data. $2p \leq 0.05$ was considered the level of significance in all tests.

RESULTS

The results of univariate survival analyses of 71 patients with ductal and 53 patients with lobular carcinoma of the breast are shown in Table 1.

Table 1. Single-factor prognostic analyses of clinicopathologic and quantitative histopathologic variables in breast cancers of the ductal and lobular types.

Variable	Ductal carcinoma 2p-value	Lobular carcinoma 2p-value
Clinical stage of disease	0.02	0.009
Lymph node status	0.005	0.03
Tumor diameter	0.005	0.08
Patient age	0.02	–
Menopausal status	–	0.18
Estrogen receptor status	0.74	0.74
Histologic grade*	0.42	–
$\bar{v}_V$(nuc)	0.03	0.03
$\bar{a}_H$(nuc)	0.02	0.25
V_V(nuc/tis)	0.14	0.002
ND	0.20	0.02
MF	0.16	0.0004

– Not analyzed.

* The grade originally assessed at the diagnosis.

The relative significance of the variables was tested in multivariate analyses. For patients with ductal carcinoma, information about lymph node status, tumor diameter, age, histologic grade, $\bar{v}_V$(nuc), ND and MF was entered. Of these, only the lymph node status and the $\bar{v}_V$(nuc) were of significant, *independent* prognostic value. In a similar multivariate analysis regarding patients with lobular carcinoma, information about the clinical stage of disease, $\bar{v}_V$(nuc), V_V(nuc/tis), ND, and MF, was entered. For patients with this type of breast cancer, all included variables, except the V_V(nuc/tis), were of significant, *independent* prognostic value.

DISCUSSION

Constituting approximately 70% of all breast cancers, the ductal type is by far the most frequent. The lobular type is the second-most common, constituting 10-20%. In agreement with larger studies (Aaltomaa & Lipponen, 1992), we found a prognostic value of the lymph node status and the tumor diameter. Naturally, the clinical stage of disease, which, in essence, is a combination of the two above-mentioned factors, was of significant value. Thus, these relatively easily accessible parameters are of great clinical value. It must be stressed, however, that the two current studies are not completely comparable, because the criteria for inclusion and the underlying population were somewhat different. This may, in part, explain the differing significance of the quantitative histopathologic variables in the two types of breast cancer. However, unbiased stereologic estimates of the mean nuclear volume proved its *independent* prognostic value in both breast cancer types. In addition, the results of others (Aaltomaa & Lipponen, 1992, and Baak *et al.*, 1985) and the current study of lobular carcinomas, indicate that quantitative estimates of the mitotic activity may also be strong prognostic predictors.

In conclusion, quantitative histopathologic variables may be of value for the objective malignancy grading of breast cancers. This is so, especially for carcinomas of the lobular type, as there is no generally accepted grading system for these tumors. Estimates of the mean size of cancer cell nuclei and the mitotic activity seems most successful. Estimates of the volume-weighted mean nuclear volume, can be obtained easily and efficiently from one, ordinary processed histologic section and the observer reproducibility has been shown to be excellent (Sørensen, 1992). Thus, besides being three-dimensional and unbiased, this estimator seems suitable for routine use.

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