

The Prognostic Value of DNA Ploidy Combined with Histologic Substaging for Incidental Carcinoma of the Prostate Gland

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Although current methods of histologic substaging for incidental prostatic carcinoma are useful, they offer only general indications of potential tumor behavior. To further define the biologic tendencies of stage A cancers, an examination was made of the role of DNA ploidy combined with histologic staging in archival material selected to achieve both a representative sample and long-term follow-up. With histology alone, 36% of stage A2 cancers and 9% of A1 neoplasms were progressive. Adding DNA flow cytometry to histology resulted in a significant improvement in the capacity of pathologic evaluation to predict outcome. Progression occurred in 67% of aneuploid stage A2 prostate cancers and in none of the nonaneuploid stage A1 tumors. Despite current limitations in the interpretation of DNA histograms from archival tissue, flow cytometry has significant potential in the pathologic evaluation of incidental prostatic carcinomas. (Key words: Flow cytometry and prostatic carcinoma; DNA ploidy and stage A prostate cancer; Staging of prostate cancer) *Am J Clin Pathol* 1988; 89: 370-373

CLINICALLY UNSUSPECTED prostatic carcinomas are often aggressive and it has become standard practice to subclassify them into stages A1 and A2 depending upon the estimated size or differentiation of the tumor as it appears in transurethral resection chips.¹² Histologic substaging has been accomplished by a variety of methods but none is entirely reliable in defining cancers with distinctive biologic behaviors.^{3,10,14} More sophisticated measurements, including nucleolar area, nuclear shape, and DNA ploidy have shown promising results but their practical role in diagnostic pathology remains to be elucidated.^{5,19,20} This study was designed to examine the value of DNA ploidy combined with histologic substaging as a prognostic indicator for stage A carcinomas of the prostate gland.

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Materials and Methods

The surgical pathology files of Baptist Memorial Hospital (BMH) were reviewed for the years 1965-1968 to achieve a follow-up period of 18 to 20 years. Cases were included provided that (1) there was no clinical suspicion of prostatic cancer when the resection was performed; (2) the lesions appeared in transurethral resection chips; (3) there was sufficient tissue for histologic review and DNA ploidy analysis; and (4) patients with prostatic cancers had been followed in the BMH tumor registry. In all, 39 cases of stage A carcinoma were selected. Controls comprised 18 cases of nodular hyperplasia. The original tissue sections were reviewed (by T.L.M., W.M.M.) and subclassified as stage A1 or A2 according to the area density method of Cantrell and associates.³ In this scheme, all areas of carcinoma are outlined on the glass slides with a marking pen. The areas encompassed by the ink are then visually compared to the amount of remaining nonneoplastic prostatic tissue. If the cancer occupies $\leq 5\%$ of the total area of chips, the tumor is classified as stage A1; if $> 5\%$, it is classed as stage A2. Additional freshly prepared sections, taken from all paraffin blocks before and after tissue for DNA ploidy analysis, were examined (by D.S., W.M.M.) to document the presence or absence of neoplasia in the material used for flow cytometry. Cancers were graded according to the scoring method of Gleason and associates.⁹

Flow cytometry for DNA ploidy was performed according to modifications of the method of Hedley and co-workers.^{4,11,13} Briefly, 30 to 50 μm sections from formalin-fixed blocks were deparaffinized and digested with 0.5% pepsin (Sigma) in 0.9% NaCl at pH 1.5 for 30 minutes at 37 °C. The resulting suspension was washed and filtered through a 41 μm nylon mesh and the nuclei

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were adjusted using a hemocytometer to a concentration of $2 \times 10^6/L$. Nuclei were then incubated with 0.1 g/L ribonuclease A (Sigma), stained with propidium iodide and analyzed in a Coulter EPICS V flow cytometer equipped with an argon ion laser operating at 200 mw and 488 nm. The instrument was calibrated with either normal human peripheral blood or cells recovered from deparaffinized human lymph nodes. Normal prostatic cells present in every test specimen served as additional internal controls. A minimum of 10,000 nuclei per specimen were processed. Computer generated histograms were created using electronic gating such that the amount of DNA/nucleus was recorded on the X axis and the number of nuclei were represented on the Y axis (Figs. 1 and 2). Due to the possibility that histograms with broad coefficients of variation might conceal small populations of abnormal nuclei, only cases with distinctly separate nuclear populations represented by at least two unequivocal peaks were considered aneuploid (An). All other interpretable cases were classed as non-aneuploid (NAn) for the purposes of this study. Histograms were analyzed independently by four of us (T.L.M., J.S.C., R.W.C., W.M.M.) without knowledge of the histologic diagnoses and all discrepancies in interpretation were discussed. If a consensus could not be reached, the histograms were considered uninterpretable, a situation primarily related to nuclear distributions with large hypodiploid fractions and indistinct peaks.

Clinical outcome for cancer patients was obtained from the files of the BMH tumor registry. Progression (P) was defined as death from prostatic cancer or documentation of metastatic disease. Patients dead of other causes or alive with or without localized prostatic tumors were classified as no progression (NP). Followup for nodular hyperplasia patients was obtained from hospital and physicians' records.

Correlations of DNA ploidy with progression and DNA ploidy as an independent variable within each subgroup were analyzed statistically by the 2×2 chi-

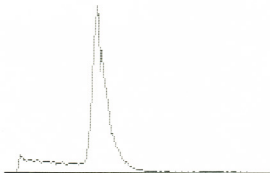


FIG. 1. DNA histogram from paraffin embedded specimen of nodular hyperplasia displaying a nonaneuploid pattern. Data displayed on linear plot where number of nuclei are counted on Y-axis and DNA fluorescence is recorded on X-axis.

square test for independent variables. Log linear models were used for determination of DNA ploidy as an adjunct to staging for prediction of progression.

Results

The results are summarized in Tables 1 and 2 and in Figures 1 and 2. Mean coefficients of variation for DNA histograms were 11.9% for aneuploid tumors and 13.2% for nonaneuploid tumors compared to 2% for deparaffinized lymph node controls. Of the 39 cancer patients, 14 were classified as stage A1. Patients with stage A1 cancer were aged 57 to 90 years (median age, 74 years). Histograms from three patients were considered uninterpretable. Median crude survival was 71 months. Of the 11 cases suitable for DNA ploidy analysis, 1 had aneuploidy. This patient subsequently died of metastatic prostatic carcinoma 20 years after the initial diagnosis (Tables 1 and 2). No progression was noted among the remaining ten nonaneuploid cases.

The 25 patients with stage A2 prostatic cancer were aged 53 to 84 years (median age, 73 years). Uninterpret-

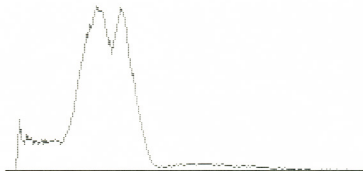


FIG. 2. DNA aneuploidy in a specimen from stage A2 prostatic carcinoma.

Table 1. DNA Ploidy in Deparaffinized TURPs (%)

	No.	An	NAn
CA-A1	(11)	9	91
CA-A2	(22)	41	59
BPH	(15)	13	87

An: aneuploidy; NAn: no aneuploidy; BPH: benign prostatic hyperplasia; TURPs: transurethral resection prostate.

able histograms occurred in three cases. Median crude survival was 70 months. DNA aneuploidy was recognized in 9 of 22 cases (Table 1) and 6 of these suffered progressive disease (Table 2). Progression of prostate cancer was noted in only 2 of the 13 nonaneuploid cases. Overall, median survival among cancer patients with DNA aneuploidy was 47 months compared to 81 months for cancer patients with no aneuploidy.

The addition of DNA ploidy analyses to histologic substaging further stratified patients with incidental prostatic cancer into nonaggressive and aggressive groups. DNA aneuploidy was significantly associated with progression ($P < 0.02$). When added to substaging for A2 cancers, DNA aneuploidy increased the predictive value of a positive pathologic evaluation ($P < 0.05$). Results were further confirmed by log linear models analyzing the three factors—substage, ploidy, and progression ($P < 0.01$).

The scoring by Gleason and group⁹ among carcinomas was the following: 2 = 0%; 3 = 0%; 4 = 10%; 5 = 6%; 6 = 55%; 7 = 6%; 8 = 22%; 9 = 0%; 10 = 0%. Strong correlations with outcome occurred only when comparing substage to scores > 7 . Perhaps more importantly, this system failed to detect the progressive stage A1 case and would have indicated a poor prognosis for one nonprogressive stage A1 patient with NAn nuclei.

The 18 patients with nodular hyperplasia (BPH) were aged 49 to 79 years (median age, 67 years). Uninterpretable histograms occurred in three cases. DNA aneuploidy occurred in two cases. Long-term follow-up among many individuals with hyperplasia could not be obtained, but the development of prostatic carcinoma had not been documented in the available records of any

patient in the BPH group. The two individuals with aneuploid specimens had not developed prostate cancer when last seen, 14 and 15 years, respectively, after the positive specimen was obtained. Although there was marked chronic inflammation in the tissues in each case, no peculiar histologic abnormalities were identified.

Discussion

Previous studies in prostatic carcinoma have confirmed the clinical value of subclassifying stage A tumors according to their size and differentiation, but neither of these histologic parameters is completely satisfactory in predicting tumor behavior. While poorly differentiated carcinomas are almost always aggressive, they are usually not incidental (*i.e.*, stage A) and the pathologic diagnosis of stage A2 generally connotes a relatively large tumor of moderate differentiation.^{3,10,14} In previous reports, at least 32% of stage A2 lesions progressed over time compared to 16% of stage A1 lesions.^{3,6} Recent studies using image analysis to measure nuclear shape (nuclear roundness factor) have shown that the predictive value for progression of stage A2 lesions can be increased if additional parameters are included in the diagnostic evaluation, but the imaging technique has been time consuming and not readily available.⁵ In contrast, flow cytometry is not only less labor-intensive and more readily available, but the adaptation of ploidy analyses to paraffin-embedded material has greatly expanded the potential usefulness of this technique in diagnostic pathology.

Our data confirm previous reports that DNA ploidy determination by flow cytometry can offer valuable information regarding tumor behavior in stage A prostate cancers.^{8,20} When ploidy analyses were combined with histologic substaging for A2 cancers, the predictive value of a positive pathologic test for disease progression almost doubled (from 36 to 67%). In contrast, no patient with a stage A1, nonaneuploid lesion suffered tumor progression and only 15% of stage A2 nonaneuploid cancers progressed over follow-up periods up to 20 years. Positive correlations with progression could be obtained using the scoring of Gleason and associates⁹ only if values < 7 were excluded (*i.e.*, only for poorly differentiated neoplasms) and even then their scores were not helpful in stage A1 cases.

Despite encouraging results from this pilot study, the routine implementation of flow cytometry on archival material from transurethral prostate resections requires further refinements. As illustrated in Figure 1, even histograms from BPH cases had rather broad coefficients of variation. Similar problems in interpretation of DNA ploidy on paraffin-embedded tissues have been reported

Table 2. Prognostic Value of Flow Cytometry for Stage A Prostate Cancer (%)

	P	NP
A1(11)	9	91
An	100	0
NAn	0	100
A2(22)	36	64
An	67	33
NAn	15	85

P: progression; NP: no progression; An: aneuploidy; NAn: no aneuploidy.

by others and ascribed either to variations in the cellular composition and thickness of individual sections or to poor fixation and specimen preservation.^{2,7,17} Suboptimal tissue fixation and paraffin infiltration are especially common in transurethral prostatic resections and may account for most of the apparent DNA heterogeneity in our samples. Real variations in the cellular composition of tissues containing benign and neoplastic glandular elements, stromal cells, and inflammatory debris should not be entirely excluded, however.

The appearance of significant DNA abnormalities in histologically benign prostate tissues cannot be adequately explained but was not unexpected since similar results have been reported by others.^{1,15,16} Although it is tempting to discount these changes as technical artifacts, they may reflect the heterogeneity of glands with significant benign proliferating components. Increased labeling indices for DNA have been reported in chronic prostatitis and some authors have cautioned against relating high cell turnover rates to cancer unless the morphology of each replicating cell can be examined.¹⁸ Discrepancies of DNA ploidy in nodular hyperplasia do not negate the value of flow cytometry for distinguishing groups of stage A cancers, however, since the determination of carcinoma was made via histology.

In conclusion, a combination of DNA ploidy and histologic sub staging can further define the aggressive tendencies of stage A prostatic carcinomas. Incidental prostatic cancers lacking aneuploidy are unlikely to progress. The apparent heterogeneity of prostatic hyperplasia and carcinoma in tissue stored in paraffin compared to that of other similarly preserved cancers is a significant hindrance to DNA ploidy analysis in archival material and further refinements are desirable before the technique can be widely applied. DNA aneuploidy in cases of prostatic hyperplasia may not be completely artifactual, although the significance of this finding is unknown.

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