

Prognostic Significance of DNA Quantitation in Stage D1 Prostate Carcinoma with the Use of Image Analysis

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Background. A characteristic feature of prostatic adenocarcinoma is its great variation in biologic behavior. This variation and the observation that most carcinomas are of intermediate grade make standard histologic grading of limited value in determining the prognosis of a patient.

Methods. DNA quantitation with the use of computer-assisted image analysis on Feulgen-stained nuclei was performed on the metastatic lymph nodes from patients with Stage D1 prostate carcinoma to determine whether ploidy was a useful predictor of survival or progression. The Gleason histologic score of the primary tumor, the number and extent of lymph node metastases, and the progression and survival intervals were documented. Treatment modalities included pelvic lymph node dissection, radical prostatectomy, external beam radiation therapy, and iodine 125 implantation.

Results. DNA ploidy quantitation showed that 65% (33 of 51) of cases were aneuploid, 2% (1 of 51) were tetraploid, and 33% (17 of 51) were in the diploid range. Progression to Stage D2 disease occurred in 76% of the patients with aneuploid cases and 53% of those with cases in the diploid range.

Conclusion. There was a significant difference in progression between the two ploidy groups (Cox regression analysis, $P < 0.05$). *Cancer* 1992; 70:1159-1165.

Prostate adenocarcinoma is characterized by wide variations in biologic behavior and time to disease progression.¹ Currently, clinical and pathologic staging are the most important parameters in determining therapy and

predicting patient prognosis.^{1,2} Histologic grade may provide additional information regarding prognosis. However, patients with a given stage and grade may have considerable individual variation in response to treatment, time to disease progression, and ultimate survival.³

Chromosome analysis and DNA quantitation studies in various tumors confirmed that neoplastic transformations have specific "marker" chromosome aberrations associated with the malignant alteration. Additional "nonmarker" chromosome changes that may be nonspecific result in measurable increases in DNA cellular content.^{4,5} The normal human somatic cell contains 46 chromosomes (23 pairs) and is referred to as diploid. A cell with identifiable deviation from 46 normal chromosomes is aneuploid and may include deletions, translocations, or duplications of an entire chromosome or a portion of the chromosome. Previous studies have suggested that the biologic potential of prostate carcinoma is related to DNA content as measured by flow cytometry or image analysis.³⁻⁶ Zetterberg and Esposti found that prostate tumors with DNA content in the normal (diploid) range have a better prognosis than aneuploid tumors and also have a higher response rate to estrogen treatment.⁶ Other studies, using flow cytometry or microspectrophotometry to measure DNA content, have confirmed these results.⁷⁻¹⁰ However, there are conflicting data in the literature, with studies of cellular DNA measurements not confirming the predictive value of ploidy.¹¹

Because of these controversies, we chose to study a group of patients in whom Stage D1 disease was identified by surgical exploration performed for staging purposes. Lymph node metastases were examined for DNA cellular content and extent of metastatic disease, which was evaluated against time to progression to Stage D2 disease and for overall survival.

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Accepted for publication November 15, 1991.

Materials and Methods

Patient Population

Patients with Stage D1 prostate carcinoma diagnosed by pelvic lymph node dissection between January 1975 and December 1985 were examined. Patient assessment included physical examination, determination of serum acid phosphatase levels, bone scan, and, in some cases, computed tomography scan of the pelvis or lymphangiogram. Pelvic lymph node dissection and intraoperative bimanual examination were performed on all patients to identify extension or lymphatic metastases. Patients then were treated by external-beam radiation therapy (RT), interstitial iodine 125 implantation, or radical prostatectomy. Adjuvant therapy was used in some cases and consisted of either external-beam RT or hormonal manipulation. Patients were examined every 3 months for 1 year and subsequently at 6-month intervals. Physical examination and serum acid phosphatase determinations were performed at each visit. Bone scans were performed every 6–12 months, when symptoms developed, or when elevated serum acid phosphatase levels were observed. All patients were observed until 1988 or death. Progression to Stage D2 disease was said to occur when a patient had a bone scan with abnormal findings consistent with metastatic tumor or biopsy-proven distant metastasis.

Cytochemical Procedures

Hematoxylin and eosin-stained slides of the needle biopsy specimens were reviewed, and histologic grading of the adenocarcinoma was performed as described by Gleason and Mellinger.² In addition, the lymph nodes were evaluated, with quantitation of the total number and site of origin of nodes examined and the extent of tumor metastases in the lymph node. Representative paraffin blocks of the metastatic deposit were selected for each patient and 8–10- μ m-thick sections prepared. This tissue thickness resulted in minimal nuclear overlap and allowed optimum DNA quantitation by image analysis. Each slide was deparaffinized in xylene, hydrated, and stained according to the modified Feulgen technique (acid hydrolysis—5 N hydrochloric acid, 28°C, 60 minutes).¹² The slides were evaluated at a magnification of 400 and intact nonoverlapping nuclei identified. DNA quantitation was performed with a computerized digital imaging system consisting of a Hewlett-Packard Vecta ES (Hewlett-Packard, Sunnyvale, CA), Sony monitor (Sony, Parkridge, NJ), and Logitech mouse (Logitech, Fremont, CA). A Dage/MTI 70 series camera (Dage/MTI, Michigan City, IN) was mounted on an Olympus BH-2 microscope (Olympus, Kokomo, IN). The software program for DNA quantitation was provided by Microsciences, Inc. (Federal Way, WA).

DNA Histogram Generation

The cytophotometric measurements of stained cell nuclei were performed with the use of a 560-nm monochromatic light. Control cells consisted of lymphocytes. The metastatic foci of adenocarcinoma were outlined on the hematoxylin and eosin-stained section and the corresponding area on the Feulgen-stained sections analyzed. Tumor cells were selected randomly from various areas of the neoplasm and the control lymphocytes from the adjacent normal lymph node. Quantitation of nuclear DNA was performed by selecting a window that was larger than the nucleus to be measured and free of any contact with adjacent nuclei. The DNA content was the summation of all gray levels in the nucleus computed against the background intensity after correction for television camera black level. The summation of these gray levels then was expressed in arbitrary units. For each patient, a minimum of 100 control nuclei and 200 tumor nuclei were measured. The DNA content distribution was plotted in arbitrary units of Feulgen-stained DNA versus cell number and displayed in histograms. The mean ($2n$) and standard deviation (SD) and the coefficient of variation were calculated for the control cells and represented the instrument sensitivity, operator consistency, sample quality, and inherent variation of control cells. Lymphocytes have been shown to take 10–15% less Feulgen than other cells, such as fibroblasts. A correction value of 0.12 was determined by comparing the DNA content of fibroblasts with that of lymphocytes for any given patient. This correction value was used to determine the true $2n$ or control value for each patient.

DNA Histogram Interpretation

Our laboratory has defined diploidy or a normal DNA range and aneuploidy or an abnormal DNA cellular content using a modified distribution as described by Auer et al.¹³ This method compares the mean DNA content of the major G₀/G₁ tumor peak with the control cell peak. Four different patterns of DNA distribution in image analysis histograms are used (Figs. 1–4):

1. Type I. There is a single distinct DNA modal value in the diploid or near-diploid range. When the tumor mean falls within the $2n \pm 2$ SD range of control cells, the tumor DNA content is considered normal or diploid ($2n$ is the control cell mean, and the SD = coefficient of variation $\times 2n/100$).
2. Type II. There is a distinct DNA modal value in the tetraploid region of control cells ($4n$), with or without an identifiable peak in the diploid region. Few intervening DNA values (synthesis phase) are pres-

ent. The tumor is classified as tetraploid if the major peak mean ± 2 SD contains the $4n$ value.

3. Type III. The DNA histogram may have two peaks that are statistically different. The tumor is classified as aneuploid or abnormal if the mean of the major G0/G1 peak falls outside the control cell ($2n \pm 2$ SD) range and is nontetraploid.
4. Type IV: A histogram has a wide distribution of DNA values, often exceeding the tetraploid region ($4n$). This type of histogram may or may not have prominent modal populations. The most important criterion that we apply is identification of cells with a DNA content exceeding $5n$ (non-type II or tetraploid histograms). This implies there are aneuploid

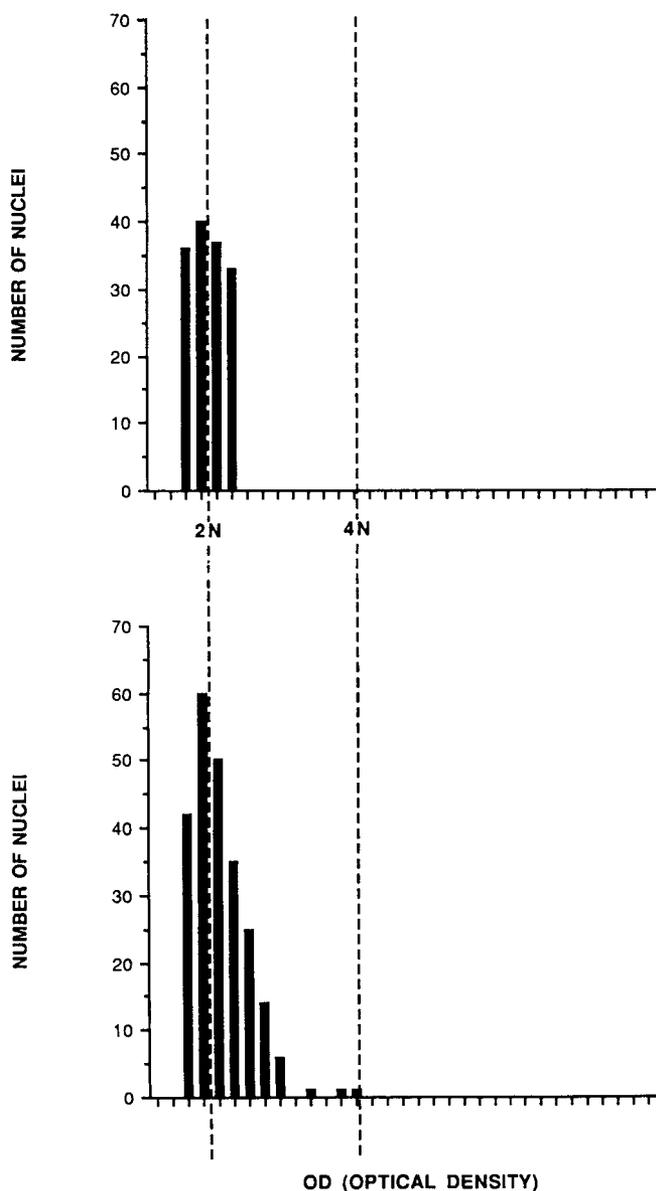


Figure 1. Type 1 histogram with a single distinct DNA modal value in the diploid or near-diploid range.

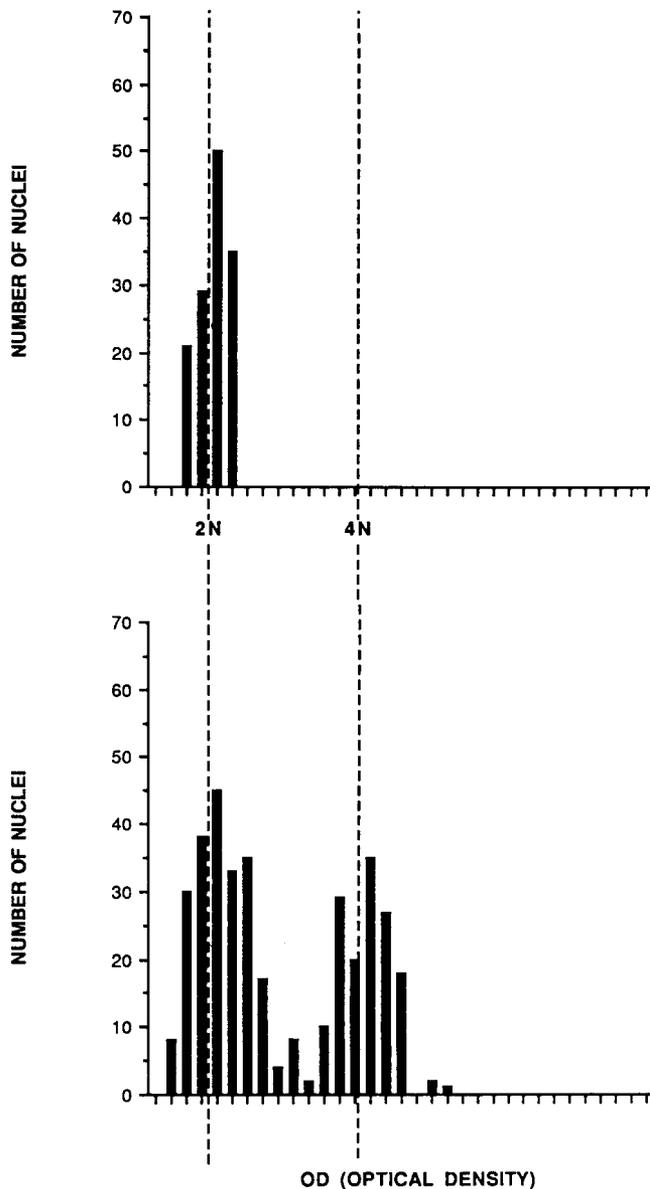


Figure 2. Type II histogram with a distinct DNA modal value in the tetraploid region of control cells ($4n$).

values exceeding $5n$ or hidden modal values between $2n$ and $4n$ (aneuploid) in which only the S (synthesis) or G2/M components can be identified beyond the $5n$ values.

Results

Clinical Data

Seventy-six cases of Stage D1 disease were diagnosed during the study period. Twelve patients were excluded because of lack of follow-up and 13 patients because of the inability to access pathologic material from outside institutions, leaving 51 assessable patients. The clinical

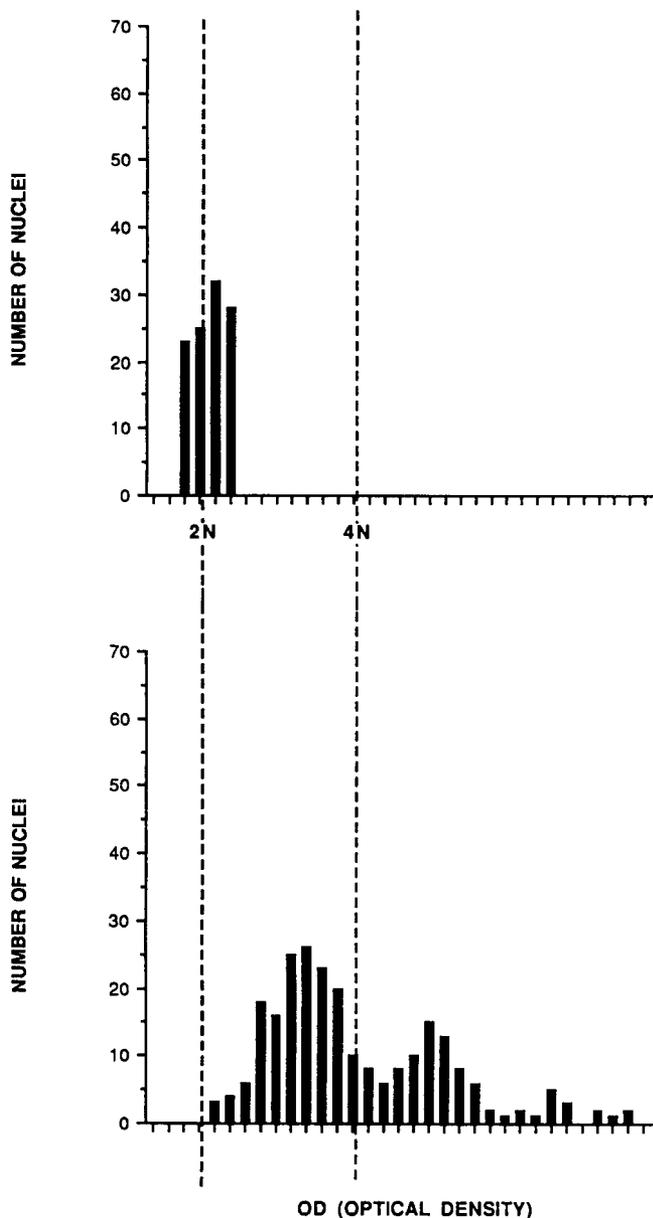


Figure 3. Type III histogram may have two peaks that are statistically different. Major G0/G1 peak falls outside the control cell range and is nontetraploid.

and pathologic characteristics of the study population are outlined in Table 1. The median follow-up was 52 months (range, 11–161 months), and patient ages ranged from 53 to 74 years. Progression to Stage D2 disease (bone or soft tissue metastasis) occurred in 35 of 51 patients (69%), with a median time to progression of 25 months.

Treatment Modalities and Progression

All patients had staging pelvic lymph node dissection with additional therapy consisting of external-beam

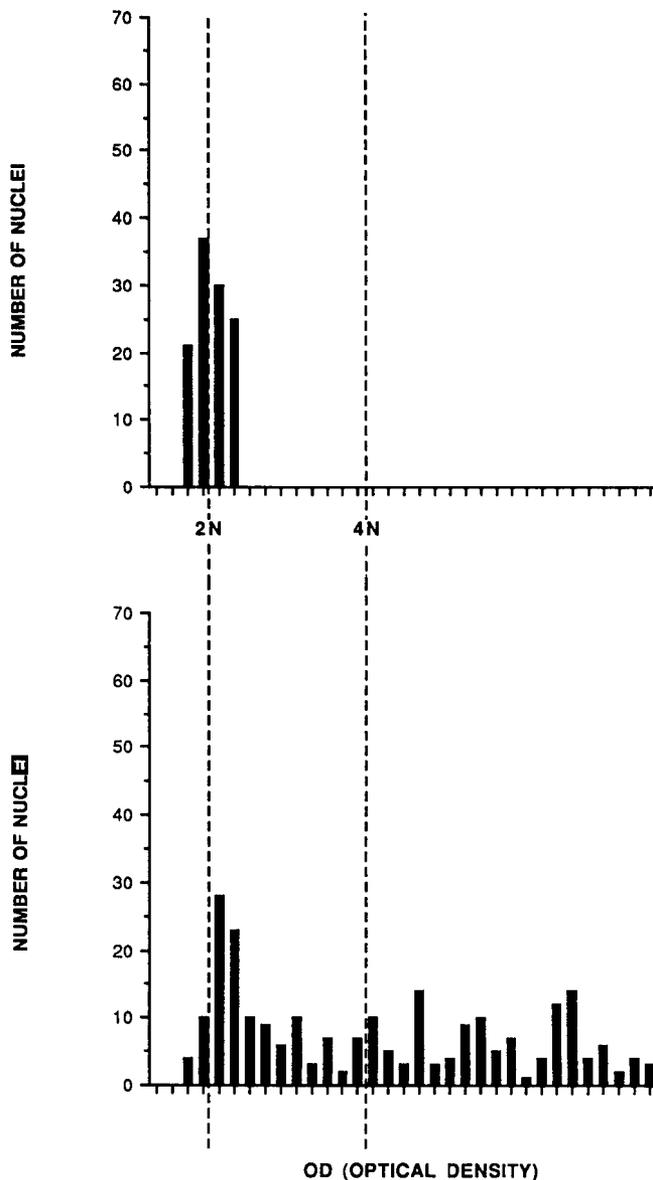


Figure 4. Type IV histogram has wide distribution of DNA values, often exceeding 4n.

RT, iodine 125 implantation with or without RT, and radical retropubic prostatectomy with or without RT. Progression occurred in 7 of 10 (70%) of patients treated with external-beam RT alone, 22 of 34 (65%) of patients treated with iodine 125 implantation with or without RT, and 6 of 7 (86%) of those treated with radical prostatectomy with or without RT. These find-

Table 1. Patient Demographics

	Total no. of patients	Median follow-up (mo)	Gleason score (mean)
Progression	35	52 (range, 11–159)	7.71
No progression	16	50 (range, 30–161)	7.56

Table 2. Treatment Versus Progression*

Treatment	Progression	No progression	Total
Radical prostatectomy ± RT	6† (86%)	1 (14%)	7
Iodine 125 ± RT	22 (65%)	12 (35%)	34
RT	7 (70%)	3 (30%)	10

RT: radiation therapy.
 * Chi-square test, $P > 0.51$.
 † Number of patients.

ings are outlined in Table 2. The time to progression among the three treatment groups was not statistically significant with log-rank testing ($P > 0.24$).

Gleason Grade and Lymph Node Status

The mean Gleason grade for patients with disease progression was 7.71, compared with 7.56 for those without progression. Gleason grade was not a statistically significant prognostic factor when Cox regression analysis was performed. Evaluation of the lymph nodes included determination of microscopic or macroscopic disease, presence of bilateralism, and extent of node replaced by metastatic disease. The lymph node status is shown in Table 3. Overall, 18 patients had bilateral lymph node deposits and 33 had unilateral disease. Of the 33 with unilateral disease, 11 had microscopic disease, defined as less than 25% of the lymph node involved with tumor. Progression to Stage D2 disease was present in 14 of 18 (78%) patients with bilateral lymph nodes, 21 of 33 (64%) patients with unilateral disease, and 6 of 11 (55%) patients with focal microscopic disease.

DNA Quantitation and Tumor Progression

The relationship between DNA ploidy pattern and progression to Stage D2 disease was studied. Rates of tumor progression according to ploidy pattern are demonstrated in Table 4. Of 17 patients with diploid tumors, 9 (53%) had progression of disease, whereas 25 of 33 (76%) patients with aneuploid tumors had progression.

When the time interval from pelvic lymph node dissection to tumor progression was analyzed, a differ-

Table 3. Lymph Node Status

	Lymph node bilateral	Lymph node unilateral	
		Gross	Microscopic*
Total	18	22	11
Progression	14/18† (78%)	15/22 (68%)	6/11 (55%)
No progression	4/18 (12%)	7/22 (32%)	5/11 (45%)

* Less than 25% replacement of a solitary lymph node by tumor.
 † Number of patients/total number of patients.

Table 4. DNA Content Versus Progression

	Ploidy		
	Aneuploid	Diploid	Tetraploid
Progression	25/33* (76%)	9/17 (53%)	1/1 (100%)
No progression	8/33 (24%)	8/17 (47%)	0/1 (0%)
Median to progression (mo)	31	84	14

* Number of patients/total number of patients.

ence was found. For all patients, the median time interval to progression was 43 months. The median time to progression for tumors with DNA diploid histograms was 84 months (range, 6–126 months); however, it was 31 months (range, 4–74 months) for tumors with aneuploid histograms. Kaplan–Meier survival analysis (Fig. 5) showed a statistically significant difference between the two ploidy groups, with progression occurring significantly earlier in patients with aneuploid tumors as compared with patients with diploid tumors (log-rank, $P < 0.02$). The Cox proportional hazards survival analysis confirmed the effect of aneuploidy versus diploidy on progression by controlling for other potentially confounding variables (lymph node status, Gleason grade, and treatment) ($P < 0.05$).

DNA Quantitation and Patient Survival

Forty-nine percent (25 of 51) of the patients in the study group died during follow-up, and 23 of these patients had disease progression at the time of death (Table 5). Of the patients with disease progression who died, 65% (15 of 23) had aneuploid, 4% (1 of 23) had tetraploid, and 30% (7 of 23) had diploid tumors. The median time until death for patients with aneuploid tumors was 63 months (range, 14–74 months), compared with 84 months (range, 11–159 months) for patients with diploid tumors. Although a correlation was found, this did not reach statistical significance with the Kaplan–Meier

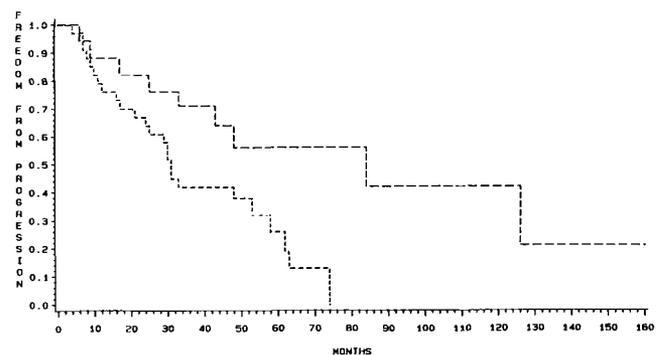


Figure 5. A significant difference is seen in time to progression between the two ploidy groups using Kaplan–Meier survival analysis (log-rank, $P = 0.02$). ----: Aneuploid; — — —: diploid.

survival analysis ($P > 0.07$) (Fig. 6). The Cox proportional hazards survival analysis was used to compare the survival length between the two ploidy groups after accounting for the other covariates of interest (type of treatment, presence of bilateral lymph nodes, presence of focal disease, and Gleason grade). With this analysis, the addition of the ploidy variable was not a statistically significant improvement in predicting time to survival compared with the other covariates alone ($P > 0.10$). However, there is an indication that patients with diploid tumors may tend to have a longer survival time than patients with aneuploid tumors if the other covariates of interest are held constant.

Discussion

Prostatic adenocarcinoma is a tumor with considerable biologic variability. The prognostic significance of lymphatic metastasis is well recognized and indicates a potential for systemic spread. In most patients with lymph node metastasis, distant metastases develop within 5 years. In addition, in many patients this spread is not affected by treatment. It has been shown by Prout et al. that the probability of survival at 5 years is significantly greater for patients without nodal disease compared with those with nodal metastasis (84% and 34%, respectively).¹⁴ In addition, it has been suggested that the extent of lymphatic involvement also may be a prognostic determinant. Smith and Middleton reported on a group of 73 patients with positive nodes, who were observed for 5 years.¹⁵ Fifteen percent of patients with gross nodal metastases, 27% with multiple microscopic metastases, and 44% with a single positive node survived 5 years without progression. In our study, lymphatic metastasis was not the principal prognostic determinant for progression or survival. The ploidy status of the tumor was a better predictor of future disease progression.

The probability of distant metastases in patients with nodal disease is affected only to a minor degree by the type of regional therapy they receive. Metastases developed in 85% of patients who were treated with

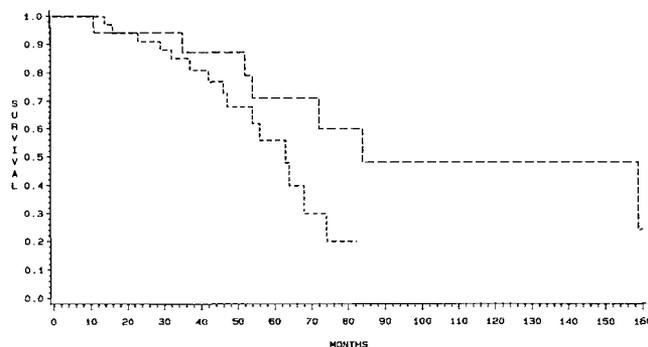


Figure 6. Kaplan-Meier survival analysis of patients with aneuploid and diploid tumors (log-rank, $P = 0.07$). ----: Aneuploid; - - - -: diploid.

external-beam RT,¹⁶ compared with 70% of those who had an ¹⁹⁸Au implant¹⁷ and 75% of those who had iodine 125 implantation.¹⁸ Our study found similar results, with no relationship between survival or progression and the treatment received.

DNA quantitation has been shown to be a useful prognostic determinant in various tumors.^{4,5} Numerous studies using both flow cytometry and image analysis have shown that the ploidy status of the tumor can add useful prognostic information regarding future disease progression.⁶⁻⁸ Winkler et al. studied patients with Stage D1 disease using flow cytometry for the primary tumor.¹⁹ All patients were treated with radical prostatectomy and observed for 5-19 years. The DNA pattern was diploid in 42%, tetraploid in 45%, and aneuploid in 13%. Only 15% of the diploid tumors progressed, whereas 75% of tumors with aneuploid or tetraploid DNA patterns progressed. We found a much higher percentage of aneuploid tumors in our study (65%), in which DNA quantitation was performed on the metastatic deposit rather than the primary tumor. In addition, progression occurred in 53% of diploid tumors in our study compared with 15% in the study reported by Winkler et al. We also found a much lower percentage of tetraploidy: 2% in our study compared with 45% in the study by Winkler et al. These differences may result in part from the technique used for DNA quantitation and the samples chosen (i.e., primary tumor versus metastatic deposit).

In a study by Stephenson et al., 82 patients with Stage D1 disease, treated with iodine 125 implantation, had DNA quantitation on the metastatic deposit with the use of flow cytometry.²⁰ Only patients with a minimum of 50% replacement of the lymph node were studied. Forty-nine patients (60%) had aneuploid and 33 (40%) had diploid tumors. The median survival times of the patients with aneuploid and diploid tumors were 5.0 and 8.8 years, respectively. The incidence of aneuploidy in this series, in which DNA quantitation was

Table 5. DNA Content Versus Survival*

Ploidy	No. of deathst	Median time to death (mo)‡
Aneuploid	15/23§ (65%)	63
Diploid	7/23 (30%)	84
Tetraploid	1/23 (4%)	56

* Log-rank test, $P > 0.07$.

† Two patients died without evidence of disease progression and were excluded from this analysis.

‡ Median survival time of all patients was 68 months.

§ Number of patients/total number of patients.

performed on the metastatic deposit, more closely approximates our findings.

DNA quantitation provides us with a useful, objective prognostic determinant that gives information above and beyond that indicated by histologic grading or pathologic staging. It is well known that patients with nodal metastases have a worse prognosis than those without nodal involvement. DNA quantitation helps us to stratify this group and predict future progression. It appears that the sensitivity of DNA quantitation is decreased when the metastatic deposit is measured, as opposed to the primary tumor. This may result in part from dedifferentiation of the tumor as metastasis proceeds. Additional studies are being performed to answer these questions.

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