Stage B Prostate Adenocarcinoma

Flow Cytometric Nuclear DNA Ploidy Analysis

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• **Over a 16-year period (1966 to 1981), 349 patients underwent radical retropubic prostatectomy for pathologic stage B adenocarcinoma of the prostate. Nuclear DNA content was measured by flow cytometry on available archival material of 283 patients. Two hundred sixty-one patients (92%) had high-quality histograms. The ploidy distribution was as follows: DNA diploid, 177 (68%); DNA tetraploid, 74 (28%); and DNA aneuploid, 10 (4%). The average follow-up was 9.4 years. At the time of follow-up, 53 patients (20%) within the study group had developed tumor progression: 22 local, 23 systemic, and 8 both. The ploidy distribution of the population that developed tumor progression was 27 DNA diploid (51%), 16 DNA tetraploid (30%), and 10 DNA aneuploid (19%). This ploidy distribution is significantly different from that found for the nonprogression group with stage B disease. Overall, 31% of patients with DNA nondiploid tumors had tumors that progressed compared with 15% of patients with DNA diploid tumors. All (100%) DNA aneuploid tumors progressed. The DNA ploidy distribution of all pathologic stage B prostate cancers differs significantly from that found in more advanced stages (C and D1) previously reported for the same time interval. However, the ploidy distribution of stage B tumors that progressed closely resembles that of the stage C and D1 tumors. These results further support the working hypothesis that nuclear DNA content has marked prognostic significance for patients with adenocarcinoma of the prostate. It seems to us that analysis of ploidy by flow or static cytometry will become an essential tool for treating patients with localized prostate cancer.**

(*Arch Surg.* 1990;125:327-331)

In 1989, prostate adenocarcinoma has become the most common noncutaneous malignant disease diagnosed in American men. The increasing incidence of prostate carcinoma is due in part to the increasing longevity of the American male population and also to use of newer laboratory tests such as serum prostate specific antigen assay and transrectal ultrasound imaging, which now permit earlier detection of prostate malignancy. The optimal treatment of men with localized prostate cancer remains highly controversial. Indeed, a recently published series carried out in Sweden followed up men with clinically localized prostate carcinoma with no treatment at all. The majority of the 223 men in the study did well with no treatment (mean follow-up, 78 months).[1]

Identification of laboratory tests that could discriminate localized prostate cancers that have a favorable prognosis from those prostate cancers that are more biologically aggressive, have a potential to progress in a biologically important time frame, and have the potential to cause the death of a patient is one of the highest priorities in clinical urologic oncology research. Flow cytometric nuclear DNA ploidy analysis at present appears to be one of the most promising techniques for stratifying the malignant potential of prostate adenocarcinoma. Several previous studies suggest that patients with DNA diploid prostate carcinomas have a much lower probability of disease progression than those with DNA nondiploid tumors.[2,3] Our research group at the Mayo Clinic, Rochester, Minn, has intensively analyzed a group of patients with apparently regionally localized prostate cancer who were treated by radical prostatectomy during the period 1966 to 1981. Relatively large-sized archival tissue samples were available for flow cytometric DNA ploidy analysis using the technique of Hedley et al.[4] We have previously reported results from studying those patients with regionally localized disease with metastatic deposits in the pelvic lymph nodes (pathologic stage D1 tumors)[5] and those patients who on pathologic examination had tumors that extended through the prostate capsule but had uninvolved pelvic lymph nodes (pathologic stage C).[6] The current report completes the description of this cohort of patients by describing nuclear DNA ploidy results and the association between ploidy and other clinical variables for patients with prostate adenocarcinoma treated by radical retropubic prostatectomy who on pathologic examination had tumors confined within the prostate capsule, pathologic stage B.

MATERIALS AND METHODS

Over a 16-year period (1966 to 1981), 349 Mayo Clinic patients underwent radical retropubic prostatectomy and bilateral pelvic lymphadenectomy for pathologic stage B adenocarcinoma of the prostate. Two hundred eighty-three patients in this group had sufficient properly fixed paraffin-embedded tissue available for laboratory analysis. Nuclear DNA content of tumor cells was measured by flow cytometric analysis of nuclei extracted and stained with propidium

Accepted for publication November 10, 1989.

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Read before the annual meeting of the Society of Surgical Oncology, San Francisco, Calif, May 25, 1989.

Reprint requests to Department of Urology, Mayo Clinic, Rochester, MN 55905 (Dr Lieber).

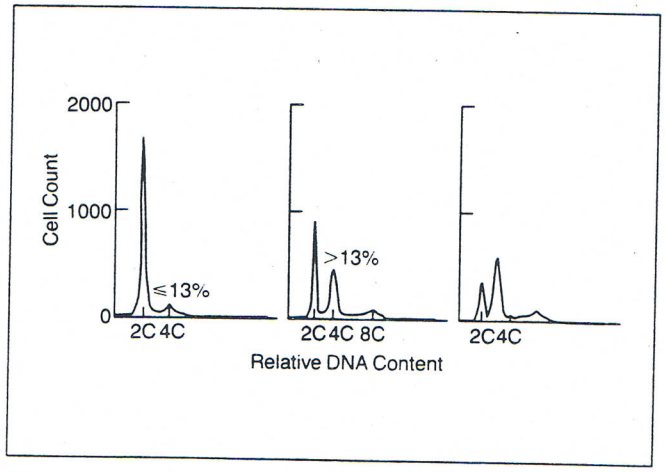

Fig 1.—Nuclear DNA histogram patterns: left, DNA diploid; center, DNA tetraploid; and right, DNA aneuploid.

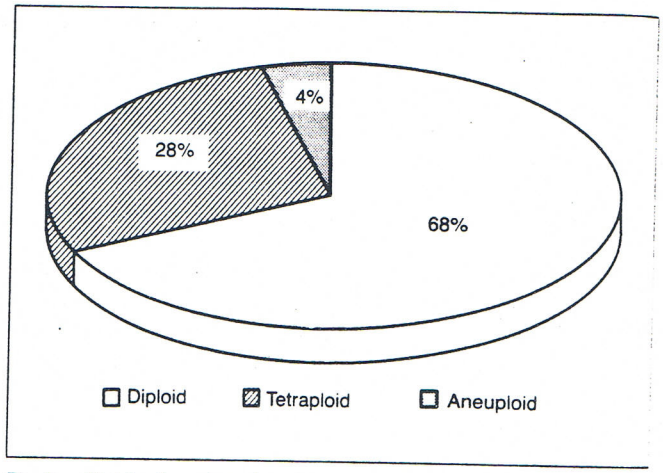

Fig 2.—Distribution of nuclear DNA ploidy patterns for 261 cases of pathologic stage B prostate adenocarcinoma.

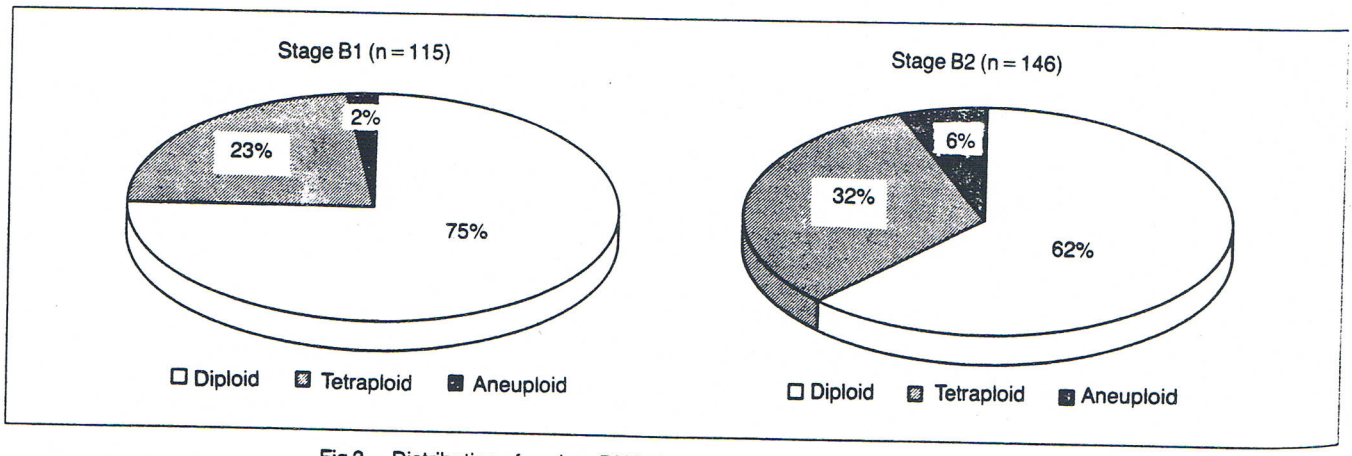

Fig 3.—Distribution of nuclear DNA ploidy patterns according to local stage.

iodide using techniques described by Hedley et al[4] and Vindelov et al.[7] Detailed methods used in the current study are presented elsewhere.[5] Nuclear DNA content was measured on a flow cytometer (FACS IV, Becton Dickinson, Sunnyvale, Calif) equipped with a 5-W argon laser at a wavelength of 514 nm. Standardization of the flow cytometer was accomplished using Fullbright Fluorospheres (Coulter Electronics Inc, Hialeah, Fla). Histograms were generated by analysis of greater than 20 000 nuclei per sample. "Cell cycle" analysis of the histograms was performed using a computer program designed by Dean and Jett.[8]

Criteria for classification of histograms had been previously established by a study of 60 specimens of human benign prostatic hyperplasia.[5] The 60 specimens had a mean ± SD percentage of nuclei in the 4C (G2) peak of 7.87% ± 1.53%. Normal or DNA diploid histograms were classified as those with less than 13% (mean ± 3 SDs of the percent nuclei found in the 4C or G2 peak). Histograms with greater than or equal to 13% nuclei in the 4C peak were classified as DNA tetraploid. A DNA aneuploid designation was assigned to those tumors with a distinct third peak separate from the 2C or 4C peaks (Fig 1).

Hematoxylin-eosin–stained histologic sections were reviewed by the study pathologist (G.M.F.). Uniform grading was carried out according to the Mayo Clinic[9] and Gleason[10] grading systems. Pathologic stage B tumor stage was based on information in the Mayo Clinic pathologic report and clinical record. This included a normal bone scan and chest roentgenogram, a serum acid phosphatase level within the normal range, and prostate cancer confined within the prostatic capsule as reported on the original pathologic report. For this study, stage B1 denotes a primary tumor less than 2 cm in greatest dimension and confined to one lobe of the gland. Stage B2 is a tumor greater than 2 cm in greatest dimension and/or one involving both lobes without evidence of capsular penetration. A complex multivariate statistical analysis of the generated data was performed using the proportional hazards regression and Cox regression models. Tumor progression and survival data curves were generated utilizing the Kaplan-Meier method.[11] Certain statistical analyses included the groups of patients for whom tissue was inappropriately fixed or unavailable for analysis and those whose histogram data were unclassifiable based on the low quality of the histogram. Comparisons of the various nuclear DNA ploidy subgroups with respect to pathologic stage, Mayo Clinic grade, Gleason score, and local or systemic progression, as well as crude and disease-specific survival, were included in the multivariate analyses. These data also were statistically compared by the χ^2 test.

RESULTS

Among the 349 patients undergoing radical retropubic prostatectomy for pathologic stage B prostate cancer during the study period, 283 patients had specimens analyzed for nuclear DNA content by flow cytometry. Sixty patients were excluded from study because either a very small focus or no residual tumor was found in the paraffin block specimen examined. An additional 6 patients were excluded from analysis due to prior treatment of the paraffin block specimen with either cupric sulfate or trinitrophenol, which made ploidy analysis technically impossible with the methods used. Of the specimens analyzed, 261 (92%) had high-quality interpretable DNA histograms. The ploidy distribution observed was 177 (68%) DNA diploid, 74 (28%) DNA tetraploid, and 10 (4%) DNA aneuploid (Fig 2).

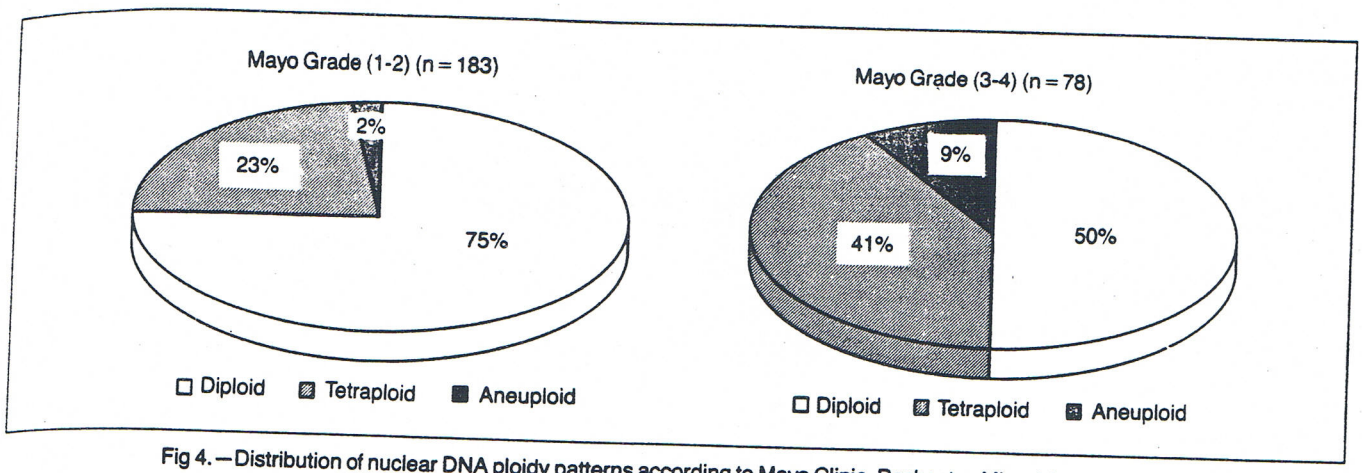

Fig 4.—Distribution of nuclear DNA ploidy patterns according to Mayo Clinic, Rochester, Minn, histologic grade.

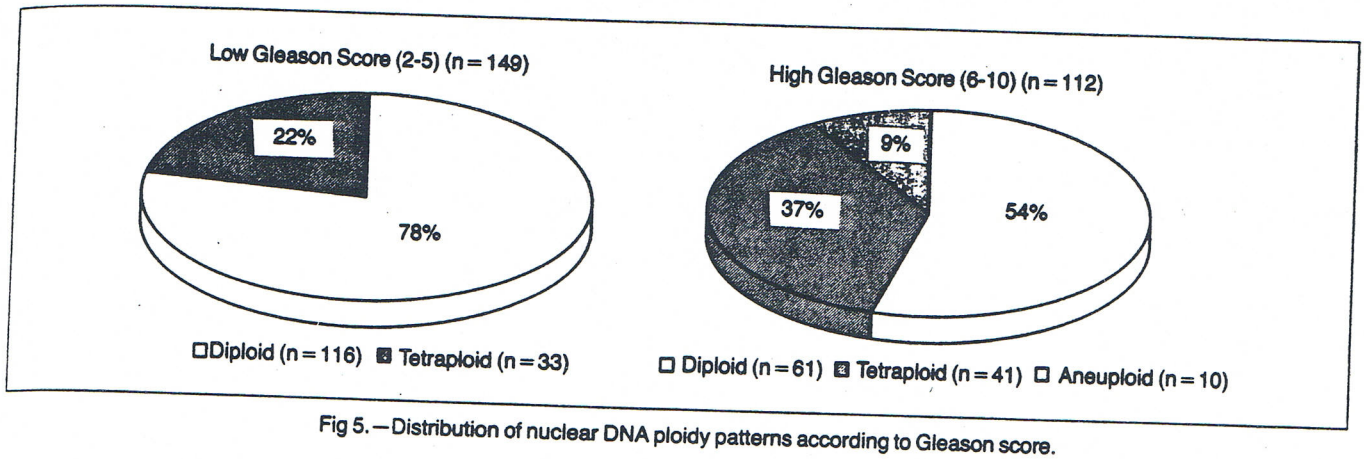

Fig 5.—Distribution of nuclear DNA ploidy patterns according to Gleason score.

Ploidy and Stage

There were 115 patients (44%) with tumors designated as stage B1 and 146 (56%) with tumors designated stage B2. The distribution of DNA ploidy for each stage is presented in Fig 3. Approximately 75% of patients with stage B1 tumors had DNA diploid patterns. In contrast, 80% of patients with DNA aneuploid tumors were found within the group of patients with stage B2 disease.

Ploidy and Tumor Grade

Histologic low-grade tumors were found in 183 patients (70%). Among the low-grade tumors, 75% were DNA diploid. This compares with 50% DNA diploidy in Mayo Clinic high-grade tumors. The DNA ploidy distributions for both high- and low-grade tumors are presented in Fig 4. Seventy percent of DNA aneuploid tumors are found within the Mayo Clinic high-grade group. Analysis of tumor grade by Gleason[10] score identified 149 patients (57%) within a high Gleason score (6 to 10) group. All DNA aneuploid tumors were found within the high Gleason score group. The distribution of nuclear DNA ploidy patterns according to low and high Gleason scores is shown in Fig 5.

Ploidy and Tumor Progression

At the time of most recent follow-up, 53 patients (20% of those within the study group) had developed a local or systemic recurrence. Eight patients developed both. For the group of patients with recurrent disease, 27 tumors (51%) were DNA diploid, 16 tumors (30%) were DNA tetraploid, and 10 tumors (19%) were DNA aneuploid. Overall, only 15% of patients with DNA diploid tumors developed tumor progression. Tumor progression occurred for 22% of DNA tetraploid tumors and 100% for DNA aneuploid tumors. Progression rates for DNA diploid and DNA tetraploid tumors are not significantly different. The progression rate for DNA aneuploid tumors was significantly higher ($P<.0001$, log rank). Nonprogression curves are presented in Fig 6. No significant difference in progression was noted for patients with tumors with inadequate tissue blocks or low-quality histograms ($P=.09$, log rank).

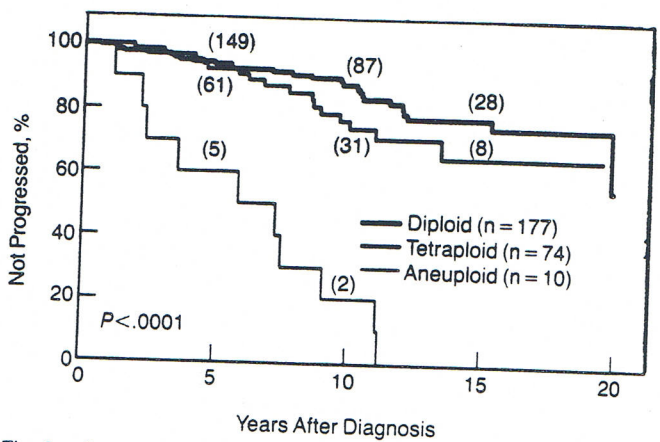

Fig 6.—Postoperative probability of nonprogression of pathologic stage B prostatic carcinoma according to DNA ploidy pattern.

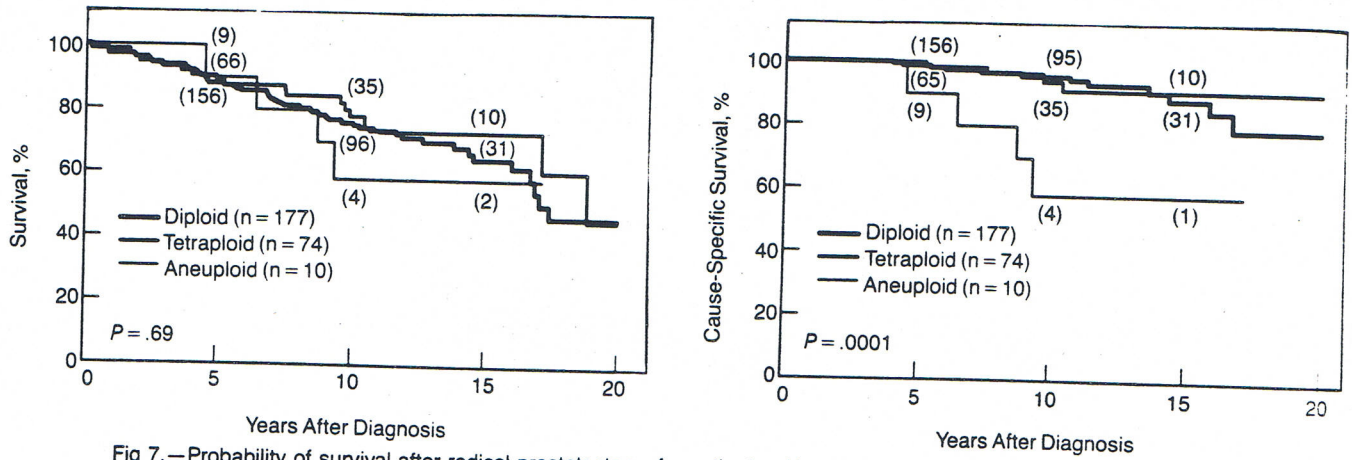

Fig 7.—Probability of survival after radical prostatectomy for patients with pathologic stage B prostatic carcinoma: left, crude survival; right, cause-specific survival.

Ploidy and Survival

Kaplan-Meier survival curves were generated for both cause-specific survival and crude survival (Fig 7). Crude survival curves failed to demonstrate any statistically significant difference between the DNA ploidy subgroups ($P = .69$, log rank). By contrast, analysis of cause-specific survival curves demonstrated the significance of DNA aneuploidy as a bad prognostic indicator ($P = .0001$). Moreover, all patients with DNA aneuploid tumors who died, died of prostate cancer.

Multivariate Analysis

Because of the association of ploidy with stage and grade, it is necessary to examine the strength of the association of ploidy with progression and survival after adjustment for stage and grade in particular, and for other patient variables. A proportional hazards model was fit using diploidy vs not, aneupleudy vs not, grade 1 to 2 vs grade 3 to 4, Gleason score of 2 to 5 vs 6 to 10, and stage for each of progression, overall survival, and cause-specific survival. No variable or combination of variables was found to be significant for predicting overall mortality. However, for both progression and cause-specific survival, DNA aneuploidy was found to be the most significant predictor, with no significant difference between diploid and tetraploid patterns seen. Significance is retained after adjustment for any one of the other variables. After adjustment of the other variables for aneuploidy, however, only a high Gleason score (6 to 10) adds significant further prediction.

COMMENT

This report completes presentation of nuclear DNA ploidy analysis for the group of patients with clinically localized adenocarcinoma of the prostate treated at the Mayo Clinic between 1966 and 1981 by radical retropubic prostatectomy and bilateral pelvic lymphadenectomy. This cohort of patients and tumors numbers nearly 500 with pathologic stages B, C, and D1 tumors. These coordinated studies demonstrate that, using the technique of Hedley et al[4] and propidium iodine staining, nuclear DNA ploidy information can be obtained from archival formalin-fixed paraffin-embedded tissue of prostate carcinoma in a very high percentage of tumors studied.

Patients with pathologic stage B tumors overall have a different distribution of ploidy patterns from those patients with pathologic stages C and D1 disease. Sixty-eight percent of patients with pathologic stage B tumors are DNA diploid. In contrast, for patients with pathologic stages C and D1 disease, only 42% to 45% are DNA diploid. For all stages of prostate carcinoma studied, patients with DNA diploid tumors have the most favorable prognosis. The high percentage of patients with DNA diploid tumors (75% in patients with stage B1 tumors) to a certain extent can "account for" the favorable prognosis historically associated with patients having pathologic stage B disease treated by radical retropubic prostatectomy and also found in this study.

Patients with tumors with DNA aneuploidy characterized by the unequivocal presence of a third stem cell line with neither 2C nor 4C ploidy were found to have the worst prognosis among all patients with pathologic stages C and D1 prostate cancer.[5,6] A similar result was found in this study. For pathologic stage B tumors, only 4% were DNA aneuploid, but all of these patients subsequently developed tumor progression and many of these patients died of prostate cancer during the period of observation. All of the tumors with DNA aneuploidy were high Gleason score (6 to 10) tumors. Therefore, a search for patients with pathologic stage B tumor who have an unfavorable prognosis in the future may be most efficiently confined to those with high-Gleason score tumors. Patients with DNA aneuploid tumors appear to have very early metastatic dissemination of their tumors. In this series, only patients with tumors confined within the prostate capsule and who had no evidence of metastatic deposits in the pelvic lymph nodes were included. Nevertheless, all of these patients with DNA aneuploid tumors subsequently developed prostate cancer progression. Such data suggest that those patients with DNA aneuploid pathologic stage B prostate cancer cannot be adequately treated by radical retropubic prostatectomy alone. Some form of active systemic adjuvant treatment seems necessary to improve prognosis for this group of patients.

In the previous Mayo Clinic studies of patients with pathologic stage C and D1 prostate carcinoma, those patients with DNA tetraploid tumors had an intermediately poor prognosis compared with patients with either DNA diploid or DNA aneuploid tumors.[5,6] In the current study of patients with pathologic stage B prostate tumors, the unfavorable prognostic association of DNA tetraploidy was not found. While there was some small increased probability of tumor progression for patients with DNA tetraploid compared with the DNA diploid tumors, this difference in prognosis did not achieve the level of statistical significance. Thus, DNA tetraploidy, found in 28% of patients with stage B prostate carcinoma, does not appear to be an unfavorable prognostic factor in this cohort.

For all stages of surgically treated prostate cancer studied at the Mayo Clinic, nuclear DNA ploidy pattern is a highly significant and independent prognostic variable for progression. This result was found herein for the group of patients with pathologic stage B prostate cancer even though the vast majority of these patients were well treated by their surgery. In the multivariate statistical analysis, DNA aneuploidy was a highly significant independent variable that could not be replaced by any other common pathologic variable. Nevertheless, for patients with pathologic stages B and C prostate cancer, tumor histologic grading by Gleason score (or for the patients with stage C disease also by Mayo Clinic nuclear histologic grading[6]) was also an independent prognostic variable. Based on results obtained so far, we suggest that within a given pathologic stage, histologic studies assessed by both nuclear grading or Gleason score and the nuclear DNA ploidy pattern will be necessary to most accurately stratify patients for probability of tumor progression. Indeed, to members of our research group, it appears that for many patients nuclear DNA ploidy pattern and histologic grade/Gleason score are more important than tumor pathologic stage in forecasting tumor progression over clinically important time intervals.

Finally, for patients with prostate carcinoma clinically localized to the pelvic region, nuclear DNA ploidy analysis provides highly significant, important, and independent prognostic information that cannot be determined by routine histologic or other clinical variables. We believe this new test, which is objective, should be profitably used to stratify patients in future clinical research studies. The test can be routinely performed now and is relatively simple and economical. It is no longer a research laboratory curiosity. In our opinion, assessment of prognosis and analysis of clinical series of localized prostate carcinoma without taking into account the nuclear DNA ploidy patterns of patients with prostate adenocarcinoma would be neglecting objective information of important biological significance.

We thank Mary Adams for her technical assistance.

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Invited Commentary

This is an important article that presents a more sophisticated method of determining what subset of patients with apparent early-stage prostate cancer are at risk for developing metastatic disease following radical prostatectomy. Enhanced patient and physician awareness of prostate cancer, improved diagnostic techniques including prostatic ultrasound, and the increasing prevalence in an aging society will significantly increase the number of patients in whom the disease can be detected at an early, potentially curable stage. Traditionally, pathologic stage with histologic grading has provided rough determinants of prognosis. In this article, the authors have provided us with more discriminatory technology that may allow the distinction of patients suitable for clinical trials of adjuvant therapy designed to improve survival from patients destined to live out their lives without fear of developing metastatic prostate cancer. As indicated by the authors, however, application of this test is currently best limited to those patients with apparently confined prostate cancer but high Gleason score on histologic study.

DONALD G. SKINNER, MD
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Medical Malpractice Experience of Physicians: Predictable or Haphazard?

Frank A. Sloan, PhD; Paula M. Mergenhagen, PhD; W. Bradley Burfield, MA; Randall R. Bovbjerg, JD; Mahmud Hassan, MBA, PhD

This study uses a large malpractice database from Florida to assess the concentration of losses among physicians, predictability of claims experience, characteristics of physicians with favorable vs unfavorable experience, and effects of claims experience on physicians' practice decisions and on actions taken by the state's licensing board. Most payments by insurers involved a comparatively small number of physicians. Physicians with relatively prestigious credentials had no better, and on some indicators, worse claims experience. If anything, physicians with adverse claims experience were less likely to make subsequent changes in their practice, such as quitting practice or moving to another state. Physicians with very poor claims histories were more likely to have complaints filed against them with the Florida licensing board, but the sanctions against physicians with either poor or excellent histories were not severe. Physicians with adverse claims experience from incidents that arose between 1975 and 1980 had appreciably worse claims experience from incidents that arose during 1981 to 1983 (*JAMA*. 1989;262:3291-3297).

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Stage B prostate adenocarcinoma. Flow cytometric nuclear DNA ploidy analysis.

Montgomery BT, **Nativ O**, **Blute ML**, **Farrow GM**, **Myers RP**, **Zincke H**, **Therneau TM**, **Lieber MM**.

Department of Urology, Mayo Clinic, Rochester, MN 55905.

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