DNA Ploidy and Prostate-Specific Antigen as Prognostic Factors in Clinically Resectable Prostate Cancer

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Prostate-specific antigen (PSA) and DNA ploidy as measured by flow cytometry were compared with conventional prognostic indicators in 112 patients who underwent radical prostatectomy for clinically resectable prostate cancer. The variables examined included age, race, prostatic acid phosphatase (PAP), Gleason score of the radical prostatectomy specimen, and pathologic stage. No significant relationships were found between DNA ploidy and age, mean PAP value, and absolute PAP value. Of the 112 patients, 65 (58.0%) had disease limited to the prostate (pathologic Stages A and B); 47 (42.0%) had extraprostatic disease (pathologic Stages C and D1). The stage was related to the Gleason score ($P < 0.0001$) where extraprostatic disease was associated with a Gleason score of 6 to 10. Nineteen (17.0%) patients had aneuploid tumors, and 93 (83.0%) had diploid tumors. DNA ploidy significantly correlated with pathologic stage ($P = 0.04$); aneuploidy was identified more frequently in patients with Stages C and D1 tumors. Aneuploid tumors occurred more frequently than diploid tumors in patients with a Gleason score of 6 to 10 ($P = 0.034$). Mean PSA values were higher in patients with aneuploid tumors ($P = 0.078$), extraprostatic neoplasms ($P = 0.00001$), and cancers with a Gleason score of 6 to 10 ($P = 0.0004$). Furthermore, PSA values greater than 10.0 ng/ml were associated with extraprostatic disease and a Gleason score of 6 to 10 ($P < 0.05$ and $P < 0.001$, respectively). Significant racial differences were found with respect to DNA ploidy, mean DNA indices, and mean PSA values. The 18 black patients had more DNA aneuploid tumors ($P = 0.043$), a higher mean DNA index ($P = 0.017$), and a higher mean PSA value ($P = 0.043$) than the 94 white patients. Both PSA and DNA ploidy analysis by flow cytometry appear to be valuable indicators in the evaluation of patients with prostatic carcinoma.
***Cancer* 67:3014–3023, 1991.**

ALTHOUGH PROSTATE CANCER, with an estimated 122,000 new cases in 1991, is the most common neoplasm in American men, the natural history of this malignancy is unpredictable.[1] The clinical spectrum of this disease may range from untreated patients with prolonged asymptomatic survival who eventually die of unrelated causes to others with rapid, fatal disease progression despite treatment. Tumor grade and pathologic stage have been used as prognostic indicators in patients with prostate cancer.[2–5] These are subjective indicators which may vary due to differences in the extent of specimen evaluation and histologic interpretation. Tumor grade

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Supported by grants from The Medical Research and Development Fund of The Ohio State University, The Bremer Foundation, and The Ohio State University Urology Research and Education Foundation.

The authors thank Willet F. Whitmore, Jr, MD, for his insight concerning interpretation of the data, Jane F. DeCenzo, MT, for her technical support with the flow cytometric analysis of the tissue specimen, and Providence F. Badalament for her assistance with data collection.

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Accepted for publication November 15, 1990.

appears to be a more sensitive prognostic indicator in patients with low-grade or high-grade tumors. Unfortunately, up to 70% of patients have an intermediate-grade (Gleason score, 5 to 7) carcinoma.[2] Thus, conventional prognostic factors (such as tumor grade and pathologic stage) alone are unable to predict the biologic behavior in most cases of prostate cancer.

Recently, prostate-specific antigen (PSA)[6-9] and DNA ploidy[10-21] were studied to evaluate their usefulness as prognostic factors in patients with prostate cancer. In 1979, Wang and associates[22] identified PSA, which is produced exclusively by normal and neoplastic prostate tissue. Several recent studies show that the magnitude of PSA elevations were proportional to increasing pathologic stage.[6,10,11,15,17,19]

Flow cytometry permits the rapid determination of DNA content in large numbers of cells. Several reports examining DNA ploidy in patients with Stage D prostate cancer have shown that patients with aneuploid tumors had an inferior response to hormonal therapy and shorter survival than patients with diploid neoplasms.[18,20,21,23] The current study examines the relationship of PSA and DNA ploidy as measured by flow cytometry to conventional prognostic factors in 112 patients who underwent radical prostatectomy for the treatment of surgically resectable prostate cancer.

Materials and Methods

Patient Characteristics

We studied 112 men who underwent radical retropubic prostatectomy for surgically resectable prostate cancer at The Ohio State University between March 1986 and May 1989. Clinical staging studies on all patients included a PSA and/or a prostatic acid phosphatase (PAP), chest radiograph, bone scan, and digital rectal examination. Additionally, most patients also underwent transrectal ultrasonography and computed tomography of the pelvis. After clinical staging, 98 (87.5%) patients had organ-confined disease (clinical Stages A or B), and 14 (12.5%) patients had limited extraprostatic disease (clinical Stage C) believed to be resectable by radical prostatectomy. There were 94 (83.9%) white patients and 18 (16.1%) black patients. The mean age was 65.3 years (age range, 48 to 76 years).

Variables Examined

Each patient had the following variables examined: age, race, PSA, and/or PAP, DNA ploidy analysis as measured by flow cytometry, Gleason score of the radical prostatectomy specimen, and pathologic stage. The Gleason score represents the sum of the primary and secondary Gleason histologic patterns.[24] As a result, it may range from 2 to 10. In the current study no patient was identified with a Gleason score of 2 or 10; therefore, a range of 3 to 9 was observed. The pathologic stage was analyzed by both the modified Whitmore-Jewett staging system (ABCD)[25] and the tumor-node-metastasis (TNM) staging system as proposed by The American Joint Committee on Cancer.[26] The two pathologic staging systems were highly correlated with nearly identical *P* values. The statistical significance of comparisons using the ABCD staging system were identical to comparisons using the TNM systems. For conciseness, only the data using the ABCD staging system will be presented. The ABCD pathologic staging system is summarized in Table 1. Both Gleason score and pathologic tumor stage were based on the original pathologic reports done routinely without subsequent review or revision.

Prostate-Specific Antigen and Prostatic Acid Phosphatase Determinations

Each patient had a serum PSA (98 of 112; 87.5%), PAP (68 of 112; 60.7%), or both (59 of 112; 52.7%) obtained by venipuncture before rectal examination and prostatic biopsy and within 1 month of radical retropubic prostatectomy. The PAP was measured on serum samples that were separated immediately from cells on arrival at the Clinical Chemistry Laboratory; if measurements were not done immediately, a capsule of sodium acetate (to stabilize acid phosphatase activity) was added to the specimen. No PAP level was run later than 2 hours after the specimen was received by the laboratory. The PSA specimens were processed either on the day they were received, or they were frozen at −20°C until measurements were completed. No determination was made more than 4 days after receipt of the specimen.

The PSA was quantified using the TANDEM-R PSA assay method (Hybritech, San Diego, CA) which is a solid-

TABLE 1. Modified Whitmore-Jewett Staging Classification of Prostate Cancer

Stage A: Clinically unrecognized, pathologically intracapsular
A1 <5% of prostatic tissue neoplastic
A2 >5% of prostatic tissue neoplastic, all high-grade tumors
Stage B: Clinically intracapsular, pathologically intracapsular
B1 Nodule <2 cm surrounded by palpably normal tissue
B2 Nodule >2 cm or multiple nodules
Stage C: Pathologically extracapsular, localized to periprostatic area
C1 Minimal extracapsular extension
C2 Large tumors involving seminal vesicles and/or adjacent structures
Stage D: Metastatic disease
D1 Pelvic lymph node metastases
D2 Distant metastases to bone, viscera, or other soft tissue structures

phase, two-site immunoradiometric assay.[27] The PAP was measured with thymophthalein monophosphate hydrolysis as adapted for the Dupont Automatic Clinical Analyzer (Clinical Systems Division, Wilmington, DE).[28]

Flow Cytometry Sample Preparation and Analysis

Our laboratory's methods (as previously described[29]) were used for tissue preparation of the paraffin-embedded specimens. The propidium iodide-stained nuclei were analyzed on a Coulter Epics Profile flow cytometer with an Omnichrome argon-ion air-cooled laser (Coulter, Hialeah, FL). The nuclei were analyzed at a rate of 200 to 500 events per second, and at least 20,000 nuclei were analyzed per specimen. The mean coefficient of variation for each specimen was 2.8 (range, 1.1 to 5.5). A DNA aneuploid tumor was defined as one with an additional population, excluding the diploid population, of nuclei and tetraploid populations of nuclei greater than or equal to 15%.

Statistical Analysis

We analyzed frequency data using chi-square tests. In the case of continuous data, we compared groups with analysis of variance.[30] Using a 0.05 level of significance, correlations were examined using parametric and nonparametric Spearman rank-correlation methods.

Results

Comparison of Clinical and Pathologic Staging

Of the 112 patients studied, the pathologic stages were Stage A1 in one (0.9%) patient, A2 in ten (8.9%) patients, B1 in 15 (13.4%) patients, B2 in 39 (34.8%) patients, C in 29 (25.9%) patients, and D1 (16.1%) in 18 patients. Thus, 65 (58.0%) patients had disease limited to the prostate (pathologic Stages A and B), and 47 (42.0%) patients had extraprostatic disease (pathologic Stages C and D1). Since 98 patients had clinical Stages A or B prostate cancer and 65 patients had pathologic Stages A or B disease, the clinical accuracy of detecting disease limited to the prostate was 66%.

TABLE 2. Comparison of Pathologic Stage and Gleason Score (n = 112)

Pathologic stage	No.	Gleason score 2	3	4	5	6	7	8	9	10
A1	1	—	—	1	—	—	—	—	—	—
A2	10	—	1	4	4	—	—	1	—	—
B1	15	—	—	8	4	1	2	—	—	—
B2	39	—	1	1	14	10	11	1	1	—
C	29	—	—	—	6	3	10	4	6	—
D1	18	—	—	—	1	3	7	3	4	—
Total	112	0	2	14	29	17	30	9	11	0

Gleason score 2–5 *versus* 6–10 ($P < 0.0001$, chi-square).

TABLE 3. Comparison of DNA Ploidy and Pathologic Stage (n = 112)

Pathologic stage	No.	Aneuploid (%)	Diploid (%)
A and B	65	7 (10.8)	58 (89.2)
A	11	0 (0.0)	11 (100.0)
B	54	7 (13.0)	47 (87.0)
C and D1	47	12 (25.5)	35 (74.5)
C	29	9 (31.0)	20 (69.0)
D1	18	3 (16.7)	15 (83.3)
Total	112	19 (17.0)	93 (83.0)

DNA ploidy compared with Stages A or B *versus* Stages C or D1 ($P = 0.04$, chi-square).

Comparison of Gleason Score and Pathologic Stage

Traditionally, patients have been divided into low-grade, intermediate-grade, and high-grade tumors by a Gleason score of 2 to 4, 5 to 7, and 8 to 10, respectively. However, statistical significance was greatest when patients were divided into two groups: one with a Gleason score of 2 to 5 and the other with a score of 6 to 10. Pathologic stage was significantly related to Gleason score ($P < 0.0001$), and grade increased with stage (Table 2). Of the 45 patients with a Gleason score of 2 to 5, 38 (84.4%) had pathologic Stage A or B disease, and seven (15.6%) had pathologic Stage C or D cancer. Of the 67 patients with Gleason scores of 6 to 10, 27 (40.3%) had Stage A or B tumor, and 40 (59.7%) had Stage C or D neoplasm.

DNA Ploidy Analysis by Flow Cytometry

Flow cytometric analysis revealed DNA aneuploid and diploid tumors in 19 (17.0%) and 93 (83.0%) patients, respectively. DNA ploidy significantly correlated with pathologic stage ($P = 0.04$, Table 3). Of the 19 patients with DNA aneuploid tumors, seven (36.8%) had Stage A or B disease, and 12 (63.2%) had Stage C or D1 tumors. In contrast, among the 93 patients with DNA diploid tumors, 59 (63.4%) had Stage A or B tumors, and 34 (36.6%) had Stage C or D1 disease.

DNA ploidy also was related significantly to Gleason score ($P = 0.034$, Table 4). Of the 19 patients with DNA aneuploidy, three (15.8%) had a Gleason score of 3 to 5, and 16 (84.2%) had a Gleason score of 6 to 9. Of the 93 patients with DNA diploid tumors, 42 (45.2%) had a Gleason score of 3 to 5, and 51 (54.8%) had a Gleason score of 6 to 9.

Prostate Specific Antigen

Each patient had serum levels of PSA (n = 98), PAP (n = 68), or both tumor markers (n = 59). Although PAP

TABLE 4. Comparison of Gleason Score and DNA Ploidy (n = 112)

Gleason score	No.	Aneuploid (%)	Diploid (%)
3	3	0 (0)	2 (100.0)
4	14	0 (0)	14 (100.0)
5	29	3 (10.3)	26 (89.7)
6	17	2 (11.8)	15 (88.2)
7	30	8 (26.7)	22 (73.3)
8	9	1 (11.1)	8 (88.9)
9	11	5 (45.5)	6 (54.5)
3–9	112	19 (17.0)	93 (83.0)

Gleason score 2–5 *versus* Gleason score 6–10 ($P < 0.01$, chi-square).

TABLE 6. Comparison of Absolute PSA Value to DNA Ploidy (n = 98)

PSA value (ng/ml)	No.	Aneuploid (%)	Diploid
0.0–4.0	32	5 (15.6)	27 (84.4)
4.1–10.0	26	2 (7.7)	24 (92.3)
10.1–20.0	21	3 (14.3)	18 (85.7)
>20.0	19	6 (31.6)	13 (68.4)
Total	98	16 (16.3)	82 (83.7)

PSA: prostate-specific antigen.
DNA ploidy *versus* PSA ≤20 ng/ml and PSA >20 ng/ml ($P < 0.05$, chi-square).

was related significantly to PSA ($P < 0.001$), PAP was not associated significantly to any of the other variables studied. Additionally, PSA was not related significantly to age. When mean PSA values were compared with DNA ploidy, statistical significance was approached ($P = 0.078$, Table 5). The mean PSA values of the 16 patients with DNA aneuploid tumors and DNA diploid tumors were 24.30 ng/ml and 19.43 ng/ml, respectively. However, patients with a PSA values of less than or equal to 20.0 ng/ml had a greater proportion of DNA diploid tumors *versus* aneuploid tumors ($P < 0.05$, by chi-square test, Table 6). Of the 79 patients with PSA values less than or equal to 20.0 ng/ml, 69 (83.3%) had DNA diploid tumors, and ten (12.7%) had DNA aneuploid tumors. Of the 19 patients with PSA values greater than 20.0 ng/ml, 13 (68.4%) had DNA diploid tumors, and six (31.6%) had DNA aneuploid tumors.

The PSA was related significantly to pathologic stage. As Table 7 demonstrates, there was a step-like relationship between the mean PSA value and pathologic stage. The mean PSA value of the 11 Stage A patients was 4.82 ng/ml; of 46 Stage B patients was 8.86 ng/ml; of 25 Stage C patients was 20.71 ng/ml; and of 15 Stage D1 patients was 32.65 ng/ml ($P = 0.0001$). When considering Stages A and B jointly and Stages C and D1 together, the mean PSA values were 8.07 ng/ml and 26.1 ng/ml, respectively ($P = 0.00001$).

Table 8 shows that when patients were divided by absolute PSA values into four groups (0.0 to 4.0 ng/ml, 4.1 to 10.0 ng/ml, 10.1 to 20.0 ng/ml, and >20.0 ng/ml), a significant relationship between PSA and pathologic stage was also observed ($P = 0.0021$). Furthermore, PSA values less than or equal to 10.0 occurred more frequently in patients with Stage A or B prostate cancer than in those with Stage C or D1 tumors ($P < 0.05$, by chi-square testing). Of the 57 patients with Stages A and B disease, 40 (70.2%) had PSA values less than or equal to 10.0 ng/ml, and 17 (29.8%) had PSA values greater than 10.0 ng/ml. Of the 40 patients with Stage C or D1 prostate cancer, 18 (45%) had PSA values less than or equal to 10.0 ng/ml, and 22 (55%) had PSA values greater than 10.0 ng/ml.

A significant difference was noted when patients with a Gleason score of 2 to 5 were compared with those with a score of 6 to 9. The mean PSA values were 6.7 ng/ml and 21.8 ng/ml, respectively ($P = 0.0004$, Table 9). When

TABLE 5. Comparison of DNA Ploidy to Mean PSA and PAP Values

	PSA*		PAP†	
DNA ploidy	No.	Mean value (ng/ml) ± SD	No.	Mean value (U/l) ± SD
Diploid	82	13.7 ± 19.3	55	0.72 ± 1.00
Aneuploid	16	24.3 ± 31.4	13	0.50 ± 0.30
Total	98	15.5 ± 21.7	68	0.68 ± 0.91

PSA: prostate-specific antigen; PAP: prostatic acid phosphatase; SD: standard deviation.
* $P = 0.0782$, chi-square.
† $P = 0.434$, chi-square.

TABLE 7. Comparison of Pathologic Stage to Mean PSA and Mean PAP Values (n = 98)

	PSA*		PAP†	
Pathologic stage	No.	Mean value (ng/ml) ± SD	No.	Mean value (U/l) ± SD
A and B	57	8.1 ± 8.7	35	0.54 ± 0.64
A	11	4.8 ± 4.0	6	0.30 ± 0.11
B	46	8.9 ± 9.3	29	0.59 ± 0.70
C and D1	41	25.8 ± 29.8	33	0.83 ± 1.12
C	26	20.4 ± 26.7	23	0.91 ± 1.31
D1	15	35.1 ± 32.6	10	0.65 ± 0.45
Total	98	19.8 ± 19.8	68	0.68 ± 0.91

PSA: prostate-specific antigen; PAP: prostatic acid phosphatase; SD: standard deviation.
* Mean PSA of Stage A and B *versus* Stage C and D1 ($P = 0.0001$, chi-square).
† Mean PAP of Stage A and B *versus* Stage C and D1 ($P = 0.430$, chi-square).

TABLE 8. Comparison of Pathologic Stage to Absolute PSA Value (n = 98)

Pathologic stage	No.	PSA (ng/ml) 0.0–4.0 (%)	4.1–10.0 (%)	10.1–20.0 (%)	>20.0 (%)
A and B	57	24 (42.1)	16 (28.1)	14 (24.6)	3 (5.3)
A	11	7 (63.6)	2 (18.2)	2 (18.2)	0 (0.0)
B	46	17 (37.0)	14 (30.4)	12 (26.1)	3 (6.5)
C and D1	41	8 (19.5)	10 (24.4)	7 (17.1)	16 (39.0)
C	26	6 (23.1)	7 (26.9)	6 (23.1)	7 (26.9)
D1	15	2 (13.3)	3 (20.0)	1 (6.7)	9 (60.0)
Total	98	32 (32.7)	26 (26.5)	21 (21.4)	19 (19.4)

PSA: prostate-specific antigen.
$P = 0.0021$, overall chi-square.

More patients with PSA ≤10.0 ng/ml had Stage A and B prostate cancer than Stage C and D1 prostate cancer ($P < 0.05$, chi-square).

patients were divided by absolute PSA value into the four groups described, PSA increased with Gleason score (Table 10). The PSA values less than or equal to 10.0 occurred more frequently in patients with a Gleason score of 3 to 5 (34/58; 58.6%) than in patients with a score of 6 to 9 (six of 40; 15.0%) ($P < 0.001$).

Racial Differences of Prognostic Factors

Of the 112 patients, there were 18 (16.1%) black and 94 (83.9%) white patients. There were no significant racial difference in regard to age, mean PAP value, Gleason score, and pathologic stage. A higher incidence of DNA aneuploid tumors and a greater mean PSA value were found in black patients compared with white patients ($P = 0.043$). Of the 18 black patients, six (33.3%) had DNA aneuploid tumors, and 12 (66.6%) had DNA diploid tumors (Table 11). Of the 94 white patients, 13 (13.8%) had DNA aneuploid tumors, and 81 (86.2%) had DNA diploid tumors. Furthermore, a significant difference between race and mean DNA indices was also observed. The mean DNA indices of black and white patients were 1.28 (standard deviation, 0.26) and 1.09 (standard deviation, 0.45; $P = 0.017$). Ninety-eight patients had PSA determinations; the mean PSA values for the 15 black and 83 white patients were 26.0 ng/ml and 13.6 ng/ml, respectively ($P = 0.043$, Table 12).

Discussion

Frequently, the pathologic stage has been underestimated by clinical evaluation; 14% to 85% of patients with clinically localized prostate cancer have had extraprostatic tumor extension on pathologic examination.[5,6,9,11,15,31] In the current study, the pathologic stage distribution and clinical staging accuracy of detecting disease limited to the prostate was consistent with many earlier reports. However, the clinical staging accuracy reported here (66%; 65/98) varied considerably from a previous study from our own institution (86%; 24/28).[6] This discrepancy may be related to the small number of patients studied, differences in the frequency of using transrectal prostatic ultrasonography and PSA determination, or differences in the patient population. The previous study included a select patient group: asymptomatic men screened for prostate cancer by digital rectal examination, transrectal prostatic ultrasonography, PSA, and PAP. The current study included symptomatic and asymptomatic men. In addition, not every patient had the benefit of transrectal ultrasonography and PSA determination.

TABLE 9. Comparison of Gleason Score and Mean PSA and Mean PAP Values

Gleason score	PSA* No.	PSA* Mean value (ng/ml) ± SD	PAP† No.	PAP† Mean value (U/l) ± SD
3–5	40	6.2 ± 6.7	26	0.63 ± 0.75
6–9	58	26.2 ± 26.2	42	0.71 ± 1.01
3–9	98	15.5 ± 20.6	68	0.68 ± 0.92

PSA: prostate-specific antigen; PAP: prostatic acid phosphatase; SD: standard deviation.
* $P < 0.0004$, chi-square.
† $P = 7.320$, chi-square.

TABLE 10. Comparison of Absolute PSA Value to Gleason Score (n = 98)

PSA value (ng/ml)	No.	Gleason score 1–5 (%)	6–7 (%)
0.0–4.0	32	19 (59.4)	13 (40.6)
4.1–10.0	26	15 (57.7)	11 (42.3)
10.1–20.0	21	5 (23.8)	16 (76.2)
>20.0	19	1 (5.3)	18 (94.7)
Total	98	40 (40.8)	58 (59.2)

PSA: prostate-specific antigen.
In patients with PSA ≤10.0 ng/ml, tumors with Gleason scores of 2–5 occurred more frequently than scores of 6–10 ($P < 0.001$, chi-square).

TABLE 11. Racial Distribution of DNA Ploidy (n = 112)

Race	No.	Aneuploid (%)	Diploid (%)
White	94	13 (13.8)	81 (86.2)
Black	18	6 (33.3)	12 (33.3)
Total	112	19 (17.0)	93 (83.0)

$P = 0.0434$, chi-square.

The observed Gleason score distribution was also consistent with prior reports.[2,12–15,19] Additionally, the association of Gleason score and pathologic stage confirmed a well-established relationship that grade increases with stage.[3,5,9,15,17,19,22,31] Similar to the Mayo Clinic experience,[18,20,21] statistical analysis led to two groupings by Gleason scores of less than or equal to 5 and greater than or equal to 6. Among 45 patients with a Gleason score of 5 or less, only one (2.2%) patient had regional lymph node metastases (Table 2). Of the 67 patients with a Gleason score of 6 or more, 17 (25.3%) had regional lymph node metastases. Patients with Gleason scores of 6, 7, 8, and 9 had regional lymph node metastases in 17.6% (three of 17), 23.3% (seven of 30), 33.3% (three of nine), and 36.4% (four of 11) of patients, respectively. Thus, for patients with a Gleason score of 6 or more, the percentage of patients with positive lymph nodes may be estimated by the following equation:

$$\text{Percent positive regional lymph nodes} = (\text{Gleason score})^2/2 \times 100$$

Gleason scores based on needle biopsy or transurethral prostatectomy specimens frequently underestimate those determined from radical prostatectomy specimens.[10,11,32] Therefore, if this equation is applied to Gleason scores obtained from needle biopsy or transurethral prostatectomy specimens, it should be assumed to represent the minimal estimate of positive regional lymph nodes.

Flow cytometry has been used to examine benign prostatic hyperplasia (BPH) and prostatic cancer. Three studies analyzed DNA ploidy of solid benign hyperplastic prostatic tissue by flow cytometry. A combined total of 160 patients revealed only three (1.9%) patients with DNA aneuploidy.[14,16,17]

Table 13 summarizes the data of 12 reports[10–21] plus the current study that analyzed DNA ploidy (by flow cytometry) of solid primary tumor specimens in 1157 patients with prostate cancer. Of the 1157 patients, DNA aneuploidy was documented in 221 (19.1%) patients. Stage was not specified in 230 patients. In the remaining 927 patients, DNA aneuploidy was noted in 6.5% (32/490) of patients with pathologic Stage A and B disease, 27.1% (71/262) with pathologic stage C disease, 18.5% (27/146) with pathologic Stage D1 disease, and 72.4% (21/29) with clinical Stage D2 prostate cancer. The overall DNA ploidy differences were statistically significant ($P < 0.0001$). Furthermore, DNA ploidy of each stage grouping was significantly different ($P < 0.05$). Our results correlated well with the overall data.

Possible reasons for the higher incidence of DNA aneuploidy in pathologic Stage C tumors compared with pathologic Stage D1 neoplasms includes errors in specimen sampling and primary tumor heterogeneity. However, another hypothetic explanation may be related to the cancer volume of the primary tumor. McNeal and associates[33] showed that Gleason scores increase with cancer volume. Since DNA aneuploidy appears to be related to Gleason score, the frequency of DNA aneuploidy may also increase with cancer volume. In general, pathologic Stage C tumors have larger primary tumor cancer volumes that pathologic Stage A or B tumors. Additionally, prostate cancer patients with clinically organ-confined disease but pathologic Stage D1 carcinoma constitute a highly select group; the primary tumor cancer volume may in fact be less than in patients with pathologic Stage C disease. Thus, among radical prostatectomy candidates, primary tumor cancer volume may increase from pathologic Stages A and B to pathologic Stage D1 to pathologic Stage C to clinical Stage D2 neoplasms. If the hypothesis that DNA aneuploidy increases with primary tumor cancer volume is true, the DNA aneuploid incidence rates for the different stages may be explained. That DNA aneuploidy occurs more frequently in pathologic Stage C prostate cancer than pathologic Stage D1 disease suggests that DNA ploidy status alone cannot explain the biologic potential of a primary tumor to develop regional lymph node metastases.

The relationship of DNA ploidy to Gleason score has not been well established. Several series have shown that DNA aneuploidy has been associated with a higher Gleason score;[12,15,19–21] others have not.[13,17,18] Table 14 summarizes the data from six series which have compared the Gleason score to DNA ploidy status of the solid primary

TABLE 12. Racial Distribution of Mean PSA and Mean PAP Values

	PSA*		PAP†	
Race	No.	Mean value (ng/ml) ± SD	No.	Mean value (U/l) ± SD
White	83	13.6 ± 18.1	57	0.64 ± 0.88
Black	15	26.0 ± 35.5	11	0.87 ± 1.10
Total	98	15.5 ± 21.6	68	0.69 ± 0.91

PSA: prostate-specific antigen; PAP: prostatic acid phosphatase; SD: standard deviation.

* $P = 0.0433$, chi-square.

† $P = 0.4466$, chi-square.

TABLE 13. Flow Cytometric DNA Ploidy Analysis of Solid Primary Tumor Specimen in Patients With Prostate Cancer

Author/year	Overall group Total/aneuploid (%)		Pathologic Stage A and B Total/aneuploid (%)		Pathologic Stage C Total/aneuploid (%)		Pathologic Stage D1 Total/aneuploid (%)		Clinical Stage D2 Total/aneuploid (%)	
Frankfort/1985	45	20 (44.4)	11	0 (0)	15	7 (46.7)	8	5 (62.5)	11	8 (72.7)
Pontes/1985	33	10 (30.3)	11	0 (0)	13	4 (30.8)	9	5 (55.6)	0	0 (0)
Fordham/1986*	72	37 (51.4)	—	—	—	—	—	—	—	—
Lundberg/1987†	50	12 (24.0)	—	—	—	—	—	—	—	—
Klein/1988	48	4 (8.3)	—	—	—	—	—	—	—	—
Lee/1988‡	88	51 (58.0)	—	—	59	40 (67.8)	—	—	0	0 (0)
McIntire/1988	33	10 (30.3)	33	10 (30.3)	0	0 (0)	0	0 (0)	0	0 (0)
Ritchie/1988§	109	6 (5.5)	73	4 (5.5)	—	—	20	2 (10.0)	0	0 (0)
Winkler/1988	91	12 (13.2)	0	0 (0)	0	0 (0)	91	12 (13.2)	0	0 (0)
Dejter/1989‖	69	19 (27.5)	36	1 (2.8)	—	—	—	—	18	13 (72.2)
Nativ/1989	146	11 (7.5)	0	0 (0)	146	11 (7.5)	0	0 (0)	0	0 (0)
Montgomery/1990	261	10 (3.8)	261	10 (3.8)	0	0 (0)	0	0 (0)	0	0 (0)
Badalament/1991	112	19 (17.0)	65	7 (10.8)	29	9 (31.0)	18	3 (16.7)	0	0 (0)
Total	1157	221 (14.1)	490	32 (6.5)	262	71 (27.1)	146	27 (18.5)	29	21 (72.4)

Because the information concerning staging distribution of patients is incomplete, the sum of the individual stages does not equal the cumulative total; "—" indicates where data could not be determined from published report ($P < 0.001$, overall chi-square).

* Twenty-nine patients had T1 and T2 tumors; 43 had T3 and T4 tumors.

† No staging information. All patients followed ≥5 years; 12 of 50 (24%) had metastases.

‡ Twenty-nine patients had pathologic Stage B tumor and tumors with capsular invasion considered jointly: 59 had seminal vesical invasion.

§ The 73 patients included in pathologic Stage A and B grouping probably includes eight patients with capsular invasion without capsular penetration.

‖ Fourteen patients had pathologic Stage C and D1 disease considered jointly.

tumor specimen in 492 patients with prostate cancer.[10,12,14,15,17,19] The mean Gleason score in patients with DNA aneuploid and DNA nonaneuploid tumors was 7.1 and 6.2, respectively. Additionally, patients with DNA aneuploid tumors were associated with a Gleason score greater than 7 more often than nonaneuploid tumors ($P < 0.0001$). Of patients with DNA aneuploid tumors, 38.9% (44/113) had a Gleason score less than or equal to 7, and 61.1% (69/113) had a Gleason score greater than 7. Of the patients with DNA diploid or tetraploid tumors, the Gleason score was 7 or less in 81.8% (162/198) and 7 or more in 18.2% (36/198). Therefore, our study and the literature support a significant relationship between DNA ploidy and Gleason score.

The PAP level has been shown to be useful in detecting advanced prostate cancer and in monitoring patients for disease progression.[34,35] Although as a screening test PAP has a high degree of specificity, it has a sensitivity of only 31% to 60%.[36–38] Thus, PAP is not advocated for prostate cancer screening.[6,34–38] The utility of PAP as a staging tool is undefined. Oesterling *et al.*[5] did logistic-regression analysis on 275 patients who had radical prostatectomy

TABLE 14. Gleason Score of Primary Tumor Compared With Flow Cytometric DNA Ploidy Analysis of Solid Primary Tumor Specimen in Patients With Prostate Cancer

Author/year	Total	Aneuploid No.	Aneuploid Gleason score ≤7	Aneuploid Gleason score >7	Aneuploid Mean Gleason score	Nonaneuploid No.	Nonaneuploid Gleason score ≤7	Nonaneuploid Gleason score >7	Nonaneuploid Mean Gleason score
Frankfort/1985	42	24	1	23	7.4	18	12	6	6.1
Fordham/1986	72	37	—	—	7.2	35	—	—	5.8
Lee/1988	88	51	26	25	7.6	37	30	7	5.9
Ritchie/1988	109	6	—	—	6.0	103	—	—	7.0
Dejter/1989	69	19	4	15	8.2	50	41	9	5.5
Badalament/1991	112	19	13	6	6.4	93	79	14	6.0
Total	492 (100%)	156 (31.7%)	44 (38.9%)	69 (61.1%)	7.1	336 (68.3%)	162 (81.8%)	36 (18.2%)	6.2

"—" indicates where data could not be determined from published reports. Aneuploid tumors were associated with a Gleason score >7 more often than nonaneuploid tumors ($P < 0.001$, chi-square).

TABLE 15. Probability Tests Defining Clinical Utility of PSA in Staging Patients With Prostate Cancer

Terminology	Definition	Biostatistical formula (×100)	Pathologic C and D1 PSA >10 (%)	Pathologic C and D1 PSA >20 (%)	Pathologic D1 PSA >10 (%)	Pathologic D1 PSA >20 (%)
Sensitivity	Probability of test detecting disease when present	$\frac{\text{True-pos}}{\text{True-pos + false-neg}}$	56.1	39.0	66.7	56.3
Specificity	Probability of test being negative when disease not present	$\frac{\text{True-neg}}{\text{True-neg + false-pos}}$	70.1	94.7	63.9	88.0
Predictive value of positive test	Probability of patient having disease when test is positive	$\frac{\text{True-pos}}{\text{True-pos + false-pos}}$	57.5	84.2	25.0	47.4
Predictive value of negative test	Probability of patient being free of disease when test is negative	$\frac{\text{True-neg}}{\text{True-neg + false-neg}}$	69.0	74.0	89.6	92.4

PSA: prostate-specific antigen; pos: positive; neg: negative.

for clinically localized prostate cancer. Serum PAP (Roy technique) correlated with capsular penetration ($P < 0.003$) and seminal vesicle involvement ($P < 0.01$) but not lymph node involvement ($P = 0.08$). However, Gibbons and associates[39] examined 16 patients with a preoperatively elevated PAP and a negative pelvic lymphadenectomy; none of these patients had disease progression 1 to 12 years after radical prostatectomy. Additionally, it is the practice of many urologists to proceed with radical prostatectomy for clinically localized prostatic cancer when patients have minimally elevated PAP values.[40,41]

We found that PAP was highly correlated with PSA but not with any of the other parameters examined. As Tables 5, 7, 9, and 12, show, mean PAP values were not related to DNA ploidy, pathologic stage, Gleason score, or race. Additionally, absolute PAP values were not associated significantly with these variable.

The PSA level has also been shown to be useful in detecting advanced prostate cancer and monitoring patients after definitive therapy. Although the reported sensitivity of PSA is 73% to 96%, it is a less specific tumor marker than PAP.[6,36-38,41,42] Because of its greater sensitivity, PSA appears to be a superior method than PAP for monitoring patients after therapy. However, due to its low specificity, PSA does not appear to be a useful screening test for localized prostate cancer.

In agreement with others, mean and absolute PSA values significantly correlated with pathologic stage and Gleason score.[6,10,11,15-17,19,37-41,43] However, probability tests that define the clinical utility of PSA (Table 15) demonstrate that PSA alone cannot be used for staging. Partin and associates[43] measured PSA levels in 250 men with clinically localized prostate cancer treated by radical prostatectomy and 72 men with histologically confirmed BPH. Their findings showed that PSA did not reflect tumor burden and pathologic stage accurately because of the unpredictable contribution from the BPH component of the gland and the decreased production of PSA by higher grade lesions as tumor volume increased.

Despite these limitations, preoperative PSA is still a useful adjunctive clinical staging parameter in certain situations. The accuracy of a PSA value greater than 20 ng/ml being associated with pathologic Stage D1 disease (80.6%) appears to be roughly equivalent to the accuracy of computed tomography in detecting regional lymph node metastases (73% to 81%).[44] The negative predictive value of a PSA less than or equal to 10 ng/ml being associated with pathologic Stage D1 disease (89.6) is similar to the negative predictive value of frozen-section pelvic lymph node analysis (81.4% to 96.1%).[45] When the PSA is greater than 20 ng/ml, the positive and negative predicative values for the identification of extracapsular disease are similar to those obtained by transrectal prostatic ultrasonography (84.2% *versus* 89% and 74.0% *versus* 76%, respectively).[46] Therefore, although PSA alone cannot be used to stage patients with prostate cancer, in certain situations it is as useful, less expensive, and less invasive than conventional clinical staging techniques.

American black men have the highest prostate cancer incidence rate of any racial group in the world.[47,48] Prostate cancer develops about twice as frequently in black than in white American men.[49] Although black American men have been reported with prostate cancer at an earlier age and have higher stage disease at initial diagnosis than their white counterparts, stage-for-stage, the mortality rates for both races are similar.[1,50]

In the current study, there were no significant racial differences in regard to age, mean PAP value, Gleason score, and pathologic stage. However, two significant ob-

servations were noted. DNA aneuploid tumors occurred more frequently in black than white men. Of patients with prostate cancer, black men had a higher mean PSA value than white men. The racial differences observed in DNA ploidy status suggests a genetic predisposition to prostate cancer among blacks. Additionally, elevated mean PSA levels in blacks may imply differential gene expression. However, another possible explanation may be that the higher mean PSA levels observed in blacks may be related to higher mean testosterone and free testosterone levels noted in blacks compared with whites.[51]

In summary, DNA ploidy (as measured by flow cytometry) and PSA provide objective criteria that we compared with the conventional prognostic indicators PAP, Gleason score, and pathologic stage. Both DNA ploidy status and PSA were related significantly to Gleason score, pathologic stage, and race. The data suggest that PSA is a superior determinant of prognosis than PAP. Furthermore, DNA ploidy analysis and PSA appear to provide prognostic information which may be used to enhance the accuracy of predicting the biologic behavior of patients with prostate cancer.

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