Stage C Prostatic Adenocarcinoma: Flow Cytometric Nuclear DNA Ploidy Analysis

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Flow cytometric nuclear DNA ploidy analysis was used to study pathologic stage C prostatic adenocarcinoma (pT3, N0, M0) in 146 patients who underwent radical retropubic prostatectomy and bilateral pelvic lymphadenectomy between 1967 and 1981. Of these tumors, 46% had a DNA diploid pattern, 47% had a DNA tetraploid pattern, and 7% had a DNA aneuploid pattern. Abnormal ploidy patterns were associated more frequently with histologic high-grade tumors than with low-grade tumors. Considered alone, DNA ploidy pattern showed a strong association with subsequent prognosis. The median interval to progression for tumors with DNA tetraploid and DNA aneuploid patterns was 7.8 and 3.5 years, respectively. For the DNA diploid tumors, only 23% progressed within 18 years, the longest follow-up. At 10 years, only 10% of patients with DNA diploid tumors had died of prostatic cancer, in comparison with 28% of the DNA tetraploid and 36% of the DNA aneuploid groups ($P<0.01$). By analysis of a combination of histologic tumor grade and nuclear DNA ploidy pattern, an even stronger association with prognosis was demonstrated. For the 38 patients with histologic low-grade and DNA diploid tumors, progression-free survival was 92% at 10 years, in comparison with 57% for 23 patients with low-grade DNA nondiploid tumors. Patients with high-grade tumors had a poorer prognosis whether the DNA ploidy pattern was diploid or nondiploid. Nuclear DNA ploidy pattern is an important and independent prognostic variable for patients with pathologic stage C prostatic cancer treated by radical prostatectomy.

Stage C prostatic cancer is the group of adenocarcinomas of the prostate in which the tumor has perforated the prostatic capsule and involved periprostatic tissues, including the seminal vesicles, membranous urethra, and bladder. The label "clinical stage C prostatic cancer" implies that distant metastatic lesions have been excluded; chest roentgenography, radionuclide bone scans, and computed tomographic scans of the abdomen and pelvis are the diagnostic modalities most often used in this endeavor. Pathologic stage C prostatic cancer identifies tumors with extracapsular extension of tumor proved by histopathologic evaluation and identified as pT3, N0, M0 in the UICC (Union Internationale Contre le Cancer [Inter-

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national Union Against Cancer]) staging system. Stage C prostatic carcinoma is one of the most common types of clinical prostatic cancer,[1-3] constituting approximately 40 to 50% of newly diagnosed cases.

Recent publications suggest that flow cytometric nuclear DNA ploidy analysis may be particularly useful in clarifying the biologic behavior of prostatic carcinoma and in helping to determine the prognosis for individual patients.[4-7] The ability to use formalin-fixed paraffin-embedded archival tumor samples to study nuclear ploidy by flow cytometry has greatly enhanced ploidy analysis and its association with patient prognosis. This technique was recently used to analyze tumor samples from patients who underwent radical prostatectomy and bilateral lymphadenectomy at our institution and who were found to have metastatic deposits in the pelvic lymph nodes. Among these patients with stage D1 (pT1-3, N1-2, M0) tumors and biopsy-proven nodal metastatic lesions, those with DNA diploid tumors had a remarkably favorable disease-free prognosis in comparison with those who had DNA tetraploid or DNA aneuploid pattern tumors.[8] In this current report, flow cytometric ploidy analysis of prostatic cancer was extended to a large cohort of patients with pathologic stage C prostatic carcinoma treated at the Mayo Clinic during the same time interval as the patients with stage D1 prostatic adenocarcinoma.

MATERIAL AND METHODS

Between 1967 and 1981, 159 patients with pathologic stage C prostatic adenocarcinoma underwent bilateral pelvic lymphadenectomy and radical retropubic prostatectomy at our institution. Of these patients, 13 were excluded from this study: 2 with low-quality DNA histograms, 5 with no tissue blocks available, and 6 with inadequate clinical follow-up. Therefore, paraffin-embedded archival specimens from 146 patients with pathologic stage C prostatic carcinoma were fully evaluable by flow cytometry for nuclear DNA content. The mean duration of follow-up for the entire group was 7.9 years (range, 4.8 to 17.8 years).

Histologic slides of these paraffin-embedded tumors stained with hematoxylin and eosin were reviewed by the study pathologist (G.M.F.) and were graded on the basis of the Mayo classification scheme[9] and the Gleason system.[10] Pathologic tumor volume was estimated with use of a method described by Zincke and associates.[11] Nuclear suspensions from paraffin-embedded tissue blocks were prepared by using the Hedley technique,[12] and isolated nuclei were stained by using the Vindeløv method.[13]

Technical methods used in this laboratory have been described in detail previously.[8] On the average, 1.56 tumor blocks per specimen were studied (1 block in 57 patients, 2 blocks in 83 patients, 3 blocks in 5 patients, and 4 blocks in 1 patient). In each tumor, the most abnormal ploidy pattern was recorded. Minimal sample discordance was found among the tumor blocks. Fresh and fixed samples of the same prostatic carcinoma (N = 22) have yielded identical results of ploidy pattern in our laboratory. Slightly higher percentage G2 results (number of nuclei in the 4C peak on the DNA histogram) were measured for the paraffin-embedded samples (unpublished results).

Cellular DNA content was measured on a FACS IV (fluorescence-activated cell sorter) flow cytometer (Becton Dickinson, Sunnyvale, California) equipped with a 5-W argon ion laser used at a wavelength of 514 nm. Each group of specimens was standardized with Fullbright Fluorospheres (Coulter Electronics, Inc., Hialeah, Florida), set to channel 35 on the FACS IV, to control day-to-day channel variations. Histograms of 20,000 nuclei for each sample were recorded at a maximal scanning flow rate of 1,000 nuclei per second. Cell-cycle evaluation of the DNA histograms derived by flow cytometry was obtained by using a computer program for Dean and Jett mathematical analysis.[14] Cross-classifications were assessed by using the Pearson χ^2 test. Nonprogression, crude survival, and cause-specific (from prostatic cancer-related death) survival curves were obtained with use of the Kaplan-Meier product-limit method.[15] For statistical comparisons between survival curves, the log-rank test was used.[16]

To quantitate the number of nuclei normally found in the nontumor G2 (or 4C) peak on the DNA histogram, we evaluated 60 specimens of benign prostatic hyperplasia. Nuclei extracted from these formalin-fixed and paraffin-embedded samples showed a mean percentage (±SD) of nuclei in the G2 (4C) peak of 7.87 ± 1.53%. On the basis of these data, an upper limit of 13% was defined as normal for the percentage of nuclei in the 4C peak, which would encompass 3 SD from the observed mean percentage. This same cutoff was used to define the limits of the DNA diploid second peak in the companion study on stage D1 prostatic adenocarcinoma.[8]

Tumor samples with a histogram similar to that seen for nuclei from the control specimens were classified as DNA diploid. Tumors that contained a significant increase in the G2 (4C) peak (more than 13% of the nuclei) were characterized as DNA tetraploid. The DNA ploidy pattern was considered DNA aneuploid if a third separate peak, different from the G0/G1 (2C) or the G2 (4C) peak, was present.

RESULTS

One hundred forty-six paraffin-embedded samples of pathologic stage C prostatic cancer were available for evaluation by flow cytometry and provided high-quality DNA histograms. The distribution of DNA ploidy patterns for the entire group was as follows: 67 (46%) had a DNA diploid pattern, 68 (47%) had a significant increase in the G2 (4C) tetraploid peak, and 11 (7%) had a distinct DNA aneuploid pattern.

Nuclear DNA Ploidy and Tumor Grade.— The distribution of nuclear DNA ploidy patterns for the various histologic grades of the tumors is shown in Figure 1 *A*. Only two patients had grade 1 tumors (both DNA diploid), and only six patients had grade 4 tumors (two of which were DNA diploid); thus, the tumor grades were grouped as 1 and 2 versus 3 and 4 for all subsequent analyses. Of the high-grade tumors, 66% had an abnormal DNA ploidy pattern, in comparison with 38% of the low-grade tumors ($P<0.004$). Similar results were found with use of the Gleason scoring system (Fig. 1 *B*). The 100 tumors with high Gleason scores (6-10) had

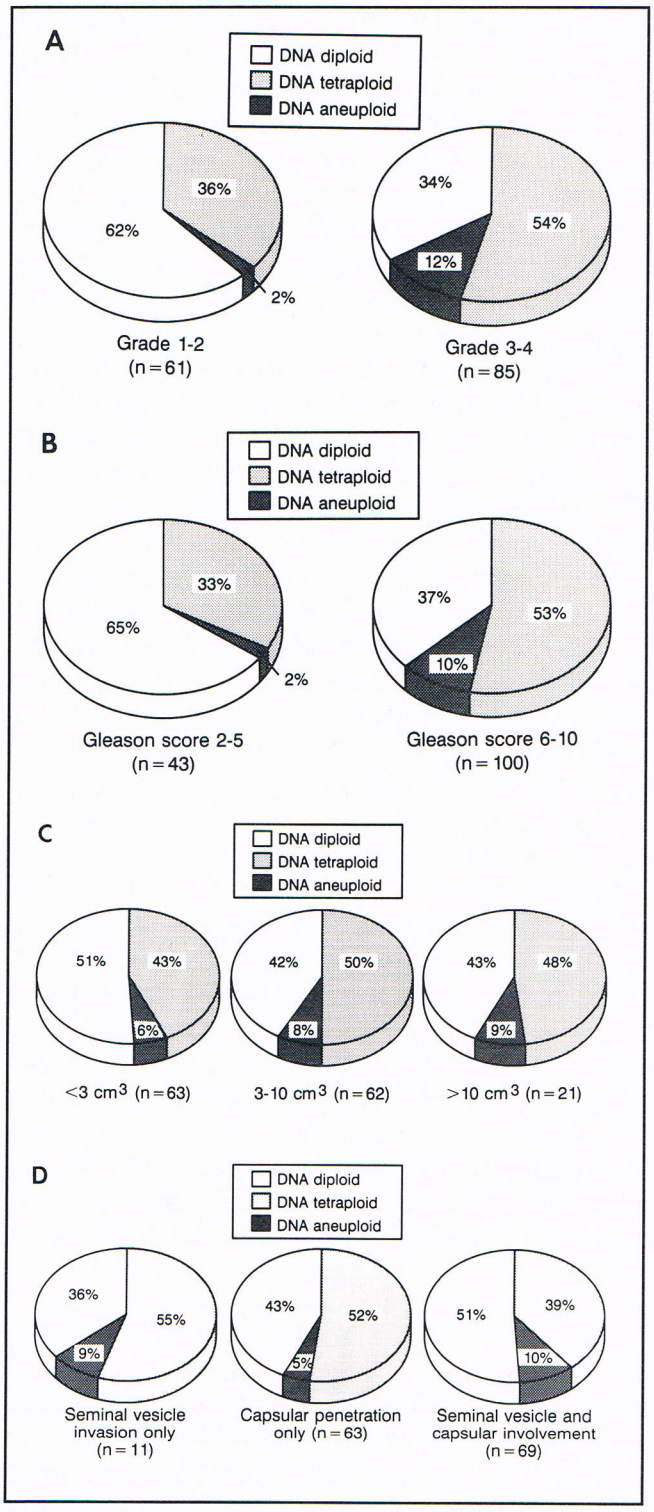

Fig. 1. Distribution of nuclear DNA ploidy patterns for stage C prostatic adenocarcinoma. *A*, Ploidy versus Mayo grade. *B*, Ploidy versus Gleason score. *C*, Ploidy versus tumor volume. *D*, Ploidy versus seminal vesicle or capsular involvement (or both).

many more DNA tetraploid and DNA aneuploid patterns than did the 43 tumors with low Gleason scores (2-5) ($P<0.002$). (Three tumors were nonevaluable for Gleason score.)

Nuclear DNA Ploidy and Tumor Volume.—The distribution of nuclear DNA ploidy patterns for small, medium, and large tumor volumes is depicted in Figure 1 *C*. Ploidy pattern did not vary significantly among the various tumor volume estimates.

Nuclear DNA Ploidy and Capsular or Seminal Vesicle Involvement.—Tumors were also classified on the basis of whether the base of the seminal vesicle was invaded, whether the prostatic capsule only was perforated by tumor, or whether both types of locally aggressive tumor spread were present in the pathologic specimen. The type of local tumor spread and the ploidy patterns are presented in Figure 1 *D*. No significant differences in distribution were observed for this group of pathologic variables.

Nuclear DNA Ploidy and Tumor Progression.—During the period of follow-up, 54 patients had recurrence of prostatic adenocarcinoma either locally (16 or 30%) or with distant metastatic lesions (38 or 70%). The DNA ploidy patterns of these two groups of patients is presented in Table 1. Seven of 11 DNA aneuploid tumors (64%), 34 of 68 DNA tetraploid tumors (50%), and 13 of 67 DNA diploid tumors (19%) demonstrated either local or systemic progression. The pattern of postoperative tumor progression (local recurrence, distant metastatic growth, or both) and its relationship to nuclear DNA ploidy patterns are depicted in nonprogression curves (Fig. 2). At 5, 10, and 15 years postoperatively, 85%, 77%, and 77%, respectively, of patients with DNA diploid tumors were clinically free of prostatic cancer. In contrast, for patients with DNA tetraploid tumors, the 5-, 10-, and 15-year nonprogression rates were 64%, 41%, and 41%, respectively. For patients with DNA aneuploid tumors, the 5-year disease-free rate was 45%. The difference between the diploid and the nondiploid curves was highly significant ($P<0.006$). The tetraploid and aneuploid curves cannot be judged as significantly different because of the small number of patients with DNA aneuploidy. The median time interval to progression for tumors with DNA tetraploid and DNA aneuploid patterns was 7.8 and 3.5 years, respectively. For the DNA diploid group of tumors, only 23% had progressed, and 18 years was the longest follow-up period.

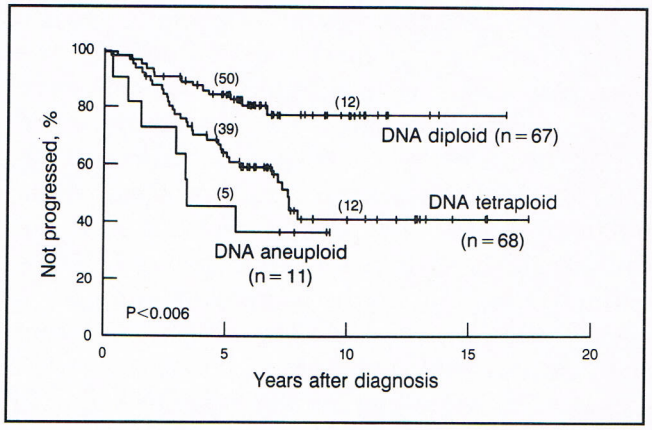

Fig. 2. Postoperative probability of nonprogression of cancer in patients with stage C prostatic adenocarcinoma for various DNA ploidy patterns ($P<0.006$, log-rank test). Numbers in parentheses denote number of patients at risk. Vertical bars represent censored cases.

Table 1.—**Nuclear DNA Ploidy Pattern and Site of Tumor Recurrence in 54 of 146 Patients With Stage C Prostatic Adenocarcinoma After Radical Retropubic Prostatectomy**

DNA ploidy pattern	No. of patients	Tumor recurrence (no.)		
		Local	Systemic	Total
Diploid	67	5	8	13
Tetraploid	68	7	27	34
Aneuploid	11	4	3	7
Total	146	16	38	54

For this cohort of patients with prostatic cancer, the histologic grade of the tumor showed an excellent correlation with progression of the lesion (Fig. 3 *A*). Patients with low-grade tumors (grades 1 and 2) had a good prognosis, whereas patients with high-grade tumors (grades 3 and 4) had relatively rapid tumor progression. Therefore, determining whether ploidy adds independent prognostic information is of interest, especially in light of the association of ploidy

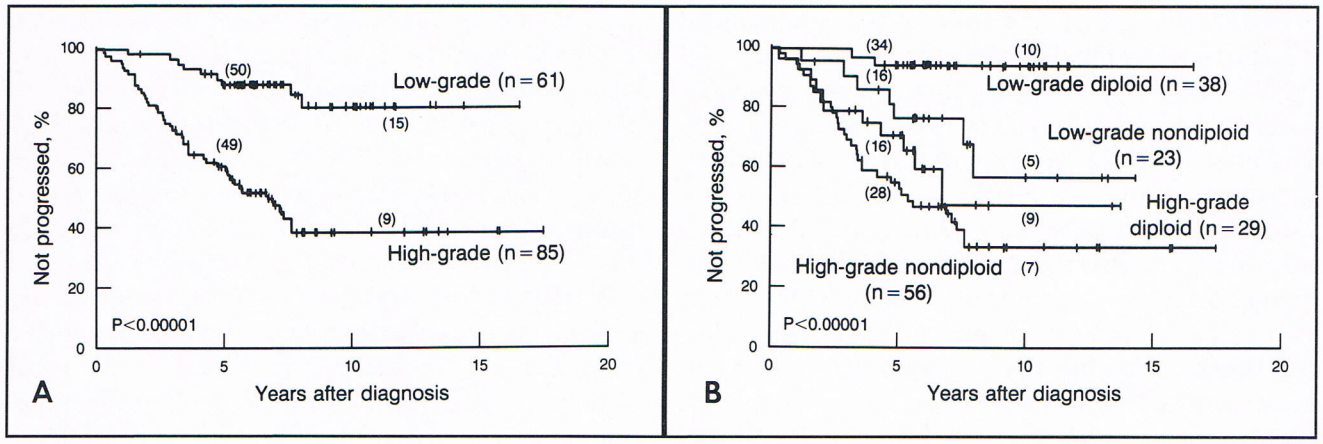

Fig. 3. Postoperative probability of nonprogression of cancer in patients with stage C prostatic adenocarcinoma. *A*, Probability of nonprogression for low-grade versus high-grade tumors ($P<0.00001$, log-rank test). *B*, Probability of nonprogression related to tumor grade and ploidy ($P<0.00001$, log-rank test). Numbers in parentheses denote number of patients at risk. Vertical bars represent censored cases.

and grade displayed in Figure 1 *A*. Figure 3 *B* shows that ploidy provides significant separation in patients with low-grade tumors ($P<0.008$), whereas it apparently does not in patients with high-grade tumors ($P = 0.23$). Only 8% of the patients with low-grade diploid tumors had recurrence at 10 years, in comparison with 43% of the patients with low-grade nondiploid tumors and 62% of those with high-grade tumors.

Nuclear DNA Ploidy and Patient Survival.—In an analysis of survival based on DNA ploidy pattern of the tumors (Fig. 4 *A*), no significant differences were noted between the various ploidy groups for crude survival. The survival curves constructed for disease-specific or prostatic cancer-related death (Fig. 4 *B*), however, showed a notable association of ploidy pattern with patient survival. At 10 years, only 10% of patients with DNA diploid tumors had died of prostatic cancer, in comparison with 28% of the DNA tetraploid and 36% of the DNA aneuploid groups ($P<0.01$).

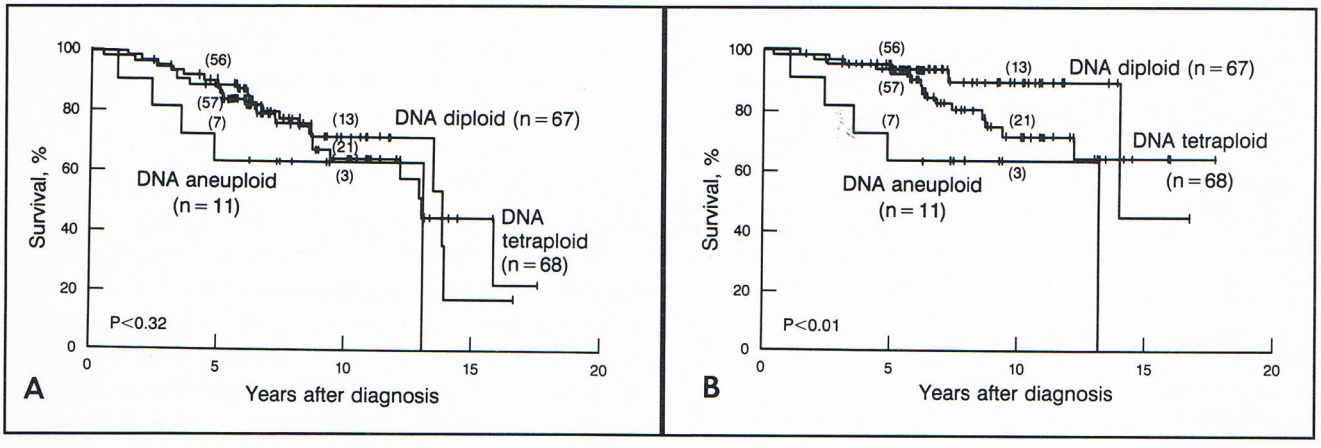

Fig. 4. Postoperative probability of survival for patients with stage C prostatic adenocarcinoma. *A*, Crude survival for various DNA ploidy patterns ($P<0.32$, log-rank test). *B*, Cause-specific survival for various DNA ploidy patterns ($P<0.01$, log-rank test). Numbers in parentheses denote number of patients at risk. Vertical bars represent censored cases.

Nuclear DNA Ploidy and Adjuvant Therapy.—Of the 146 patients in this series, 33 (23%) received early adjuvant therapy after radical prostatectomy. The distribution of ploidy patterns for the various adjuvant treatment groups is shown in Table 2. Twenty-four patients received early endocrine therapy: orally administered diethylstilbestrol in 14 and bilateral orchiectomy with or without diethylstilbestrol in 10. Thirteen patients underwent external-beam radiotherapy: x-ray therapy only in nine and x-ray therapy plus early endocrine treatment in four. Overall, the distribution of ploidy patterns among the adjuvant treatment groups was similar to that seen for the entire group of patients with pathologic stage C prostatic cancer. The nonprogression and survival curves for patients with adjuvant treatment were compared with those for patients who did not have adjuvant treatment. Although the curves seemed to separate after 8 years, further follow-up is needed to confirm or refute this suggestion. Thus, the presence or absence of adjuvant therapy does not seem likely to have had a major influence on the results obtained in the current study.

Early adjuvant therapy was strongly correlated with tumor grade. In patients with high-grade lesions, the treating physicians were more likely to prescribe early endocrine therapy than in those with low-grade lesions. After adjustment for tumor grade, no significant or even apparent association was found between early adjuvant therapy and tumor volume, type of local extension, or recurrence. The effect of ploidy pattern was most apparent for the low-grade tumors; only 3 of 61 patients with such tumors received early adjuvant therapy. Treatment of this small fraction of the patients could have had little effect on the overall results.

Table 2.—**Adjuvant Therapy and DNA Ploidy Pattern in 33 Patients With Stage C Prostatic Adenocarcinoma After Radical Prostatectomy**

Adjuvant therapy*	DNA ploidy pattern			
	Diploid	Tetraploid	Aneuploid	Total
DES	2	7	3	12
Orchiectomy ± DES	4	3	1	8
X-ray therapy only	6	3	...	9
X-ray therapy + DES	...	2	...	2
X-ray therapy + orchiectomy	1	1	...	2
Total	13	16	4	33

*DES = diethylstilbestrol.

Multivariate Analysis.—Multivariate interactions for tumor progression were investigated by using a Cox statistical model. The most important variable found, as previously mentioned, was tumor grade (1 and 2 versus 3 and 4), and the Gleason score acted as a surrogate for this partition. After incorporation of tumor grades, the *P* values for addition of other factors to the model were as follows: DNA diploid versus DNA nondiploid, 0.02; small volume (less than 3 cm^3) versus large volume of tumor (greater than 10 cm^3), 0.09; age, 0.17; capsular penetration alone versus other types of local extension, 0.18; and early endocrine therapy versus no early endocrine therapy, 0.83. If ploidy status was not entered into the model as a factor, no other variable or combination of variables besides tumor grade had any predictive value.

In addition to this overall Cox model, separate analyses were done on the subgroups with high-grade and low-grade tumors. Within the low-grade subgroup, ploidy was the only significant variable ($P = 0.002$); for all other variable groups, the difference was not significant ($P > 0.14$). Within the high-grade subgroup, ploidy status was not a significant variable ($P = 0.23$), nor was any other variable or combination of variables significant.

DISCUSSION

Flow cytometric nuclear DNA ploidy analysis with use of the Hedley technique was routinely applicable to archival samples of stage C prostatic adenocarcinoma. The overall distribution of nuclear DNA ploidy patterns found in this group of patients with stage C prostatic cancer was virtually identical to that noted in the group of patients with stage D1 adenocarcinoma of the prostate treated at the Mayo Clinic during the same period.[8] High-grade tumors had a statisti-

cally significant increase in abnormal ploidy patterns. In contrast, the distribution of ploidy patterns did not vary significantly among the various tumor volumes or the types of extracapsular extension.

Almost two-thirds of the DNA aneuploid tumors, 50% of the DNA tetraploid tumors, and approximately 20% of the DNA diploid tumors progressed locally or systemically or both during the observation period in the current study, a difference that was statistically significant. Moreover, nonprogression curves and disease-specific survival curves also demonstrated a highly significant difference in prognosis for those patients who had DNA diploid tumors in comparison with those patients who had DNA nondiploid tumors.

In this study of pathologic stage C prostatic cancer, the histologic grade of the tumor had an important association with patient prognosis (Fig. 3 *A*). This association was not observed previously in the group of patients with metastatic deposits in the pelvic lymph nodes.[8] Of importance, the coupling of nuclear DNA ploidy pattern measured by flow cytometry with histologic grading to identify certain groups of patients with pathologic stage C prostatic cancer may have prognostic implications. Of the 146 patients in this study, 38 (26% of the overall group) had histologic low-grade and DNA diploid tumors. These patients experienced an excellent prognosis when treated by radical retropubic prostatectomy and pelvic lymphadenectomy. During the follow-up period with a minimum of 5 years of observation, 92% remained free of disease. Those patients who had well-differentiated but nondiploid tumors had a significantly poorer prognosis, only 57% being disease-free during the period of follow-up ($P<0.02$). Those patients with histologic high-grade tumors fared less well. For patients with grade 3 or 4 tumors, differences in prognosis for those with DNA diploid pattern tumors and those with DNA nondiploid pattern tumors were not apparent. Thus, approximately a fourth of the patients with pathologic stage C prostatic carcinoma treated in this series—those with histologic low-grade DNA diploid tumors—had an excellent prognosis. Such tumors seem to be biologically different from similar tumors that are DNA nondiploid or from tumors with higher histologic grades. These results support acceptance of DNA ploidy pattern as an important variable for analyzing the prognosis of patients with locally advanced prostatic cancer.

Patients with low-grade DNA diploid stage C prostatic adenocarcinoma have an excellent prognosis when treated by radical prostatectomy alone. Patients with higher grade tumors and DNA nondiploid tumors have high rates of both local and systemic recurrence despite surgically proven negative pelvic lymph nodes. These data suggest that, after radical prostatectomy, patients who have high-grade and DNA nondiploid tumors might benefit from early aggressive adjuvant treatment, including local radiotherapy, hormonal treatment, cytotoxic chemotherapy, or combinations thereof in prospective trials. The ability to determine the DNA ploidy patterns accurately before and after surgical treatment of patients with apparently localized prostatic cancer may thus be important in selecting appropriate therapy.

The percentage with nonprogression, the crude survival, and the disease-specific survival among patients with pathologic stage C or pathologic stage D1 prostatic adenocarcinoma treated during the same time interval are shown in Figure 5. (The patients with stage D1 prostatic cancer were described previously in this journal.[8]) These data clearly show that, for nonprogression and disease-specific survival, DNA diploidy is associated with a favorable prognosis for patients with both stage C and D1 prostatic cancer. Indeed, for nonprogression and disease-specific survival, the curves for patients with DNA diploid tumors are similar in the two groups, as are those for patients with DNA nondiploid tumors. In this report and in the previous study of stage D1 prostatic cancer, nuclear DNA ploidy measured by flow cytometry was an independent prognostic variable when studied by standard statistical techniques. Ploidy pattern cannot be correlated simply with previous clinical variables such as histologic grade or tumor volume alone.

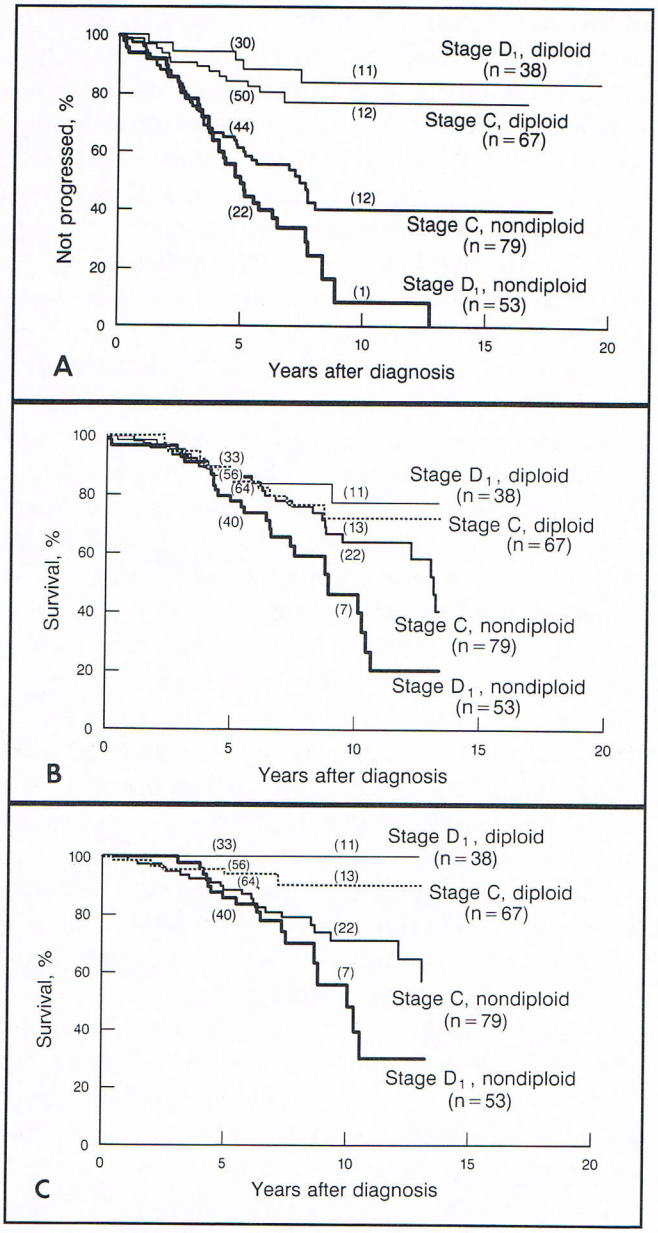

Fig. 5. Outcome of patients with stage C and D1 prostatic adenocarcinoma after radical prostatectomy. *A*, Probability of nonprogression of cancer related to tumor stage and ploidy. *B*, Crude survival related to tumor stage and ploidy. *C*, Cause-specific survival related to tumor stage and ploidy. Numbers in parentheses denote number of patients at risk.

A major study in which ploidy was correlated with outcome in a large group of patients with stage C prostatic cancer was recently published.[17] In that group, 42% of the tumors were DNA diploid and 58% were DNA nondiploid, similar to the ploidy distribution found in the current study (46% and 54%, respectively). Like us, those authors concluded that the nuclear DNA ploidy pattern was significantly correlated with postoperative tumor recurrence.

These data indicate to members of our research group that nuclear DNA ploidy must be considered in future clinical research studies and treatment deliberations for patients with locally advanced or regionally metastatic prostatic carcinoma.

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REFERENCES

1. De Vere White R, Paulson DF, Glenn JF: The clinical spectrum of prostate cancer. J Urol 117:323-327, 1977
2. Catalona WJ, Scott WW: Carcinoma of the prostate: a review. J Urol 119:1-8, 1978
3. Kirk D: Prostatic carcinoma. Br Med J 290:875-876, 1985
4. Fordham MVP, Burdge AH, Matthews J, Williams G, Cooke T: Prostatic carcinoma cell DNA content measured by flow cytometry and its relation to clinical outcome. Br J Surg 73:400-403, 1986
5. Benson MC, Walsh PC: The application of flow cytometry to the assessment of tumor cell heterogeneity and the grading of human prostatic cancer: preliminary results. J Urol 135:1194-1198, 1986
6. Tribukait B: Flow cytometry in assessing the clinical aggressiveness of genito-urinary neoplasms. World J Urol 5:108-122, 1987
7. Stephenson RA, James BC, Gay H, Fair WR, Whitmore WF Jr, Melamed MR: Flow cytometry of prostate cancer: relationship of DNA content to survival. Cancer Res 47:2504-2507, 1987
8. Winkler HZ, Rainwater LM, Myers RP, Farrow GM, Therneau TM, Zincke H, Lieber MM: Stage D1 prostatic adenocarcinoma: significance of nuclear DNA ploidy patterns studied by flow cytometry. Mayo Clin Proc 63:103-112, 1988
9. Utz DC, Farrow GM: Pathologic differentiation and prognosis of prostatic carcinoma. JAMA 209:1701-1703, 1969
10. Gleason DF, Mellinger GT, Veterans Aministration Cooperative Urological Research Group: Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 111:58-64, 1974

11. Zincke H, Fleming TR, Furlow WL, Myers RP, Utz DC: Radical retropubic prostatectomy and pelvic lymphadenectomy for high-stage cancer of the prostate. Cancer 47:1901-1910, 1981
12. Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA: Method for analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. J Histochem Cytochem 31:1333-1335, 1983
13. Vindeløv LL, Christensen IJ, Nissen NI: A detergent-trypsin method for the preparation of nuclei for flow cytometric DNA analysis. Cytometry 3:323-327, 1983
14. Dean PN, Jett JH: Mathematical analysis of DNA distributions derived from flow microfluorometry. J Cell Biol 60:523-527, 1974
15. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958
16. Peto R, Peto J: Asymptomatically efficient rank invariant test procedures. J R Stat Soc [A] 135:185-206, 1972
17. Lee SE, Currin SM, Paulson DF, Walther PJ: Flow cytometric determination of ploidy in prostatic adenocarcinoma: a comparison with seminal vesicle involvement and histopathological grading as a predictor of clinical recurrence. J Urol 140:769-774, 1988