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Histologic Grade, DNA Ploidy, and Intraglandular Tumor Extent as Indicators of Tumor Progression of Clinical Stage B Prostatic Carcinoma

A Direct Comparison

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Histologic grade, DNA ploidy, and percentage tumor area were assessed in prostatectomy specimens from 73 patients with clinical stage B adenocarcinoma of the prostate and analyzed for their value as predictors of tumor progression. Further, the relationship between percentage tumor area and DNA ploidy was studied. Percentage tumor area was the indicator most strongly associated with the likelihood of tumor extension beyond the capsule of the prostate and of tumor progression as assessed in a logistic regression model. Grade was slightly superior to percentage area in predicting time to progression in a Cox model analysis. Increasing percentage tumor area was associated with an increased likelihood of aneuploidy. Little additional predictive ability was obtained with the concurrent use of two indicators in multivariate analysis, suggesting a high degree of interrelatedness of percentage tumor area, histologic grade, and DNA ploidy. DNA ploidy was not an independent predictive factor, and from a practical standpoint histologic grade and percentage tumor area were more important predictors of tumor progression than DNA ploidy.

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Carcinoma of the prostate is now the most common noncutaneous malignancy in males in the United States (28). This malignant tumor exhibits remarkably variable biologic behavior—from small, well-differentiated, localized carcinomas that are incidental findings at autopsy to large, high-grade carcinomas that metastasize widely and cause death. Therefore, it is important to establish and compare prognostic indicators for prostatic carcinoma so as to direct therapy and accurately predict prognosis.

Histologic grade, intraglandular tumor extent (as measured by tumor volume or percentage tumor area), and DNA ploidy have all been well established as prognostic indicators in prostatic carcinoma (3–13,16–18,20–27,29,30), but little is known of the relative prognostic strength of these indicators. Only one study (24) has reported a direct comparison of all three variables by multivariate analysis, but it did not include a morphometric determination of intraglandular tumor extent. A second investigation (17) directly compared, by multiple linear regression analysis, the relative ability of increasing tumor volume to predict lymph node metastasis. A direct comparison of increasing Gleason grade, aneuploidy, and the relative prognostic strength of DNA ploidy, histologic grade, and intraglandular tumor extent, as assessed by percentage tumor area, has not previously been reported. Also not clearly defined is the relationship between DNA ploidy and the prognostic indicators of histologic grade and intraglandular tumor extent. Conflicting data have been published as to whether the DNA ploidy status of the patient is significantly

linked to histologic grade and intraglandular tumor extent. For intraglandular tumor extent as measured by tumor volume, DNA ploidy has been reported to be both unrelated to intraglandular tumor volume (2,24,29) and tightly linked to tumor volume (17). In this latter study, aneuploidy was found almost exclusively (25 of 26 cases) in tumors larger than 4 cc (17). The relationship of DNA ploidy to a second commonly used measure of intraglandular tumor extent, that is, the percentage of prostatic tissue involved by tumor (percentage tumor area), has not been previously reported.

In this investigation, we evaluated by multivariate analyses the relative prognostic ability of DNA ploidy, Gleason histologic grade, and percentage tumor area in prostatectomy tissue from 73 patients with clinical stage B carcinoma of the prostate. Further, we assessed the ability of these indicators to provide additional prognostic information when present together in logistic regression and Cox model analyses. Finally, we examined the relationship between DNA ploidy and percentage tumor area.

MATERIALS AND METHODS

Patient Identification

Seventy-three patients who underwent radical prostatectomy at Duke University Medical Center between the years 1973 and 1988 were studied. All patients were clinical stage B and had clinically localized disease preoperatively by digital rectal examination, serum prostatic acid phosphatase, and bone scan. The evaluated patient population represents a subset of a previously studied group of patients (18) that was selected on the basis of adequacy of archival pathologic material for determination of percentage tumor area or percentage of prostatic tissue involved by tumor as previously described (3,16). Fifteen patients were pathologic stage B. The remaining 58 patients were pathologic stage C, 45 of whom demonstrated seminal vesicle involvement histopathologically. The ages of the cancer patients at diagnosis ranged from 36 to 77 years (mean, 64 years).

Preparation of Paraffin-Embedded Archival Specimens

Representative areas of tumor from formalin-fixed, paraffin-embedded archival specimens were selected by light microscopy, and an adjacent 50-μ section was used for flow cytometric analysis as previously described (4,18). Deparaffinization and rehydration were performed by a modification of the technique of Hedley (14). Briefly, the specimens were deparaffinized in three changes of Histoclear (National Diagnostics, Manville, NJ) followed by sequential rehydration in absolute, 95%, 70%, and 50% ethanol. The specimens were then washed in distilled water and incubated for 30 min at 37°C in a 0.5% pepsin solution, pH 1.5, with frequent vortex mixing.

Mononuclear suspensions generated were then filtered through a 41-μ nylon mesh and centrifuged at 1,500 rpm for 10 min. The pellets were then washed in phosphate-buffered saline and stained using propidium iodide in a 0.1% ribonuclease solution.

Flow Cytometric Analysis

Flow cytometric analysis of the above preparations was performed on a Coulter Electronics EPICS 753 Cell Sorter using a 5-W argon ion laser at 488-nm wavelength. For each sample, 50,000 cells were counted to generate DNA histograms. Tetraploid, aneuploid, and nontetraploid aneuploidy were defined as previously reported (18). For purposes of statistical analysis, no distinction was made between tetraploid and nontetraploid aneuploidy, and nondiploid specimens were simply designated aneuploid.

Tumor Grade and Area

Histologic grade was assigned by the Gleason method (10–12). The range of Gleason score (GS) was 2–9, with the following frequencies: GS 2, one case; GS 3, one case; GS 4, eight cases; GS 5, seven cases; GS 6, seven cases; GS 7, 23 cases; GS 8, 10 cases; GS 9, 15 cases. Percentage tumor area or the percentage of prostatic tissue involved by tumor was determined by a grid ratio method (16) and by the pathologist's percentage estimate (3), as described. A mean of 9.3 slides per case was examined; this degree of sampling has been previously demonstrated to generate percentage gland involvement values that are prognostically significant (16).

Definition of Clinical Endpoints

In the statistical analysis, we examined three clinical endpoints: binary extent of disease, likelihood of progression, and time to progression. Binary extent of disease was set at zero if the tumor was localized to the prostate (pathologic stage B) or at one if the tumor was beyond glandular confines (pathologic stage C). Progression was defined clin-

ically as development of metastases by bone scan, biopsy-proven local recurrence, or elevation of prostatic acid phosphatase to the abnormal range. Postoperatively, patients were followed regularly with physical examination, prostatic acid phosphatase determination, and bone scan on a yearly basis or when clinically indicated. Time to progression was defined in months and calculated on the basis of date of first diagnosis to date of first evidence of progression as defined above.

Only 12 patients died "with tumor." Because it was not known if they died of their tumor, we could not analyze for survival time.

Statistical Analyses

We used a logistic regression model, the LOGIST program of Harrell (Duke University Medical Center, Division of Epidemiology and Biostatistics, Durham, NC, U.S.A.) and the SAS statistical package (SAS Institute, Cary, NC, U.S.A.) to study the relationship between extent, or likelihood of progression, and the variables of grade, percentage area of tumor, and ploidy. We used the Cox proportional hazard model (and the program PHGLM of Harrell, Duke University Medical Center) to study the relationship between time to progression and the variables of binary extent, grade, percentage area of tumor, and ploidy. We also tested the relationship between ploidy and percentage tumor area with a logistic model.

RESULTS

Of the 73 patients in this study, 15 were pathologic stage B and 58 were pathologic stage C. By stage, the distribution of cases according to ploidy status is as follows: stage B, three aneuploid cases and 12 diploid cases; stage C, 36 aneuploid cases and 22 diploid cases. This measure of local tumor extent, that is, pathologic stage, was used as an endpoint in examining the relationship between the likelihood of having tumor outside the prostate and the three prognostic indicators. This data is presented in Table 1. All three indicators showed a significant relationship to binary stage of tumor, and for all three an increase in each parameter meant a greater likelihood of tumor outside the gland (aneuploidy was arbitrarily assigned a greater value than diploid state). Nevertheless, percentage area of tumor was more closely tied to stage than either histologic grade or ploidy, and the pathologist's percentage estimate was slightly better (larger chi-square value) than was the grid-ratio method.

TABLE 1. *Logistic regression model relating the likelihood of tumor outside the prostate to prognostic indicators*

Parameter	Chi-square	p Value
Percentage tumor area		
Pathologist's estimate	27.15	0.0000
Grid ratio[a]	20.72	0.0000
Ploidy[a]	8.48	0.0036
Gleason score[a]	8.42	0.0037

[a] Not significant when pathologist's percentage estimate was included in the model.

Histologic grade and ploidy did not provide additional predictive information when tumor area was present in the model.

Table 2 shows the logistic analysis of the likelihood of progression; Table 3 shows the proportional hazard analysis of time to progression. Although the likelihood of progression was significantly tied to binary extent (pathologic stage), Gleason grade, pathologist's percentage estimate area, grid ratio, and ploidy, the association was greatest for percentage tumor area, as measured by the grid ratio. Ploidy resulted in the smallest chi-square, and the pathologist's percentage estimate chi-square was close to that of the grid ratio. Again, histologic grade and ploidy did not provide additional predictive information when tumor area was in the model.

Only in the analysis of time to progression did the Gleason histologic grade surpass percentage tumor area in significance (Table 3); the best model was with both factors present (last two columns of Table 3). Ploidy was less significant, and its significance disappeared with Gleason grade and percentage tumor area present in the model.

The scatter distribution of the grid-ratio values for diploid and aneuploid cases is shown in Figure 1. The mean ratio value for the 34 diploid tumors was 0.32; for the 39 aneuploid tumors, the mean ratio value was 0.50. This association of greater-area tumors with aneuploidy was significant ($p = 0.00512$, using the grid-ratio method), as assessed

TABLE 2. *Logistic regression model relating likelihood of clinical progression to prognostic indicators*

Parameter	Chi-square	p Value
Percentage tumor area		
Grid ratio	18.69	0.0000
Pathologist's estimate[a]	17.39	0.0000
Gleason score[a]	12.29	0.0005
Binary extent[a]	9.06	0.0026
Ploidy[a]	6.20	0.0128

[a] Not significant when grid ratio was included in the model.

TABLE 3. *Cox model relating time to progression to prognostic indicators*

Parameter	Initial model		Final model[a]	
	Chi-square	p Value	Chi-square	P Value
Gleason score	17.00	.0000	8.87	.0029
Percentage tumor area				
Grid ratio	15.85	.0001	NS	
Pathologist's estimate	14.18	.0002	5.75	.0165
Ploidy	11.60	.0007	NS	
Binary extent	7.44	.0004	NS	

NS, not significant.
[a] Final model with *both* Gleason score and pathologist's percentage estimate present.

with a logistic regression analysis. The trend toward higher percentage tumor area with aneuploidy is clear, but there is also substantial overlap of individual cases.

DISCUSSION

The data presented here suggest that DNA ploidy, histologic grade, and percentage tumor area, as indicators of the clinical progression of prostatic carcinoma, may be stratified according to the relative strength of predictive capacity, which was determined by multivariate non-Gaussian regression statistical analysis, the preferred approach in retrospective studies (13). Few studies have employed statistical analyses designed to compare DNA ploidy, intraglandular tumor extent, and histologic grade as predictors of prostate cancer biologic behavior (17,24,30), and only one of these studies (24) used multivariate analysis. These studies all assessed intraglandular tumor extent by volumetric measurements; no study has previously compared DNA ploidy, histologic grade, and intraglandular tumor extent as assessed by percentage tumor area, which is a highly significant prognostic indicator that has been found to exhibit a stronger association with pathologic stage and tumor progression than tumor volume (25). Furthermore, percentage area is related to volume; as defined in quantitative microscopy texts (31), the average fractional area equals the fractional volume.

The direct comparative analysis presented here revealed percentage tumor area to be more significantly linked to both the likelihood of tumor extension beyond the capsule of the prostate gland (pathologic stage) and the likelihood of clinical progression as compared with histologic grade (Gleason score) and DNA ploidy. Analysis of time to progression showed that tumor grade outperformed percentage tumor area, followed by DNA ploidy. Overall, then, intraglandular tumor extent, as assessed by percentage tumor area, was the most significant predictor of biologic behavior of localized (stage B and C) prostatic carcinoma. This finding supports the recently reported superior predictive power for lymph node metastases of intraglandular tumor extent, as assessed by tumor volume, compared with histologic grade and aneuploidy (17). In their multiple linear regression analysis, however, local invasiveness (capsule penetration or seminal vesicle invasion) was the most significant predictor of lymph node metastases. We did not find such extension (binary extent) to be a superior predictor of clinical progression or time to progression as compared with histologic grade or intraglandular tumor extent. Also, in contrast to our results, other studies (24,30) found histologic grade (24) and DNA ploidy (30) to be the most important pathologic variables in influencing tumor progression in stage C and D patients, respectively. It is not possible to compare these data with ours, because chi-square values were not provided in one study (24) and because the second study (30) employed Kaplan-Meier survival plots rather than multivariate analysis.

Conflicting data also exist as to whether the predictive power of any of the three indicators is enhanced by the addition of any of the other variables. Our data indicate that, for the endpoints of likelihood of tumor extension beyond the prostatic capsule (Table 1) and likelihood of clinical progression (Table 2), no additional information was obtained by including a second indicator in the model. Only for the endpoint of time to progression did the two variables of grade and percentage tumor area provide additional information when both were in the model together (Table 3). In accord with this finding, tumor volume (24) and percentage tumor area (16) have previously been reported to provide additional predictive information when considered with histologic grade. The additive effect observed here and also in a separate study (16) was slight, however, suggesting that these three indicators (DNA ploidy, histologic grade, and percentage tumor area) may be so closely interrelated that separating these variables into independent indicators may not be feasible.

DNA ploidy did not provide additional information for any of the biologic behavioral endpoints after the most significant indicator was present in the model. Previous studies have reported both that DNA ploidy does (1,24) or does not (17) provide additional information. In general, though, multivariate studies have failed to demonstrate the inde-

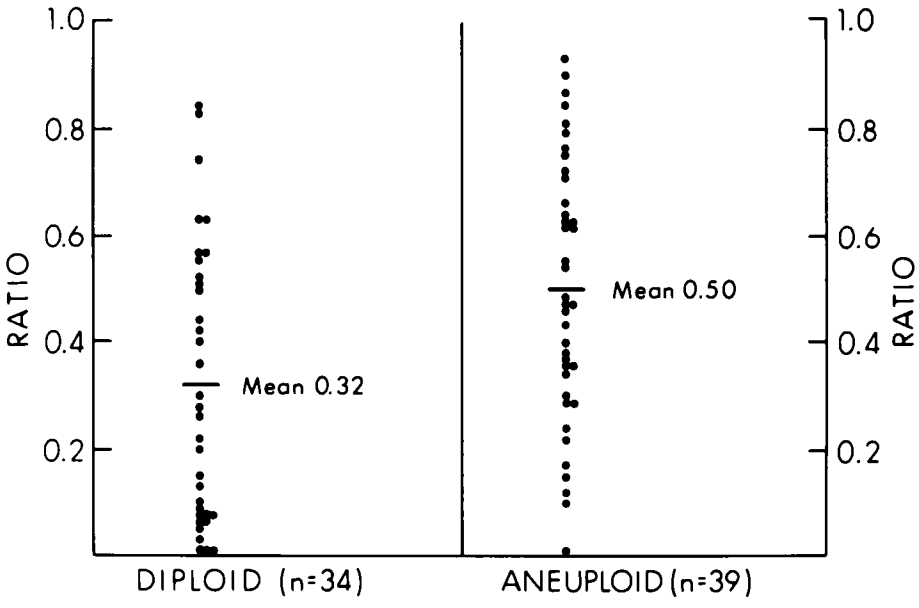

FIG. 1. Distribution of individual percentage tumor area values as a function of DNA ploidy. Percentage tumor area was determined by the grid-ratio method (16).

pendent predictive power of DNA ploidy after consideration of other prognostic indicators, such as histologic grade (13,27). DNA ploidy may, however, be useful in predicting the biologic behavior of the large category of intermediate histologic grade (GS 5–7) tumors (18). Because intermediate histologic grade tumors exhibit markedly disparate clinical courses, the development of indicators to predict the behavior of these tumors is desirable. Similar to ploidy, percentage tumor area or volume may also be useful in predicting the outcome for patients with intermediate grade tumors (15,19).

A close interrelationship between DNA ploidy and percentage tumor area is substantiated by our finding that increasing percentage tumor area was significantly linked with aneuploid status ($p = 0.0051$). The scatter distribution of individual tumor area values, however, according to diploid or aneuploid status, illustrates so significant an overlap that for an individual patient it is difficult to predict percentage tumor area based on DNA ploidy status alone. Such a scatter distribution for individual tumor area values is similar to the scatter observed by plotting individual tumor area values versus seminal vesicle invasion and tumor progression (16). Figure 1 shows that in cases with >70% tumor area, 10 of 13 tumors were aneuploid; only one of 12 tumors with area <10% were aneuploid. This latter finding is in agreement with a previous report (17) that also described a predominance of diploidy in patients with a lesser degree of intraglandular tumor extent; in that investigation, only one of 26 tumors with a volume <4 cc were aneuploid (17). Other reports (2,24,29) have failed to establish a link between tumor volume and DNA ploidy, but these measurements of tumor volume were apparently not made by microscopic assessment of tissue sections; rather, they were gross morphologic or clinical measurements that fell into rather broad categories. The different methods of measuring intraglandular tumor extent may account for the discrepancies in linkage of DNA ploidy and intraglandular tumor extent.

In summary, the results of this investigation indicate that DNA ploidy, histologic grade (Gleason score), and percentage tumor area (intraglandular tumor extent) are all significant predictors of the biologic behavior of carcinoma of the prostate. Overall, by direct multivariate analysis comparison, percentage tumor area was the best predictor of biologic behaviors. Only for one biologic behavior (time to tumor progression) did two prognostic indicators (percentage tumor area and grade) provide additional predictive information. DNA ploidy was not an independent predictor, and all three indicators—DNA ploidy, histologic grade, and percentage tumor area—are probably closely interrelated. In this study, increasing percentage tumor area was associated with a greater likelihood of aneuploidy. From a practical standpoint, histologic grade and percentage of prostatic tissue involved by carcinoma were more important predictors of tumor progression than DNA ploidy. Further multivariate studies of prognostic indicators in prostatic carcinoma will be necessary to establish firmly the relationships between, and relative clinical usefulness of, DNA ploidy, histologic grade, and tumor area. □

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