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und disease-progression

The Prostate 31:21–28 (1997)

# The Significance of DNA-Ploidy and S-Phase Fraction in Node-Positive (Stage D1) Prostate Cancer Treated With Androgen Ablation

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**BACKGROUND.** The prognostic significance of primary tumor DNA-ploidy and S-phase fraction (SPF) was evaluated in patients treated with androgen ablation for regionally localized node-positive prostate cancer.

**METHODS.** All patients were diagnosed with lymph node involvement by pelvic lymphadenectomy between 1984 and 1992 and were treated only with androgen ablation. Median follow-up was 45 months. Adequate material for DNA/nuclear protein flow cytometric analysis was available in 33 patients.

**RESULTS.** The tumors were classified as diploid in 11, near-diploid in 4, tetraploid in 10, and aneuploid in 8 cases. Grouping the patients by nonaneuploidy (diploid and near-diploid and tetraploid) and aneuploidy revealed actuarial 4-year disease progression rates of 14 and 48% (log-rank, $P = 0.04$), and overall survival rates of 100 and 61% ($P = 0.008$); however, biochemical progression (rising prostate-specific antigen profile) rates were similar at around 70%. In contrast, SPF was not significantly related to any of the endpoints tested. Several other potential prognostic factors were examined and none correlated significantly with disease progression or survival.

**CONCLUSIONS.** The biochemical progression rates for patients with nonaneuploid and aneuploid tumors were comparable and high, while the disease progression rates were higher and survival rates lower for those with aneuploid tumors. These data indicate that the lead time from biochemical to disease progression and death was shorter with aneuploidy. That these relationships were observed in such a small patient population attest to the strength of DNA-ploidy as a prognostic factor in this cohort. *Prostate 31:21–28, 1997.*



*KEY WORDS:* **flow cytometry; DNA content; prostate neoplasm**

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Received 17 October 1995; Accepted 8 January 1996

## INTRODUCTION

Flow cytometric analysis of DNA content has been reported in a number of studies to be a strong predictor of prostate cancer patient outcome [1]. Most of these reports have revolved around patients treated with radical prostatectomy for localized disease, have focused on DNA-ploidy, and have described this parameter as an independent prognostic factor. We recently found DNA-ploidy to be a significant correlate of the combined endpoint of biochemical and disease failure in patients with stages T1–T4 prostate cancer treated with definitive radiotherapy [2]. A multivariate analysis revealed pretreatment prostate-specific antigen (PSA), tumor grade, and DNA-ploidy to be the only independent predictors of this endpoint.

The technique we used to determine DNA-ploidy involved the simultaneous staining and flow cytometric analysis of DNA and nuclear protein [3,4]. The nuclear protein parameter was valuable in recognizing artifacts from incomplete fixation and in classifying near-diploid tumor populations by reducing the overlap between diploid and near-diploid nuclei in the resultant bivariate histograms. An analysis of the significance of near-diploidy identified in this fashion revealed that prostate cancer patients with near-diploid tumors had an intermediate prognosis as compared to those with diploid or nondiploid (aneuploid or tetraploid) tumors [5]. The division of traditionally diploid tumors into diploid and near-diploid based on DNA/nuclear protein analysis remained significant in multivariate analysis.

Several investigators have also analyzed the prognostic value of tumor S-phase fraction (SPF), which is a more problematic parameter to quantify due to normal and tumor population overlapping. In some cases, SPF has been found to be more significant than DNA-ploidy [6,7]. Thus, both DNA-ploidy and SPF are potential prognostic factors that may be useful in profiling the patient with prostate cancer.

There are few series concerning the prognostic value of DNA content analysis for node-positive (stage D1) prostate cancer patients, although the results are promising [8,9]. The present report examines whether DNA-ploidy or SPF predicts therapeutic outcome in a cohort of node-positive patients treated initially with androgen ablation alone [10].

## MATERIALS AND METHODS

### Patients and Tissues

There were 181 patients treated with androgen ablation alone for regionally localized node-positive (stage D1) adenocarcinoma of the prostate between 1984 and 1992 at U.T.-M.D. Anderson Cancer Center. The outcome of these patients has been described in detail previously [10]. An attempt was made to obtain the original paraffin-embedded archival pathologic material from the referring institutions for all of the patients in this cohort. The blocks were recovered from 65 of the original 181 diagnostic specimens and, of these, 39 were felt to have enough tumor for flow cytometric analysis. The material was deemed suitable for flow cytometric analysis if at least 30% was composed of tumor tissue. Of these 39, 33 were acceptable for analysis; 5 specimens had an insufficient yield of nuclei and one histogram was uninterpretable. The 33 patients with analyzable histograms are the subject of this report.

The initial work-up has been detailed previously [10]. Lymph node involvement was confirmed pathologically by lymphadenectomy. In most cases, the lymph node metastases were identified on frozen section and the procedure was aborted. Thus, the extent of lymph node disease was not accurately assessed. All patients were clinically and radiographically free of distant metastases when androgen ablation was started. Androgen ablation was begun within 3 months of the diagnosis of node positivity. No patient underwent radical prostatectomy or received prostate radiotherapy before progression.

Pretreatment serum PSA levels were obtained in 76% of the patients with an average of 42.0 ± 9.7 (±S.E.) and a median of 32.4 ng/ml (range 4.4–247.3). The average prostatic acid phosphatase (PAP) level was 0.50 ± 0.05 (median = 0.4; range 0.2–1.4) using the enzymatic method with an upper limit of 0.8 mU/ml.

Patient age ranged from 51 to 76 years with mean and median ages of 65 and 67 years, respectively. The average follow-up for those living (n = 27) was 44 ± 4.4 months with a median of 45 months (range 4–109 months). Follow-up was typically at 3–6-month intervals.

### Disease Endpoints

Local progression was defined as biopsy-proven progression in the prostate when prompted by a rising PSA profile (biochemical progression), increasing urinary obstructive symptoms, or clinical evidence of progression on digital rectal examination. Biochemical progression was based on two or more consecutive increasing PSA values on follow-up visits, or a single increase of >1 ng/ml or 1.5-fold. Regional progression included pelvic lymph nodal progression, with or without prostate progression, by clinical-radiographic criteria. Distant metastasis was defined as para-aortic lymph node enlargement or hematogenous spread confirmed radiographically. Biopsy of

lymph nodal or distant metastasis was not required. Disease progression included local, regional, or distant progression, and did not include biochemical progression.

### Sample Preparation

All tissue material was reviewed by the study pathologist (P.T.) to ensure suitability for flow cytometric analysis. The tissue blocks for the study were selected to include the areas of the tumor with the highest histologic grade. Hematoxylin and eosin stained 3 μm sections obtained before and after the thick section(s) for flow cytometry were reviewed to determine the presence and percentage of tumor in each sample. To facilitate comparison with the parent series [10], the Gleason scores shown in the tables are those from the original pathologic review at our institution. Somewhat higher Gleason scores were noted on re-review. Of the 33 that had sufficient material for this analysis, there were 7 that were from transurethral resections (TURP) and 26 from biopsy specimens.

Nuclei were extracted from paraffin-embedded tissue using a technique described in detail previously [2–5]. Briefly, one or two 50 μm sections were deparaffinized, digested in pepsin, and treated with acid to denature the DNA in the nuclei prior to staining. The protein in the extracted nuclei was then stained with fluorescein isothiocyanate (FITC) and the DNA with propidium iodide (PI). The samples were analyzed on an EPICS 752 flow cytometer (Coulter Electronics, Hialeah, FL) with an argon-ion laser set at 488 nm. Appropriate filters were used to resolve the green FITC signal from the red PI signal.

The bivariate DNA/nuclear protein histograms were used to classify the tumors as diploid, near-diploid, tetraploid, or aneuploid. The criteria for this breakdown have been described in detail [5]. Near-diploidy was defined as a split or second G1 peak with a DNA index (DI) of less than 1.12 and greater than 1.00 as identified in the DNA/nuclear protein histograms. It was possible to resolve these near-diploid peaks because of differences in nuclear protein. The DI is the ratio of the peak red fluorescence channel of the abnormal G1 peak to the peak red fluorescence channel of the diploid G1 peak. The DI was acquired from the 256-channel single-parameter DNA histograms. The coefficient of variation (CV), calculated for the diploid peak using MODFIT-LT software (Verity Software House, Topsham, ME), averaged 3.85 ± 0.24 with a median of 3.52 (range 1.72–7.68).

Tetraploidy was defined as greater than 5% of nuclei in G2M (DI = 1.9–2.1). The 5% cut-off delineating tetraploidy was derived from the finding that normal or hyperplastic prostate consistently had ≤3% in G2M. Aggregates were gated out on the peak vs. integral fluorescence signal from PI and, therefore, contributed little to the proportion in G2M. Tumors with a DI of ≥1.12 and <1.9, or >2.1 were classified as aneuploid.

The SPF was derived for all 33 cases from the single parameter DNA histograms using MODFIT-LT software. The model used corrected for single-cut debris, approximated S-phase using rectangles, and estimated G1 and G2M using gaussians. Software-driven aggregate correction had minimal impact on the results. In general, the guidelines set forth by the 1992 DNA Consensus Conference were used [11,12]. When the DI was <1.3, a single composite SPF for the normal and tumor populations was derived. When the DI was ≥1.3, the SPFs of the overlapping diploid and aneuploid (or tetraploid) populations were modeled and an SPF estimate for each population obtained. In these cases, the SPF for the aneuploid/tetraploid population was assumed to be the most pertinent and was used in all tabulations described. No tumor was multiploid.

### Statistical Analysis

The significance of differences between proportions was assessed using the Chi-square test [13]. The Berkson-Gage method was used to calculate the actuarial curves, and the log rank test was used to calculate statistical significance [14]. The actuarial calculations were begun from the date of pelvic lymphadenectomy.

## RESULTS

From the 33 prostate cancer patients comprising this cohort there were 11 diploid, 4 near-diploid, 10 tetraploid, and 8 aneuploid tumors. The average DI for the aneuploid tumors was 1.55 ± 0.12 (± S.E.) with a median of 1.77 (range 1.12–1.86). Since the number of tumors was too small to evaluate the prognostic significance of near-diploidy, and since previously we found tetraploidy and aneuploidy to reflect the same prognosis [2], the initial analyses involved the pooling of patients with diploid and near-diploid tumors, and the pooling of those with tetraploid and aneuploid tumors. Accordingly, the group referred to as diploid consisted of those with diploid and near-diploid tumors, while the nondiploid group contained those with tetraploid and aneuploid tumors.

Table I shows the relationship of the distribution of patients by DNA-ploidy and other potential prognostic factors. The only factor that correlated with DNA-ploidy was the Gleason score. Of the 15 patients with diploid tumors, there were 4 with local progression,

**TABLE I. Distribution of Patients by DNA-Ploidy and Other Potential Prognostic Factors**

| Factor | Percent (n) | | |
|---|---|---|---|
| | Diploid | Nondiploid | *P* value |
| All patients | 45 (15) | 55 (18) | |
| T-stage | | | |
| T2 | 13 (2) | 22 (4) | |
| T3/T4 | 87 (13) | 78 (14) | 0.51* |
| Gleason score | | | |
| 5 and 6 | 47 (7) | 22 (4) | |
| 7 | 40 (6) | 28 (5) | |
| 8–10 | 13 (2) | 50 (9) | 0.04** |
| Pretreatment PSA | | | |
| ≤20 | 30 (3) | 33 (15) | |
| >20 | 70 (7) | 67 (10) | 0.86* |
| Pretreatment prostatic acid phosphatase | | | |
| ≤0.4 | 47 (7) | 67 (12) | |
| >0.4 ≤0.8 | 33 (5) | 22 (4) | |
| >0.8 | 20 (3) | 11 (2) | 0.27** |
| TURP in T3/T4 | | | |
| No | 69 (9) | 79 (11) | |
| Yes | 31 (4) | 21 (3) | 0.58* |
| SPF | | | |
| ≤0.031 | 67 (10) | 39 (7) | |
| >0.031 | 33 (5) | 61 (11) | 0.11* |

*Chi square.
**Test for linear association.

none with distant progression, 6 with any disease progression, 7 with biochemical progression, and 1 who died. Of the 18 patients with nondiploid tumors, there were 5 with local progression, 2 with distant progression, 6 with any disease progression, 6 with biochemical progression, and 5 who died. Comparisons of the distributions of patients by DNA-ploidy and treatment failure for each endpoint using the chi-square test did not reveal any statistical differences. Univariate actuarial analyses of the association of DNA-ploidy to patient outcome are displayed in Table II. While no statistically significant correlations were evidenced, 100% of those with diploid tumors were alive and 100% were free of disease progression at 4 years, as opposed to 79% and 57%, respectively, of those with nondiploid tumors. Figure 1 illustrates the actuarial curves for disease progression and overall survival. The diploid and nondiploid curves do not begin to separate until 30 months following treatment, which may explain the lack of significance. Of note, the degree of biochemical progression was striking; 78% progressed at 4 years.

The grouping of patients by diploidy and nondiploidy was based on our previous experience [2,5], however, some studies have described that tetraploidy was associated with a significantly better prognosis than was aneuploidy [15]. As a consequence, patients having diploid and tetraploid tumors are many times grouped together [9]. Hence, additional analyses were performed comparing patients with nonaneuploid (diploid + near-diploid + tetraploid) to those with aneuploid tumors. Of the 25 patients with nonaneuploid tumors, there were 6 with local

**TABLE II. Relationship of DNA-Ploidy to Patient Outcome: Diploid vs. Nondiploid†**

| | 4-Year actuarial percent | | |
|---|---|---|---|
| | Diploid | Nondiploid | *P* value* |
| Local progression | 0 | 36 | 0.56 |
| Distant metastasis | 0 | 15 | 0.23 |
| Disease progression | 0 | 43 | 0.40** |
| Biochemical progression | >86 | >65 | 0.79 |
| Overall Survival | 100 | 79 | 0.11 |

†The greater than sign (>) indicates that there were not enough patients at risk to accurately assess.
*Log rank.
**$P = 0.11$ using the Wilcoxon (Gehan) statistic.

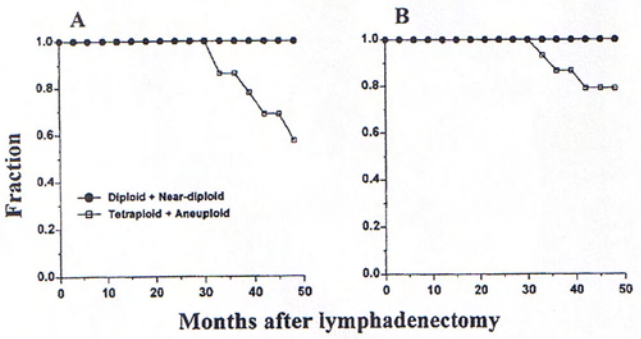


**Fig. 1.** Relationship of DNA-ploidy in terms of diploid (closed circles) vs. nondiploid (open squares), freedom from disease progression (A), and overall survival (B). **A:** Number of patients at risk at 2 and 4 years were 12 and 8 for the diploid group and 17 and 6 for the nondiploid group. **B:** The number of patients at risk were 12 and 8 for the diploid group and 17 and 9 for the nondiploid group.

progression, 1 with distant progression, 8 with any disease progression, 10 with biochemical progression, and 3 who died. Of the eight patients with aneuploid tumors, there were three with local progression, one with distant progression, four with any disease progression, three with biochemical progression, and three who died. The chi-square test did not show any significant differences based on this grouping of DNA-ploidy, although a trend was seen with survival ($P = 0.10$). The distribution of the prognostic factors displayed in Table I was also analyzed, with DNA-ploidy divided into nonaneuploidy and aneuploidy, and no significant relationships were identified using the chi-square test. Table III shows that when actuarial comparisons were made, the division of patients into nonaneuploid and aneuploid tumor groups predicted for disease progression and overall survival. Figure 2 displays the actuarial curves for these data.

The other potential prognostic factors listed in Table I were then examined in univariate analyses using disease progression as the endpoint. None of the factors tested correlated with disease progression; however, a trend was seen with the Gleason score in that there were no disease failures at 4 years when the Gleason score was ≤6, as opposed to 33% failing when the Gleason score was higher ($P = 0.13$). Likewise, none of these factors were significant when overall survival was used as the endpoint. Thus, the only significant prognostic factor for disease progression and overall survival was DNA-ploidy (Table III). Cox proportional hazards analysis was performed for disease progression, including Gleason score (≤6 vs. >6) and DNA-ploidy (nonaneuploid vs. aneuploid) as covariates. DNA-ploidy was the only significant correlate.

The mean and median SPF values were 4.57 ± 0.73 (± S.E.) and 3.06 (range 0–14.7). The mean and median SPFs by DNA-ploidy were: 5.27 ± 1.6 and 2.27 for the diploid tumors, 2.94 ± 1.7 and 1.57 for the near-diploid tumors, 4.76 ± 1.28 and 4.46 for the tetraploid tumors, and 4.20 ± 1.16 and 3.17 for the aneuploid tumors. The median SPF cut-point of 3.1 was chosen to examine the association of the distribution of patients by tumor SPF and other prognostic factors. Table IV reveals that SPF correlated with Gleason score and TURP in patients with stage T3/T4 disease. All of those having an SPF above the median had a Gleason score >6 and a TURP prior to androgen ablation. Since the calculation of SPF is compromised somewhat when the nondiploid population overlaps the diploid population due to extrapolation errors, this analysis was repeated using only those with diploid tumors. For these patients, SPF correlated only with TURP. Table V shows the prognostic value of SPF to various disease endpoints using the entire study group. SPF was not predictive of any of the endpoints tested. There were not enough patients with diploid tumors to restrict such actuarial analyses to this subset.

We recently reported that, for patients with stage T1–T4 prostate cancer, PSA-doubling time (PSADT) correlated significantly with DNA-ploidy [16]. In the series described here, there were 13 patients with biochemical progression. The PSADTs from these patients were not significantly different when divided by DNA-ploidy. The average PSADT was 8.8 months for both the nonaneuploid and aneuploid groups.

## DISCUSSION

Previously we established that DNA-ploidy was an independent predictor of biochemical or disease failure in patients with clinical stage T1–T4 prostate cancer who were treated with definitive radiotherapy [2]. The results of this study typify the majority of reports in which radical prostatectomy was the mode of treatment. Other studies, particularly those in which patients were treated solely with androgen ablation, failed to confirm that this association was independent of other prognostic factors in multivariate analyses [6,17,18]. In fact, some have described that SPF was more significantly correlated with patient outcome than DNA-ploidy. Since the available data concerning the prognostic significance of DNA content measurements for patients with stage D1 prostate cancer treated with androgen ablation are limited [9], we examined our patient population to better define these relationships.

Although the parent cohort consisted of 181 stage D1 patients [10], we were only able to obtain suffi-

**TABLE IV. Distribution of Patients by SPF and Other Potential Prognostic Factors**

| Factor | Percent (n) SPF ≤0.031 | Percent (n) SPF >0.031 | *P* value |
|---|---|---|---|
| All patients | 52 (17) | 48 (16) | — |
| T-stage | | | |
| T2 | 24 (4) | 12 (2) | |
| T3/T4 | 76 (13) | 87 (14) | 0.41* |
| Gleason score | | | |
| 5 and 6 | 65 (11) | 0 (0) | |
| 7 | 18 (3) | 50 (8) | |
| 8–10 | 18 (3) | 50 (8) | 0.0004* |
| Pretreatment PSA | | | |
| ≤20 | 36 (5) | 27 (3) | |
| >20 | 64 (9) | 73 (8) | 0.65* |
| Pretreatment prostatic acid phosphatase | | | |
| ≤0.4 | 53 (9) | 63 (10) | |
| >0.4 ≤0.8 | 29 (5) | 25 (4) | |
| >0.8 | 18 (3) | 12 (2) | 0.57** |
| TURP in T3/T4 | | | |
| No | 100 (13) | 50 (7) | |
| Yes | 0 (0) | 50 (7) | 0.003* |

*Chi square.
**Test for linear association.

**TABLE V. Relationship of SPF to Patient Outcome†**

| Endpoint | 4-Year actuarial percent SPF ≤0.031 | 4-Year actuarial percent SPF >0.031 | *P* value* |
|---|---|---|---|
| Local progression | 10 | 28 | 0.70 |
| Distant metastasis | 0 | 15 | 0.22 |
| Disease progression | 10 | 35 | 0.49 |
| Biochemical progression | >72 | >85 | 0.33 |
| Overall survival | 82 | 91 | 0.95 |

†The greater than sign (>) indicates that there were not enough patients at risk to accurately determine the actuarial percentage.
*Log rank.

association closer to the unfavorable outcome of patients with aneuploid tumors.

## CONCLUSIONS

In summary, DNA-aneuploidy in patients with regionally localized node-positive (stage D1) prostate cancer who are treated with androgen ablation alone have reduced freedom from disease progression and survival rates as compared to patients with nonaneuploid tumors. The finding that the biochemical progression rates were not associated with DNA-ploidy suggests that the major difference between the nonaneuploid and aneuploid patients was in the rapidity of growth and dissemination. Since there is evidence that local therapy, such as radiotherapy [19], substantially reduces biochemical progression rates in these patients, some may be cured with multimodality treatment.

## ACKNOWLEDGMENTS

The authors thank Ms. Joan Day, Department of Radiotherapy, for typing the manuscript. This study was supported in part by grants CA 06294 and CA 16672 awarded by the National Cancer Institute, U.S. Department of Health and Human Services, an American Cancer Society Career Development

Award (A.P.), and the Katherine T. Unsworth Lead Annuity Trust.

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