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Stage D1 Prostatic Adenocarcinoma: Significance of Nuclear DNA Ploidy Patterns Studied by Flow Cytometry

HARRY Z. WINKLER, M.D.,* LESLIE M. RAINWATER, M.D., ROBERT P. MYERS, M.D., *Department of Urology*; GEORGE M. FARROW, M.D., *Division of Pathology*; TERRY M. THERNEAU, Ph.D., *Section of Biostatistics*; HORST ZINCKE, M.D., MICHAEL M. LIEBER, M.D., *Department of Urology*

Flow cytometric analysis of nuclear DNA ploidy pattern was performed on 91 samples of prostatic adenocarcinoma from patients with stage D1 disease (metastatic deposits in pelvic lymph nodes). All patients had undergone radical retropubic prostatectomy and bilateral pelvic lymphadenectomy. Clinical follow-up ranged from 5 to 19 years. Nuclei were extracted from paraffin-embedded archival material. Isolated nuclei were stained with propidium iodide. The DNA ploidy pattern was diploid (normal) in 42% of tumors, tetraploid in 45%, and distinctly aneuploid in 13%. Only 15% of DNA diploid tumors progressed locally or systemically, whereas 75% of tumors with an abnormal DNA ploidy pattern (tetraploid or aneuploid) subsequently progressed ($P<0.0001$). Among low-grade tumors, ploidy analysis detected a subgroup associated with a poor prognosis; among high-grade tumors, a subgroup associated with a favorable prognosis was detected. None of the patients with a DNA diploid tumor died of prostatic cancer during the period of observation. In contrast, 43% of patients with DNA tetraploid tumors and 44% of those with DNA aneuploid tumors had died of prostatic cancer 10 years after surgical treatment ($P<0.001$). Determination of nuclear DNA ploidy pattern by flow cytometry provides objective, highly significant, prognostic information for patients with stage D1 prostatic carcinoma.

An important problem in the management of prostatic adenocarcinoma is predicting its malignant potential in individual patients.[1,2] Pathologic staging and histologic grading performed with standard light microscopic techniques have been the traditional and proven methods for assigning treatment and assessing the prognosis for patients with cancer of the prostate.[3-7] Nevertheless, within the subsets of patients with a given stage and grade of prostatic carcinoma, large variations in the response to treatment, time to disease progression, and patient survival are typically seen. A need exists for new laboratory tests that can more accurately predict the clinical behavior of prostatic carcinoma.

Analysis of tumor nuclear DNA content by flow cytometry now has been demonstrated to be useful in predicting the clinical course of patients with various urologic malignant lesions.[8-14] Although adenocarcinoma of the prostate is the most prevalent urologic malignant disease in the

*Current address: Beilinson Medical Center, Petah-Tiqva, Israel.

Address reprint requests to Dr. M. M. Lieber, Department of Urology, Mayo Clinic, Rochester, MN 55905.

United States, only a relatively small number of prostatic carcinoma samples have been studied thus far by flow cytometric techniques. Early studies used tumor cells from freshly excised prostatic tumor samples.[15-19] These studies demonstrated that the nuclear DNA ploidy pattern correlated well with tumor stage and grade. In 1983, Hedley and associates[20] described a method of analyzing nuclear DNA content by flow cytometry of nuclei extracted from formalin-fixed paraffin-embedded archival pathologic material. This new method obviates the need for fresh tissue and allows retrospective analysis in which the DNA ploidy pattern can be correlated with a long-term clinical course. A few studies in which the Hedley method of flow cytometry was performed on prostatic carcinoma samples have recently been published.[21-24] These studies indicated that patients with a DNA diploid ploidy pattern have a better prognosis than those patients with nondiploid ploidy patterns.

Patients with prostatic carcinoma who have biopsy-proven metastatic deposits in the pelvic lymph nodes but no evidence of tumor dissemination on radioactive bone scans are commonly classified as having stage D1 prostatic carcinoma. Such patients have a particularly indeterminate natural history; the type and timing of surgical, radiation, and hormonal treatment of such patients with stage D1 carcinoma are controversial areas in urologic oncology. At our institution, a cohort of 100 patients with stage D1 prostatic cancer was available for study; the follow-up period for these patients ranged from 5 to 19 years after treatment by bilateral pelvic lymphadenectomy and radical retropubic prostatectomy. This group of patients with stage D1 prostatic cancer and their tumor specimens seemed to be ideal for investigation by use of the Hedley technique for nuclear DNA ploidy analysis.

MATERIAL AND METHODS

Paraffin-embedded archival specimens from 91 of the 100 stage D1 (metastatic deposits in pelvic lymph nodes) prostatic adenocarcinomas resected at our medical center between February 1967 and July 1981 were evaluable by flow cytometric DNA measurements and yielded high-quality DNA histograms. Of the nine tumors with no data available and excluded from the study, three yielded low-quality histograms, three had no tissue blocks available, and three cases were discovered after the study was completed. All patients had undergone radical retropubic prostatectomy and bilateral pelvic lymphadenectomy. The histories of the 91 patients were reviewed with specific attention to pathologic grade, tumor volume, number of pelvic lymph nodes involved, additional hormonal treatment, progression as either local recurrence or metastatic lesions (or both), and survival. Hematoxylin and eosin-stained slides of these paraffin-embedded tissue blocks were reviewed by the study pathologist (G.M.F.). Pathologic grading was performed according to the Mayo classification[4] and the Gleason system.[3] The tumor volume was measured as described by Myers and associates.[25] The clinical and pathologic characteristics of the patients are shown in Table 1.

Nuclear suspensions from paraffin-embedded tissue blocks were prepared by using the technique described by Hedley and colleagues.[20] Three 40-μm-thick sections were cut with use of a standard tissue microtome. The sections were placed in 10-ml glass culture tubes, dewaxed by

Table 1.—**Clinical and Pathologic Characteristics of 91 Patients With Stage D1 Prostatic Adenocarcinoma***

Characteristic	No. of patients
Mayo grade	
1-2	49
3-4	42
Gleason score	
2-5	33
6-10	58
Tumor volume (cm^3)	
<3	17
3-10	31
>10	43
Involved lymph nodes	
1	43
2	21
3	13
≥4	14
Early endocrine therapy	
Orchiectomy	24
Diethylstilbestrol	10
Orchiectomy + diethylstilbestrol	9
None	48
Status†	
Living: Free of disease	46
With recurrence	10
Deceased: Cancer-related death	19
Other cause of death	16

*The ages of the patients at the time of surgical treatment ranged from 41 to 73 years (median, 64 years).
†The duration of follow-up for patients still living ranged from 4.8 to 19.6 years (median, 7.5 years).

using two changes of 3 ml of Histo-Clear (National Diagnostics, Somerville, New Jersey) for 10 minutes each at room temperature, and rehydrated in a sequence of 3 ml of 100%, 95%, and 70% ethanol for 10 minutes each at room temperature. The tissue was then washed twice in distilled water and resuspended in 1 ml of 0.5% pepsin (P 7012, Sigma Chemical Company, St. Louis, Missouri) in 0.9% sodium chloride, adjusted to pH 1.5. The specimens were incubated at 37°C for 60 minutes and subjected to frequent intermittent vortex mixing. The resulting nuclear suspension was centrifuged at 2,800 rpm for 10 minutes to form a nuclear pellet, and the pepsin supernatant was removed.

The isolated nuclei were stained with propidium iodide by using the method of Vindeløv and co-workers.[26] First, 0.9 ml of a solution (solution A) that contained 0.015 g of trypsin dissolved in 500 ml of stock solution [2 g of trisodium citrate · $2H_2O$, 2 ml of Nonidet P-40, 1.044 g of spermine tetrahydrochloride, and 0.121 g of tris(hydroxymethyl)aminomethane dissolved in distilled water to make a total volume of 2,000 ml], adjusted to pH 7.6, was added to 0.1 ml of nuclear suspension in citrate buffer and mixed gently for 10 minutes. Then, 0.75 ml of a solution (solution B) that contained 0.25 g of trypsin inhibitor and 0.05 g of ribonuclease A dissolved in 500 ml of stock solution, adjusted to pH 7.6, was added and mixed gently for 10 minutes. Finally, 0.75 ml of a solution (solution C) that contained 0.208 g of propidium iodide and 0.580 g of spermine tetrahydrochloride dissolved in 500 ml of stock solution, adjusted to pH 7.6, was added. The solution of propidium iodide was protected from light by use of tinfoil during the preparation, storage, and staining procedure. The solutions were mixed, and the sample was filtered through a 30-μm pore diameter nylon mesh filter to provide single nuclei and to eliminate nuclear clumps. Samples were processed on the flow cytometer within 30 minutes after the addition of propidium iodide.

Nuclear 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. Every 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 and the coefficient of variation of the diploid G0/G1 peak derived by flow cytometry were obtained by using a computer program for Dean and Jett mathematical analysis.[27]

The flow cytometric data were compared statistically by using the adjusted chi-square test. Nonprogression, overall survival, and cause-specific (from prostatic cancer death) survival curves were obtained by using the Kaplan-Meier estimator.[28] Statistical comparison of these curves for various DNA ploidy subgroups was done with use of the log-rank test.[29] The relationship of continuous variables to survival and progression was examined with use of scatterplots[30] (Fleming T, Grambsch PM, Therneau TM: Personal communication).

For quantitating the number of nuclei normally found in the nontumor 4C or G2 peak, 60 specimens of human benign prostatic hyperplasia were studied. Nuclei extracted from these 60 formalin-fixed and paraffin-embedded samples showed that the mean percentage ± SD of nuclei in the 4C or G2 peak was 7.87 ± 1.53%. On the basis of these normal tissue control 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. Tumors that contained a significant increase in G2 (4C) peak (those having more than 13% of the nuclei in the 4C peak) were categorized as DNA tetraploid. Tumor samples with a histogram similar to that seen for nuclei from sections of benign prostatic hyperplasia tissue were classified as DNA diploid (or normal).

Tumor DNA content was classified as aneuploid if a third separate peak was present that differed from the standard large diploid G0/G1 (2C) peak and the small tetraploid G2 (4C) peak. The term "aneuploidy" by convention is used to designate an abnormal DNA stemline of cells, but the absence of an abnormal DNA stemline as determined by flow cytometry does not exclude the existence of an abnormal karyotype, such as a balanced translocation.[31] A DNA index was calculated as a ratio of the peak channel (corresponding to DNA amount) of the abnormal DNA stemline of cells to the peak channel of the DNA diploid cells. By definition, the DNA index of

diploid cells with a standard large 2C and a small 4C peak is 1.0.[31] The Fullbright Fluorosphere singlet peak was always set at channel 35, whereas the Fullbright Fluorosphere doublet peak appeared at channel 76; thus, the ratio for doublet to singlet peak channels on the FACS IV instrument used was 2.17. All tissue blocks were analyzed, and histograms were classified as DNA diploid, DNA tetraploid, or DNA aneuploid without knowledge of patient survival.

RESULTS

Ninety-one paraffin-embedded specimens of stage D1 prostatic adenocarcinoma were evaluable by flow cytometry. Thirty-eight of the tumors (42%) showed histograms that closely resembled the histograms observed for nontumor control samples of human benign prostatic hyperplasia tissue; these tumors were categorized as DNA diploid or normal (Fig. 1 *A* and *B*). Forty-one tumors (45%) exhibited a significant increase (more than 13% of the total nuclei) in the 4C peak and were designated as DNA tetraploid (Fig. 1 *C*). Twelve tumors (13%) showed a distinct aneuploid peak (Fig. 1 *D*).

Ploidy and Tumor Grade.—The distribution of nuclear DNA patterns for the low-grade (Mayo grades 1 and 2) and the high-grade (Mayo grades 3 and 4) stage D1 prostatic adenocarcinomas is presented in Figure 2 *A*. The DNA aneuploid tumors were generally high grade ($P<0.003$, chi-square test); no statistical difference in tumor grading was found between the DNA diploid and tetraploid tumors. When the tumors were graded on the basis of Gleason score, no significant difference in ploidy distribution was seen between the low-score (2 through 5) and the high-score (6 through 10) tumors, although DNA aneuploid patterns were more common in the group with high Gleason scores (Fig. 2 *B*).

Ploidy and Tumor Volume.—The distribution of nuclear DNA ploidy patterns according to the volume of the primary tumor is shown in Figure 2 *C*. No significant correlation was found between the tumor DNA ploidy pattern and tumor volume. Small tumors and large tumors had similar frequencies of normal and abnormal ploidy patterns.

Ploidy and Number of Involved Lymph Nodes.—Although no statistically significant differences were found between the different ploidy subgroups and the extent of lymph node involvement, a slight trend was seen toward association of DNA aneuploidy with a higher number of involved lymph nodes. The association between number of involved lymph nodes and ploidy is depicted in Figure 2 *D*.

Ploidy and Tumor Progression.—The relationship of DNA ploidy pattern to tumor progression (local recurrence or metastatic disease) was studied for patients who had either a minimum of 5 years of follow-up postoperatively or tumor progression. The longest follow-up was 19 years 7 months (median, 7.5 years). Rates of tumor progression according to ploidy patterns and stratified for pathologic grade are presented in Table 2. Only 15% of the stage D1 prostatic adenocarcinomas that exhibited DNA diploidy

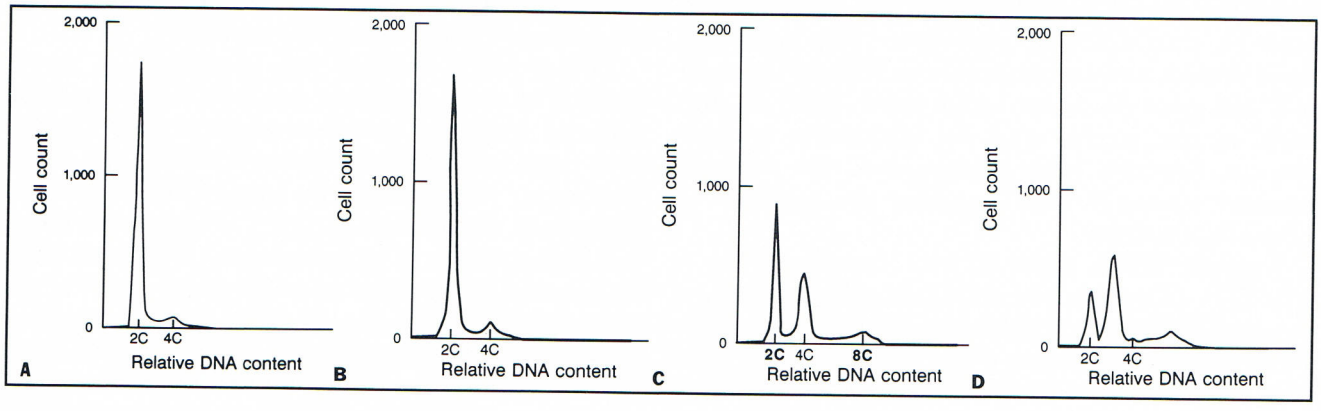
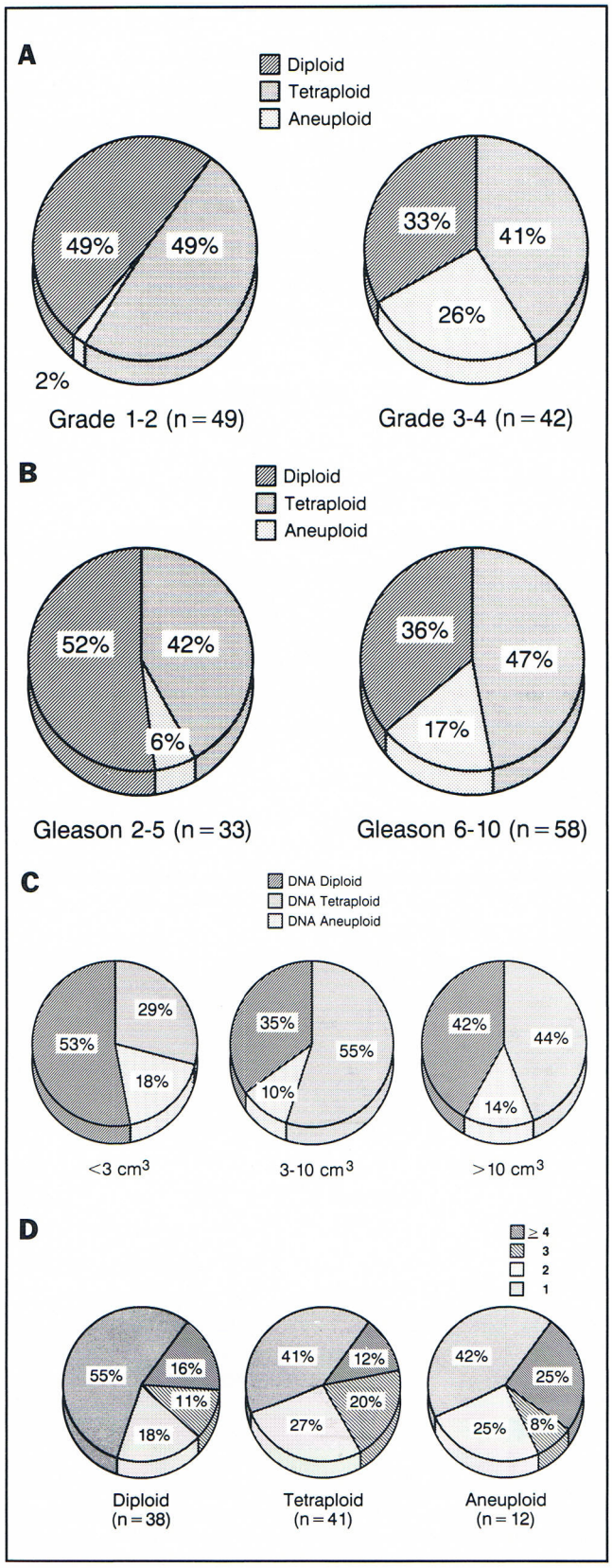


Fig. 1. Nuclear DNA histogram patterns by flow cytometry of benign prostatic hyperplasia (*A*) and prostatic adenocarcinoma (*B* through *D*). *A*, Pattern of benign prostatic hyperplasia. *B*, DNA diploid (normal) pattern. *C*, DNA tetraploid pattern. *D*, DNA aneuploid pattern.



progressed. In contrast, 75% of the tumors with an abnormal DNA ploidy pattern (tetraploid or aneuploid) showed progression either locally or systemically. This difference between the normal and abnormal ploidy groups was highly significant ($P<0.0001$, log-rank test).

Nonprogression curves constructed for the various nuclear DNA ploidy patterns are presented in Figure 3. At 5, 10, and 15 years postoperatively, 92%, 84%, and 84%, respectively, of the patients with tumors that showed DNA diploid patterns were free of disease. In contrast, patients with tumors that exhibited DNA tetraploidy had 5-, 10-, and 15-year free-of-disease rates of 48%, 9%, and 0%, respectively. Those patients who had tumors with DNA aneuploidy had 5- and 8-year free-of-disease rates of 51% and 15%, respectively. This difference between the normal and the abnormal ploidy progression curves was highly significant ($P<0.0001$, log-rank test).

In an analysis of the time interval from surgical treatment to tumor progression, we found differences between ploidy subgroups. The median time interval to progression for tumors with DNA diploid histograms was 53 months (range, 49 to 81 months), whereas for those tumors with DNA tetraploid and DNA aneuploid patterns, the median time interval to progression was much shorter—39 months (range, 4 to 150 months) and 22.5 months (range, 6 to 56 months), respectively.

Twenty-three percent of the low-grade (Mayo grades 1 and 2) tumors with DNA diploidy progressed, whereas in 77% of the patients with low-grade tumors that exhibited abnormal ploidy, either local recurrence or distant metastatic lesions subsequently developed (Table 2) ($P<0.001$). Similar differences in incidence of progression were found in tumors with low Gleason scores (2 through 5) ($P<0.001$). For high-grade (Mayo grades 3 and 4 and Gleason scores 6 through 10) tumors, the association of abnormal ploidy pattern with tumor progression was clearly seen. Whereas none of the Mayo high-grade tumors and only 18% of the tumors with high Gleason scores and DNA diploid patterns progressed, 73%

Fig. 2. Distribution of nuclear DNA ploidy patterns for stage D1 prostatic adenocarcinoma. *A*, Ploidy versus Mayo grade. *B*, Ploidy versus Gleason score. *C*, Ploidy versus tumor volume. *D*, Ploidy versus number of involved lymph nodes.

Table 2.—**Tumor Progression* Shown by DNA Ploidy Pattern and by Pathologic Grade of Prostatic Adenocarcinoma**

Outcome	Total		DNA histogram pattern: Diploid (normal)		Tetraploid		Aneuploid		Tetraploid + aneuploid (abnormal)	
	No.	%	No.	%	No.	%	No.	%	No.	%
Overall results†										
Progression	41	51	5	15	28	74	8	80	36	75
No progression	40	49	28	85	10	26	2	20	12	25
Died <5 yr postop (no progression)	10		5		3		2		5	
Results by pathologic grade										
Mayo grade 1-2‡										
Progression	22/44	50	5/22	23	16/21	76	1/1	100	17/22	77
Died <5 yr postop (no progression)	5		2		3		0		3	
Mayo grade 3-4†										
Progression	19/37	51	0/11	...	12/17	71	7/9	78	19/26	73
Died <5 yr postop (no progression)	5		3		0		2		2	
Gleason score 2-5‡										
Progression	12/30	40	2/16	12	8/12	67	2/2	100	10/14	71
Died <5 yr postop (no progression)	3		1		2		0		2	
Gleason score 6-10†										
Progression	29/51	57	3/17	18	20/26	77	6/8	75	26/34	76
Died <5 yr postop (no progression)	7		4		1		2		3	

*Studied for patients with a minimum of 5 years of follow-up postoperatively.
†$P<0.0001$, normal versus abnormal pattern group (log-rank test).
‡$P<0.001$, normal versus abnormal pattern group (log-rank test).

and 76%, respectively, of those poorly differentiated tumors with abnormal DNA ploidy patterns progressed. This important difference in tumor progression between normal and abnormal ploidy groups noted for the high-grade tumors was highly significant ($P<0.0001$, log-rank test).

Ploidy and Patient Survival.—Kaplan-Meier survival curves were constructed for the patients studied (Fig. 4 *A*). Patients with tumors that exhibited DNA diploidy had 5-, 10-, and 15-year projected survival rates of 87%, 78%, and 65%, respectively. Those patients with tumors that had abnormal ploidy patterns had significantly lower 5-, 10-, and 15-year survival rates—80%, 48%, and 12%, respectively, for tumors with DNA tetraploidy and 67%, 47%, and 0%, respectively, for tumors that exhibited DNA aneuploidy ($P<0.002$, log-rank test).

The effect of ploidy on patient survival *from prostatic cancer death* is presented in Figure 4 *B*. None of the patients with tumors that had a normal ploidy pattern died of prostatic cancer. Forty-three percent of the patients with tumors

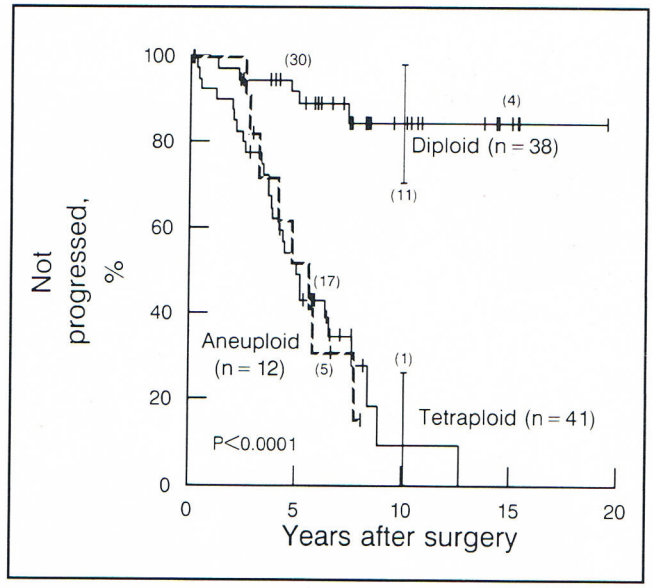


Fig. 3. Postoperative probability of nonprogression of prostatic adenocarcinoma ($P<0.0001$, log-rank test) for normal versus abnormal DNA patterns. Numbers in parentheses represent number of patients at risk. Vertical bars represent censored cases.

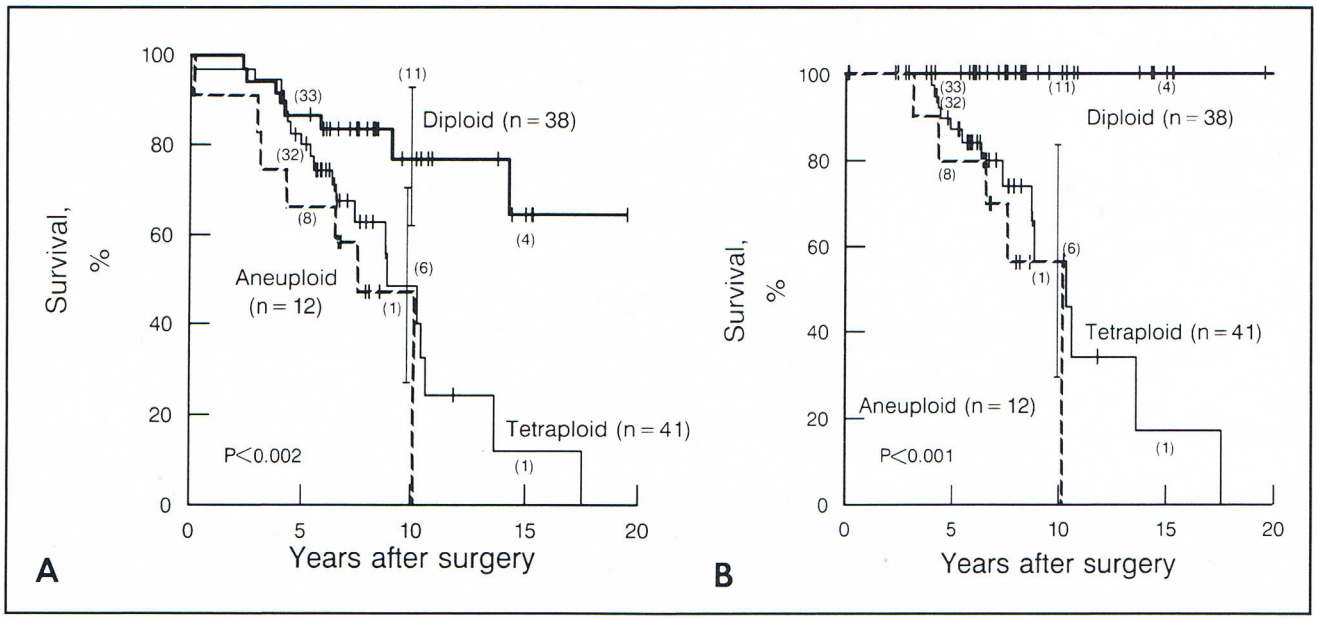


Fig. 4. Probability of survival after radical retropubic prostatectomy for patients with stage D1 prostatic adenocarcinoma, shown by various DNA ploidy patterns. *A*, Crude survival ($P<0.002$, log-rank test) for normal versus abnormal DNA patterns. *B*, Cause-specific survival ($P<0.001$, log-rank test) for normal versus abnormal DNA patterns. Numbers in parentheses represent number of patients at risk. Vertical bars represent censored cases.

that showed DNA tetraploid patterns and 44% of those with tumors that exhibited DNA aneuploid patterns had died of prostatic cancer 10 years after surgical treatment ($P<0.001$, log-rank test).

At 5 and 10 years postoperatively, the clinical outcome was determined for 91 and 46 patients, respectively (Table 3). At those intervals, 79% and 61%, respectively, of the patients with tumors that showed DNA diploid patterns were alive and free of disease in comparison with only 43% and 4%, respectively, of patients with tumors that had abnormal DNA ploidy patterns.

Percentage G2 and Prognosis.—We compared the number of nuclei in the 4C peak (percentage G2) for DNA diploid and DNA tetraploid tumors with the patient's clinical course. This analysis allowed direct assessment of "percentage G2" on the patient's risk for tumor progression or death (Fig. 5 *A* and *B*). A transition from low to high risk at values from 10 to 15% was found, above which the patient's risk for tumor progression or death significantly increased.

Table 3.—**DNA Ploidy Pattern and Clinical Outcome in Patients With Prostatic Adenocarcinoma***

DNA ploidy pattern	Disease-free survival			
	At 5 yr (N = 91)		At 10 yr (N = 46)	
	No.	%	No.	%
Diploid	30/38	79	11/18	61
Nondiploid	23/53	43	1/28	4

*All patients underwent radical retropubic prostatectomy and bilateral pelvic lymphadenectomy.

DISCUSSION

Carcinoma of the prostate is a tumor that varies considerably in biologic behavior, ranging from a state of long-standing indolence to a highly aggressive and lethal metastasizing cancer. The management of prostatic cancer in which metastatic lesions are limited to the pelvic lymph nodes is a controversial subject. Despite the belief that stage D1 disease should be considered a systemic disease, which therefore is incurable by current surgical[32] or radiotherapeutic[33] means, several reports have suggested that radical prostatectomy and pelvic lymphadenectomy for patients with stage D1 cancer of the prostate may result in prolonged disease-free survival for some patients and early tumor progression and death in others.[34-36] These differences in clinical outcome do not seem predictable on the basis of any

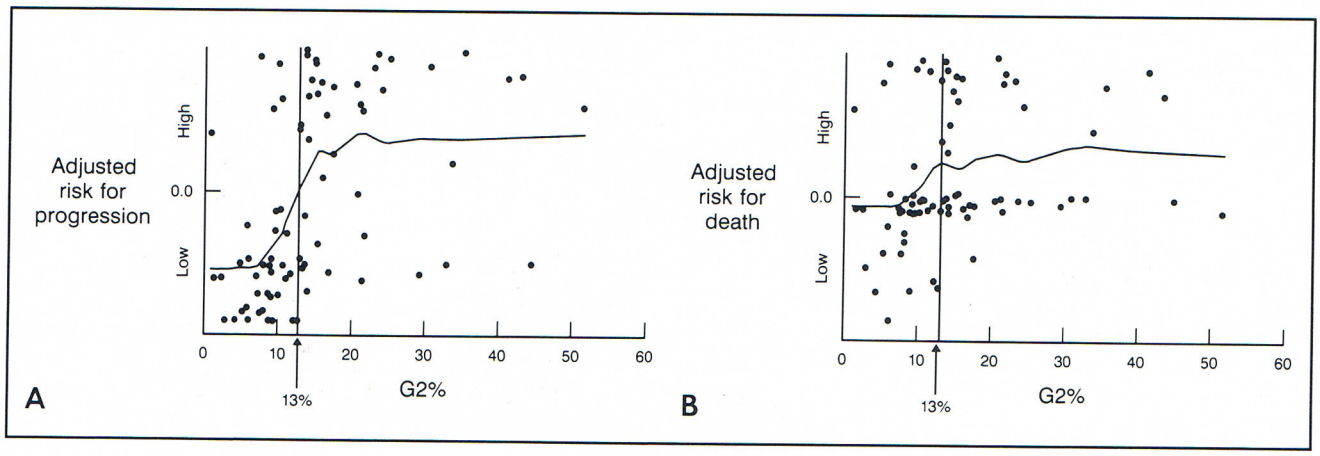


Fig. 5. Scatterplots of percentage G2 (number of nuclei in the 4C peak on the DNA histogram) versus risks for patients with prostatic adenocarcinoma of diploid or tetraploid DNA ploidy pattern. The y variable presents patients' risk for tumor progression (*A*) or death (*B*), adjusted for duration of follow-up in the study (a patient who was alive very early or who died very late in the study performed "as expected" and would receive a value near zero). Continuous curve acts as visual aid to summarize pattern of plot. Sudden transition from low to high risk at percentage G2 values of 10 to 15% was evident.

conventional tumor variable once pelvic lymph nodes are involved.[35,36]

Although correlations of histologic grade with frequency of tumor progression, time to progression, or time to cancer-related death generally demonstrate more aggressive behavior with increasing grade of the tumor, none of the standard grading systems predicts tumor behavior and its metastatic potential categorically for the individual patient or has been able to distinguish the "good" from the "bad" intermediate-grade lesions.[37]

Several studies have reported a positive correlation between tumor DNA ploidy pattern and histologic grading; well-differentiated tumors tended to show normal ploidy patterns, and poorly differentiated lesions exhibited abnormal ploidy patterns.[21,22] Lundberg and associates,[23] however, found no significant correlation between the histopathologic grade and the tumor ploidy. This study did reveal a significant association between high tumor grade and DNA aneuploidy. No significant difference in tumor grade was noted for the DNA diploid and DNA tetraploid tumors. When tumors were classified on the basis of both ploidy pattern and degree of glandular differentiation, subgroups of tumors with higher or lower malignant potential became apparent. Tumors that morphologically were similar demonstrated dissimilarity in DNA profiles and evidenced different biologic behavior. In each grade subgroup, the tumors with abnormal DNA ploidy patterns behaved much more aggressively than did those with DNA diploidy. These important biologic features are undetectable by histologic examination of the primary tumor.

Retrospective studies in which static DNA cytometry was used have shown that DNA diploid tumors are associated with a considerably better prognosis than are nondiploid tumors.[15,38] Other investigators who used flow cytometric DNA analysis recently reported a significant relationship between DNA ploidy pattern and patient survival.[22-24] The current study demonstrates that DNA diploid stage D1 prostatic adenocarcinomas are associated with a relatively much more favorable clinical outcome than are DNA tetraploid or aneuploid tumors. Patients with tumors that show either DNA tetraploidy or aneuploidy had a highly statistically significant increase in the incidence of local and systemic tumor progression and of death from prostatic cancer. Similar results have been reported recently by Stephenson and colleagues,[24] who analyzed 82 patients with stage D1 prostatic cancer treated with ^{125}I seed implants. In this latter study, measurements of DNA content were made on tumor cell nuclei recovered from pelvic lymph node metastatic lesions, not from the primary prostatic cancer. These investigators found a

much more favorable clinical course for patients with DNA diploidy than for those with "aneuploidy" in terms of disease-free survival. Furthermore, nuclear DNA measurement has been proved to be a valuable predictor of a likely response to adjuvant endocrine therapy.[15,38]

Prostatic carcinoma is known to be highly sensitive to treatment by systemic androgen deprivation. For patients with stage D1 prostatic carcinoma, previous retrospective clinical studies have demonstrated that early bilateral orchiectomy or other methods of early androgen deprivation have a significant effect on the extent and timing of tumor progression. We are currently analyzing the timing and type of endocrine treatment along with the tumor DNA ploidy pattern and the other standard clinical variables in a complex multivariate analysis to delineate the contribution of these individual factors to the course of patients with stage D1 prostatic carcinoma. These results will be published in a sequel to this current report.

CONCLUSION

The ploidy data and analysis presented herein demonstrate a definite association between DNA ploidy pattern and the clinical course of patients with stage D1 cancer of the prostate. Such results in a group of patients treated by bilateral pelvic lymphadenectomy and radical prostatectomy confirm the similar findings obtained at Memorial Sloan-Kettering Cancer Center in patients with stage D1 prostatic cancer treated by bilateral pelvic lymphadenectomy and ^{125}I seed implantation.[24] The predictive effects of the DNA tumor ploidy patterns are so striking and dramatic that it seems evident to us that this new tumor variable will need to be considered in future treatment recommendations and in future clinical research studies of patients with cancer of the prostate. Intensive ploidy studies of other groups of patients with other stages of prostatic cancer may be anticipated. Methods for accurate determination of ploidy assignment on small prostatic needle biopsy samples or aspiration specimens must be avidly pursued as well because the tumor DNA ploidy pattern, an objective measurement of a previously "invisible" property of tumor nuclei, will likely become an important factor in determining treatment recommendations for patients with prostatic carcinoma in the future.

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