Flow cytometric determination of ploidy in prostatic adenocarcinoma and its relation to clinical outcome

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Flow cytometry was used to measure the DNA content in paraffin-embedded archival specimens of prostatic adenocarcinoma. The specimens were from 49 patients who were found to have adenocarcinoma of the prostate at the time of transurethral resection of the prostate for bladder outlet obstruction. At initial presentation, 34 of these patients had clinically localized disease and 15 had metastatic disease. The authors studied the relationship of DNA ploidy to clinical stage, histologic grade, disease progression, and duration of survival. Their results indicate that regardless of the clinical stage at presentation, the mean time to disease progression is longer in a patient with a DNA diploid tumor when compared with that in a patient with a DNA aneuploid tumor (18.1 months vs 6.5 months, respectively). Additionally, mean time of survival was longer in a patient with a diploid tumor than in a patient with an aneuploid tumor (31.6 months vs 9.6 months, respectively).

(Key words: Flow cytometry, ploidy, prostate neoplasm)

A major problem in managing patients with adenocarcinoma of the prostate is predicting the tumor's malignant potential in individual patients. It is known that some tumors will run a long and uncomplicated course, the patient dying *with*, rather than *of*, the cancer. In some instances, the tumor is an incidental finding at autopsy,[1] whereas other tumors are highly aggressive, running a rapidly fatal course. The decision to treat a particular patient must be aimed at increasing life expectancy without substantially reducing the quality of the patient's life.

Historically, clinicians have relied on clinical stage and histologic grade to assess prostate tumor aggressiveness and prognosis.[2] Unfortunately, clinical staging by rectal examination in the absence of definitive palpable periprostatic extension is notorious for its propensity to underestimate tumor burden.[3] Histopathologic grading is another parameter that has been examined extensively for its ability to predict subsequent disease progression with variable effectiveness.[4,5] Because the grading

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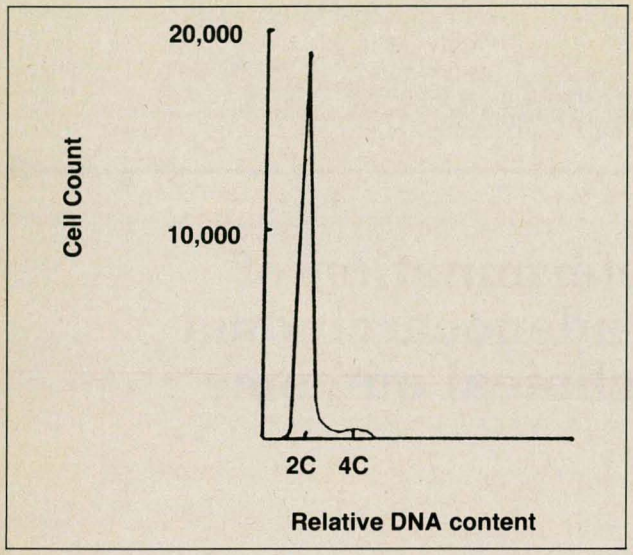

Figure 1. *Diploid histogram.*

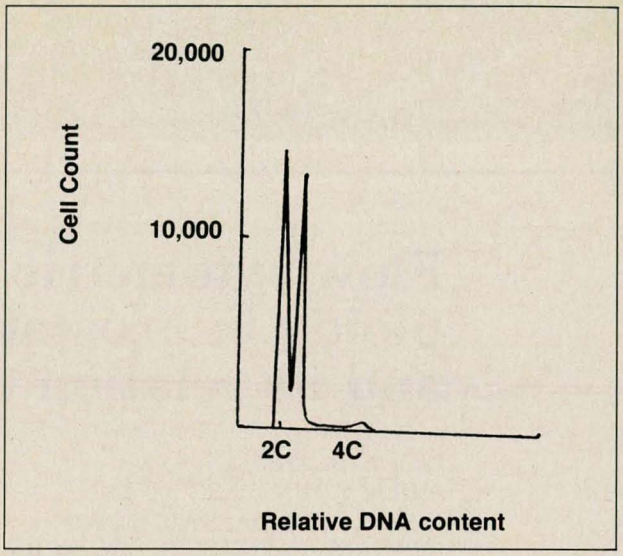

Figure 2. *Aneuploid histogram.*

of prostate carcinoma is a subjective evaluation, it may not be consistent and reproducible.

Several investigators[6-11] have demonstrated that the analysis of tumor nuclear deoxyribonucleic acid (DNA) content by flow cytometry is useful in predicting the clinical course of patients with various urologic malignant lesions. In the early studies on prostate carcinoma, the researchers[12-15] used freshly excised prostatic tumor samples. In 1983, Hedley and colleagues[16] reported a method of analyzing nuclear DNA content, or ploidy, by flow cytometry using nuclei extracted from formaldehyde-fixed, paraffin-embedded archival specimens. This method has permitted retrospective analysis of large numbers of patients with adenocarcinoma of the prostate in whom long-term outcome is already known.

We report here a study investigating the relationship of DNA ploidy (measured on paraffin-embedded prostatic tissue) to clinical stage, histologic grade, disease progression, and survival in 49 patients with adenocarcinoma of the prostate.

Materials and methods

Patient identification

Paraffin-embedded archival specimens from 49 men who had adenocarcinoma of the prostate diagnosed at the time of transurethral resection of the prostate for bladder outlet obstruction between November 1983 and December 1987 were selected for the study. These patients ranged in age from 57 to 90 years (mean, 72 years). We reviewed their clinical records with specific attention to pathologic grade, clinical stage at presentation, and clinical outcome.

Pathologic staging was based on the size of the glandular formations and on nuclear anaplasia.[17] Tumors were classified as well-differentiated, moderately differentiated, or poorly differentiated. Clinical stage was assessed on the basis of findings of a digital rectal examination, serum acid phosphatase determination, radionuclide bone scanning, and computed tomography (CT).

For the purposes of this study, we used the Whitmore-Jewett system[18,19] for clinical staging:

- A stage A lesion is defined as a tumor that is not suspected on digital rectal examination but is detected on pathologic examination of the prostatectomy specimen. Stage A1 is further designated as focal, well-differentiated disease. Stage A2 refers to more diffuse or less differentiated but still unsuspected disease.
- Stage B lesions are palpable carcinomas confined to the prostate on digital rectal examination. Stage B1 tumors are smaller than 1.5 cm in diameter and are confined to one lobe, whereas stage B2 tumors are larger.
- Stage C carcinoma extends beyond the capsule of the prostate but is confined to the pelvis.
- Stage D comprises prostatic carcinoma with demonstrable metastasis either to pelvic lymph nodes (stage D1) or more widespread disease (stage D2).

All patients were seen for regular follow-up vis-

its in the postoperative period or until death, a period ranging between 1 and 89 months (mean, 30.9 months).

Fourteen (29%) of the 49 patients had clinical stage A disease; 4 of these patients had tumors staged as A1, and 10 patients had clinical stage A2 disease. Six of these 10 patients were treated with external beam radiation therapy. Seven (14%) of the 49 patients had clinical stage B lesions, all of which were thought to be stage B2. All seven of these patients were treated with external beam radiation therapy. Thirteen (27%) of the 49 patients had clinical stage C prostate cancer on presentation; they also were treated with external beam radiation therapy at the time of diagnosis. On presentation, 15 patients (31%) had stage D disease; all were found to have distant metastasis and were treated with hormonal manipulation.

Preparation of paraffin-embedded archival specimens

Nuclear suspensions from paraffin-embedded tissue blocks were prepared by using an adaption of the technique described by Hedley and coworkers.[16] Five 25-μm thick sections were cut with the use of a standard microtome. The sections were placed in 10-mL glass culture tubes, dewaxed by immersion of the tissue in three changes of 15 mL of xylene for 30 minutes at room temperature (25°C), followed by sequential rehydration of 10 mL of 100%, 95%, 85%, and 50% ethanol solutions 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 (Sigma Chemical Co, St Louis, Mo) 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 2800 rpm for 10 minutes to form a nuclear pellet, and the pepsin supernatant was removed. After each sample had been diluted with 5 mL of Hank's buffered saline solution (HBSS) and filtered through a 37-μm pore nylon mesh, the cells were centrifuged at 1000 rpm for 5 minutes at 25°C, and the supernatant was discarded.

Nuclear ribuonucleic acid (RNA) was removed by incubating the cell suspension in 1 mL of 0.05% RNase solution (Sigma Chemical Co). After this incubation, the suspension was stained with 1 mL of 0.05% propidium iodide (Sigma Chemical Co). This stain binds specifically to the DNA and in direct proportion to the amount of DNA present. Cellular DNA content was measured using a Coulter Model EPICS V flow cytometer (Coulter Diagnostics, Hialeah, Fla) using a 488 rpm argon laser excitation beam, counting 10,000 to 20,000 cells per sample. Human peripheral blood lymphocytes were stained in the same manner and measured before each sample as a control.

The data were then displayed as a plot of relative DNA content versus number of nuclei measured, and integrated computer analysis of the DNA histogram was used to calculate the number of cells in the G0-G1, S, and G2-M compartments.

Two basic patterns were obtained from the cell suspension prepared from the carcinomas on the flow cytometer. The first type, diploid tumors, have a tumor cell DNA content in the same range as benign cells that contain a normal diploid (2C) chromosomal complement. Diploid tumors exhibit a prominent single G0-G1 peak (*Figure 1*). The second pattern is produced by tumor cells with high values of DNA and are called aneuploid tumors. These tumors are characterized by the presence of an additional separate, distinct G0-G1 peak (*Figure 2*). Histograms were classified as tetraploid only if apparent subpopulations of G0-G1 cells in the 4C region had a corresponding visible S and G2-M population of cells.

Results

Patient distribution

A total of 49 paraffin-embedded specimens of prostatic adenocarcinoma were evaluable by flow cytometry. Thirty-nine (80%) of the tumors showed DNA histograms that were categorized as diploid. Ten tumors (20%) were classified as DNA aneuploid. The distribution of patients by clinical stage, pathologic grade, and nuclear DNA patterns is shown in *Tables 1, 2,* and *3*.

Patient outcome

The overall survival rate of the 49 patients during the study period was 74%. The majority of the patients who died with disease had clinical stage D adenocarcinoma of the prostate (*Table 4*), a highly significant finding ($P = .0002$, χ^2-test). As *Table 5* shows, 95% of patients with a well-differentiated tumor and 73% of patients with a moderately differentiated tumor were alive during the study period compared with 47% of patients with a poorly differentiated tumor ($P = .0002$, χ^2-test).

Table 6 examines patient survival related

Table 1
Patient Distribution by Clinical Stage and DNA Ploidy Pattern

Clinical stage	Total No.	DNA ploidy pattern: Diploid, No. (%)*	Aneuploid, No. (%)*
A	14	13 (93)	1 (7)
B	7	6 (86)	1 (14)
C	13	11 (85)	2 (15)
D	15	9 (60)	6 (40)

*Numbers in parentheses are % of total No.

Table 2
Patient Distribution by Pathologic Grade and Clinical Stage

Pathologic grade	Total No.	Clinical stage: Stage A, No. (%)*	Stage B, No. (%)*	Stage C, No. (%)*	Stage D, No. (%)*
Well-differentiated	19	14 (73)	2 (11)	2 (11)	1 (5)
Moderately differentiated	15	0 (0)	4 (27)	6 (40)	5 (33)
Poorly differentiated	15	0 (0)	1 (7)	5 (33)	9 (60)

*Numbers in parentheses are % of total No.

Table 3
Patient Distribution by Pathologic Grade and DNA Ploidy Pattern

Pathologic grade	Total No.	DNA ploidy pattern: Diploid, No. (%)*	Aneuploid, No. (%)*
Well-differentiated	19	18 (95)	1 (5)
Moderately differentiated	15	11 (73)	4 (27)
Poorly differentiated	15	10 (67)	5 (33)

*Numbers in parentheses are % of total No.

to pathologic grade and DNA ploidy. Of interest, but not statistically significant ($P = .4$, χ^2-test), the majority (89%) of the patients who survived were DNA diploid regardless of the histologic grade of their tumor. *Table 7* illustrates the distribution of DNA ploidy patterns and patient outcome. It is significant that of the patients who died with disease during the study, 18% had a diploid tumor as compared with 60% who had an aneuploid tumor (($P = .01$, Fisher's exact test).

Table 8 compares the survival figures by clinical stage and indicates that more patients with a diploid tumor survived the study period than those patients with an aneuploid tumor regardless of the clinical stage at presentation.

Table 4
Patient Outcome by Clinical Stage

Clinical stage	Total No.	Outcome: Survived, No.	(%)*	Outcome: Died, No.	(%)*
A	14	14	(100)	0	(0)
B	7	5	(71)	2	(29)
C	13	12	(92)	1	(8)
D	15	5	(33)	10	(66)

*Numbers in parentheses are % of total No.
$P = .0002$ (ordered χ^2-test).

Table 5
Patient Outcome by Pathologic Grade

Pathologic grade	Total No.	Outcome: Survived, No.	(%)*	Outcome: Died, No.	(%)*
Well-differentiated	19	18	(95)	1	(5)
Moderately differentiated	15	11	(73)	4	(27)
Poorly differentiated	15	7	(47)	8	(53)

*Numbers in parentheses are % of total No.
$P = .002$ (ordered χ^2-test).

Table 6
Survival Related to Pathologic Grade and DNA Ploidy Pattern

Pathologic grade	Total No. of survivors	DNA ploidy pattern: Diploid, No.	(%)*	Aneuploid, No.	(%)*
Well-differentiated	18	17	(94)	1	(6)
Moderately differentiated	11	9	(82)	2	(8)
Poorly differentiated	7	6	(86)	1	(14)

*Numbers in parentheses are % of total No. of survivors.
$P = .4$ (ordered χ^2-test).

Disease progression

Of the 49 patients studied, 19 (39%) had progression of disease as defined by documented metastasis or death. Thirty-four (69%) of the 49 patients had localized prostate carcinoma (clinical stage A, B, and C) at presentation; disease progressed in 9 (26%) of these 34 patients during the study. Seven (78%) of these 9 patients were DNA diploid and 2 (22%) were DNA aneuploid. The mean time from diagnosis to documented metastasis was 18.1 months for patients with a DNA diploid tumor versus 6.5 months for patients with a DNA aneuploid tumor (*Table 9*). All patients received hormonal manipulation treatment at the time metastasis was diagnosed.

At presentation, 15 (31%) of the 49 patients had metastatic prostate carcinoma (clinical stage D); 10 (67%) of these 15 patients had disease progression and died of disease during the study. Five (50%) of these 10 patients had DNA diploid tumors and 5 (50%) had DNA aneuploid tumors.

The mean time from diagnosis to death for the 13 patients who died of disease during the study was 27.4 months for patients with a

Table 7
Patient Outcome by DNA Ploidy Pattern

DNA ploidy pattern	Total No.	Outcome			
		Survived, No.	(%)*	Died, No.	(%)*
Diploid	39	32	(82)	7	(18)
Aneuploid	10	4	(40)	4	(60)

*Numbers in parentheses are % of total No.
$P = .01$ (Fisher's exact test).

DNA diploid tumor and 12 months for patients with a DNA aneuploid tumor. *Table 10* compares the mean time from diagnosis to death related to histologic grade and DNA ploidy and shows that regardless of histologic grade at the time of diagnosis, patients with a DNA diploid tumor had a longer time of survival from diagnosis to death than did patients whose tumors were DNA aneuploid.

Discussion

Carcinoma of the prostate is the most common malignancy in American men and is the third leading cause of cancer deaths in the United States.[20] Currently, carcinoma of the prostate is diagnosed in approximately 103,000 patients each year and accounts for 29,500 deaths annually.[20] Effective methods for early detection and prevention and proper treatment would undoubtedly contribute to lowering these dismal statistics. Despite the wide range of treatment modalities used, survival statistics have not improved significantly in the past 20 years.

Flow cytometry has emerged as an important tool in the diagnosis and prognosis of many neoplasms. It is a technique that allows measurements of specific nuclear and cellular properties from individual cells in fluid suspension after staining with a specific metachromatic fluorescent dye and excitation with an argon laser. The amount of fluorescence is objectively quantified and used to determine relative DNA content. The proportion of cells in each cell cycle compartment is calculated to enable determination of the presence of diploid versus aneuploid neoplasms.

Previous investigators[12,13,21,22] have shown that flow cytometry can differentiate between benign and malignant lesions of the prostate. Several studies[23,24] 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. Other investigators[25,26] have shown that the frequency of aneuploidy increases significantly with advancing clinical stage of tumor. Additionally, retrospective studies using flow cytometry have reported a significant relationship between DNA ploidy pattern and disease progression[27,28] as well as patient survival.[24,29,30]

We studied the flow cytometry pattern of 49 paraffin-embedded transurethral prostatectomy specimens of men with newly diagnosed prostate carcinoma. The survival rate during the study was 74% with the majority (77%) of cancer deaths occurring in patients who had advanced clinical stage disease when first seen (*Table 4*). With regard to pathologic grade, 62% of patients who died with disease had a poorly differentiated tumor. *Table 7* illustrates that patients with DNA diploid tumor had a favorable survival, with 82% of them surviving during the study as compared with 40% of the patients with DNA aneuploid tumor.

We also examined disease progression in relation to ploidy. A total of 19 patients (39%) had progression of disease during the study. Nine of these patients had progression from localized disease at presentation to documented metastatic disease during the study. The remaining 10 patients had metastatic disease at presentation and died during the study period. It is interesting to note that although 7 of the 9 patients who had localized disease at presentation and had disease progression during the study period had DNA diploid tumors; the mean time to progression of disease was 18.1 months for a DNA diploid tumor versus 6.5 months for a DNA aneuploid tumor. Likewise, DNA ploidy pattern is a significant prognostic indicator for patients who were initially seen with metastatic disease and died during the study period. Five of the 10 patients who had metastatic disease at initial diagnosis and who died during the study had DNA diploid tumor; however, the mean time from

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Table 8
Survival Related to Clinical Stage and DNA Ploidy Pattern

Clinical stage	Total No. of survivors	DNA ploidy pattern			
		Diploid, No.	(%)*	Aneuploid, No.	(%)*
A	14	13	(93)	1	(7)
B	5	5	(100)	0	(0)
C	12	10	(83)	2	(17)
D	5	4	(80)	1	(20)

*Numbers in parentheses are % of total No. of survivors.
$P = .3$ (ordered χ^2-test).

Table 9
Progression Time* Related to Clinical Stage and DNA Ploidy Pattern

Clinical stage	Total No. of patients with progression	Diploid		Aneuploid	
		Mean progression time, mo	No. of patients	Mean progression time, mo	No. of patients
A	. . .	. . .	. . .	. . .	. . .
B	4	6.0	3	12.0	1
C	5	27.1	4	1.0	1
D	10	31.6	5	9.6	5

*Defined as progression from local to metastatic disease, or from metastatic disease to death.
$P = .1$ (ranking test).

Table 10
Time From Diagnosis to Death Related to Pathologic Grade and DNA Ploidy Pattern

Pathologic grade	Total No. of cancer deaths	Diploid		Aneuploid	
		Mean time to death, mo	No. of patients	Mean time to death, mo	No. of patients
Well-differentiated	1	5.0	1	. . .	. . .
Moderately differentiated	4	45.0	2	14.5	2
Poorly differentiated	8	24.3	4	10.8	4

$P = .02$ (ranking test).

diagnosis to death for these patients was 31.6 months versus 9.6 months for those with a DNA aneuploid tumor (*Table 9*). Also significant is the fact that of the 13 cancer deaths occurring during the study, the mean time from diagnosis to death was 27.4 months for patients with a DNA diploid tumor and 12 months for patients with a DNA aneuploid tumor.

Conclusion

Our data show that DNA ploidy as determined by flow cytometric analysis of deparaffinized specimens of prostatic adenocarcinoma correlates well with clinical stage, pathologic grade, and clinical outcome. Although our study is limited by being retrospective rather than prospective and comprises a small patient population over a short study period, it appears that flow cytometry may be a useful adjunct to routine pathologic grading and clinical staging. The addition of DNA ploidy may improve the prognostic evaluation of prostatic tumors and, as such, help to determine which patients should have earlier and more aggressive treatment.

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