0022-5347/90/1446-1404$02.00/0
THE JOURNAL OF UROLOGY
Copyright © 1990 by AMERICAN UROLOGICAL ASSOCIATION, INC.
Vol. 144, December
Printed in U.S.A.

THE PROGNOSTIC VALUE OF MODAL DEOXYRIBONUCLEIC ACID IN LOW GRADE, LOW STAGE UNTREATED PROSTATE CANCER

JAN ADOLFSSON, LARS RÖNSTRÖM, PER-OLOV HEDLUND, TORSTEN LÖWHAGEN, JOHN CARSTENSEN AND BERNHARD TRIBUKAIT

From the Departments of Urology, Pathology (Section for Cytology) and Medical Radiobiology, Karolinska Hospital, Stockholm, and Department of Oncology, University Hospital, Linköping, Sweden

ABSTRACT

We selected for a prospective surveillance study 167 patients with untreated grades 1 and 2 prostate cancer. The tumors were classified regarding modal deoxyribonucleic acid value (ploidy) by flow cytometry, cytological grade by transrectal fine needle aspiration biopsy and local tumor stage. Of the patients 146 could be evaluated. Mean followup was 50 months.

The initial ploidy was statistically correlated to cytological grade but not to initial local tumor stage, prostatic acid phosphatase activity or patient age. Initial ploidy and cytological grade had a prognostic value regarding local tumor progression when considered as single predictors and in combination. Two patients with diploid and 8 with nondiploid tumors initially had metastases during the surveillance. Five patients (1 with diploid and 4 with nondiploid disease) died of prostatic cancer. Modal deoxyribonucleic acid value and cytological grade were of prognostic value in untreated prostate cancer. (*J. Urol., 144: 1404–1407, 1990*)

The natural course of prostate cancer is highly variable. Several parameters have been evaluated in attempts to predict the course of the disease. The tumor grade, assessed either by histopathology or cytology studies, and local tumor stage are the basic prognostic parameters used, although the significance of these parameters has been challenged.[1] The modal deoxyribonucleic acid (DNA) value of prostate cancer cells also has been suggested to be of prognostic value. Single cell studies on slides and flow cytometry studies during recent years have correlated a nondiploidy of the prostate cancer cells to a progressive anaplasia and a more advanced local tumor stage.[2] In some investigations a prognostic significance of the DNA value was found,[3, 4] while in others the additional value of the DNA value to that of tumor stage and grade has been questioned.[5] In these studies the course of the prostate cancer was influenced by different types of treatment. We report the results of followup in a selected group of untreated patients with prostate cancer during surveillance to assess the prognostic value of the modal DNA value.

MATERIAL AND METHODS

From 1978 to 1982 all patients with newly diagnosed prostate cancer at the department of urology of our hospital were characterized with respect to the modal DNA value, cytological grade, local tumor stage and status of metastatic lesions. In 4 patients the observation was started before 1978. The diagnosis of cancer was made by transrectal fine needle aspiration biopsy in all patients. The tumors were classified as well (grade 1), moderately (grade 2) and poorly (grade 3) differentiated according to the criteria described by Esposti.[6] The material used for DNA analysis was obtained by fine needle aspiration biopsy at the same time as specimens for the cytological diagnosis. The modal DNA value was assessed by flow cytometry performed by our routine method described previously.[7, 8] The DNA content in the tumor cells was classified as diploid (normal, 2c), tetraploid (4c) or nontetraploid aneuploid.

The local tumor category was assessed by digital rectal examination and classified according to the International Union Against Cancer.[9] The digital rectal examination finding was described in a standard drawing in the patient record. Skeletal metastases were assessed with isotope scanning. Prostatic acid phosphatase (PAP) activity was analyzed by a tartrate inhibited enzymatic assay. Patients with normal bone scan and normal value of PAP were considered to be without metastasis (M0). The PAP values were characterized as low normal (less than 50% of upper normal value) and high normal (50 to 100% of upper normal value).

Of a large number of patients with newly detected tumors 167 with low stage, low grade tumors without metastases were selected for close surveillance without treatment. On review of the records of these patients 21 had either failed followup (11) or the records were incomplete (10). Thus, 146 patients with a median age of 68 years (range 38 to 89 years) could be evaluated. Initially, 69 patients had diploid, 68 tetraploid and 9 nontetraploid aneuploid tumors. Since there were only 9 patients with nontetraploid aneuploid tumors and the clinical course did not differ from those with tetraploid tumors, these groups were combined in the analysis. The majority of the tumors were grade 1 (71%) and of category T2 (67%, table 1).

The patients were followed with digital rectal examination and analysis of PAP every 3 to 6 months. In the majority of patients followup was performed by 2 physicians. Bone scan, transrectal fine needle aspiration biopsy for cytology and flow cytometric analysis were performed every 12 to 18 months. A positive bone scan was required to classify the tumor as metastasized during followup of the patient. Patients with rapid local progression and/or metastatic disease received antitumoral treatment.

Evaluation of the patient records was done by 1 of the investigators in October 1986. Significant local tumor progression was defined as a 25% or greater increase in the largest diameter of the palpable tumor infiltrate as judged from the statements and serial standard drawings of the tumor in the patient record. The flow cytometry results were not available to us at evaluation of the patient records. The analysis in our series regards the period during which the patients were kept under surveillance. End points in the analysis of the study were significant local progression of the tumor or development of distant metastases.

The prognostic value of the different variables determined at diagnosis was analyzed by Cox's proportional hazards regres-

Accepted for publication November 22, 1989.
Supported by Grants from the Research Foundation of Karolinska Institute, Cancer Society in Stockholm and King Gustav V. Jubilee Foundation, Stockholm, Sweden.

TABLE 1. *Relations of DNA ploidy and cytological grade to other variables*

Variable Category	No. Pts.	% Nondiploid	Statistical Significance (p value)	% Moderately Differentiated	Statistical Significance (p value)
Grade:					
Well differentiated	104	46		—	
Moderately differentiated	42	69	0.012	—	—
Tumor stage:					
T1	13	38		8	
T2	98	50		30	
T3	35	66	0.056*	34	0.12*
PAP:					
Low normal	122	52		29	
High normal	24	54	0.88	29	0.96
Pt. age (yrs.):					
<70	80	46		25	
≧70	66	61	0.085	33	0.27

* Test for trend.

sion model.[10] Rate ratios were estimated as e^{β} and 95% confidence intervals at $e^{\beta} \pm 1.96 \times$ S. E., where β is the regression coefficient of the Cox model and S. E. is the standard error of the regression coefficient. The cumulative probability of not progressing was estimated and plotted with the actuarial method described by Berkson and Gage.[11] The patients dead of intercurrent disease also were included in the analysis but were considered as censored data.

RESULTS

During a median observation of 50 months (range 6 to 135 months) 99 patients (64%) remained untreated while 57 (36%) received treatment due to either rapid local progression or development of metastasis. Patients with no treatment throughout the observation period were observed for a median of 56 months (range 7 to 135 months), while the median observation without treatment for the patients who were subsequently treated was 39 months (range 6 to 89 months).

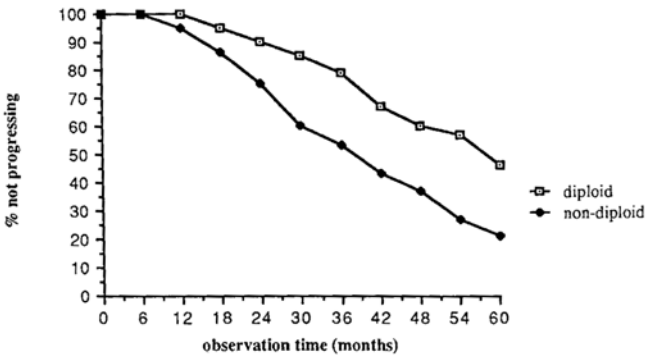
Of the 146 patients observed 77 (53%) had locally progressive disease and 10 (7%) had metastases while still untreated. Of the patients with metastatic disease 2 had initially diploid and 8 had nondiploid tumors. In addition, 5 patients had metastatic disease after treatment had been initiated due to rapid local progression. Five patients (3%) died of prostate cancer, while 24 (16%) died of disease unrelated to prostate cancer during the observation period. One patient who died of prostate cancer had a diploid tumor while the other 4 had nondiploid tumors initially. Of the patients who died of intercurrent disease 5 had local tumor progression before death, while none had metastasis. The numbers of patients who had metastatic disease and died of prostate cancer were too low to permit statistical evaluation.

Initially diploid tumors were predominantly grade 1, while nondiploid tumors were grade 2 and this correlation was statistically significant (table 1). However, initial local tumor stage, PAP or patient age was not correlated with initial ploidy.

A total of 33 patients with diploid and 44 with nondiploid tumors had locally progressive disease. The median interval to local progression in the diploid group was 58 months, compared to 37 months in the nondiploid group (see figure). This difference was statistically significant (table 2). Also, initial cytological grade had a statistically significant prognostic value regarding locally progressive disease (table 2). Further analyses by which the variables were compared to each other according to the multiple Cox regression analysis model, adjusting for the correlation between the variables, still showed a significant prognostic value of modal DNA values and initial cytological grade (table 3).

DISCUSSION

The majority of the patients studied had stage T2, grades 1 to 2 tumors, that is low stage, low grade cancers with a supposed generally good prognosis. All patients were initially without



Relative number of patients with diploid and nondiploid untreated prostate cancer not progressing in relation to observation interval. Numbers of patients at risk at 0, 12, 24, 36, 48 and 60 months were 69, 63, 51, 41, 24 and 16, respectively, for patients with diploid disease, and 77, 68, 45, 29, 17 and 6, respectively, for patients with nondiploid disease.

TABLE 2. *Simple Cox's regression analyses of the relations of disease progression to DNA ploidy, cytological grade and local tumor stage at diagnosis in patients with untreated prostate cancer*

Variable Category	Rate Ratio*	95% Confidence Interval	Statistical Significance (p value)
DNA ploidy:			
Diploid	1.00	—	
Nondiploid	1.89	1.2–3.0	0.006
Grade:			
Well differentiated	1.00	—	
Moderately differentiated	2.02	1.2–3.4	0.009
Tumor stage:			
T1	1.00	—	
T2	1.78	0.8–4.2	
T3	2.24	0.9–5.8	0.10†

* Ratio of the rate of patients with progression in each category to that in the reference category (the first category for each variable).
† Test for trend.

known metastases. Therefore, our investigation can be considered to be biased towards patients with a basically good prognosis regarding the initial cytological grade and the local tumor stage. This also is reflected by the outcome of the followup, with a low rate of metastatic disease and a low number of patients who died of prostate cancer. With respect to the modal DNA values at diagnosis 47% were diploid, 47% tetraploid and 6% nontetraploid aneuploid. In an earlier study by our group including 500 unselected patients with prostate cancer 31% had diploid, 44% tetraploid and 25% aneuploid disease.[8]

In several earlier investigations of prostate cancer diploid tumors have been found to be predominantly well differentiated and aneuploid tumors to be poorly differentiated when the grade of the cancer was assessed cytologically[2, 7, 12] and histo-

TABLE 3. *Multiple Cox's regression analysis of the relations of disease progression to DNA ploidy, cytological grade and local tumor stage at diagnosis in patients with untreated prostate cancer*

Variable Category	Rate Ratio*	95% Confidence Interval	Statistical Significance (p value)
DNA ploidy:			
Diploid	1.00	—	
Nondiploid	1.77	1.1–2.8	0.018
Grade:			
Well differentiated	1.00	—	
Moderately differentiated	1.99	1.2–3.4	0.013
Tumor stage:			
T1	1.00	—	
T2	1.32	0.6–3.2	
T3	1.70	0.6–4.5	0.25†

* Adjusted for other variables listed, as well as PAP and patient age.
† Test for trend.

pathologically.[13] A similar correlation between the ploidy and cytological grade of the cancer was found in our study. On the contrary, 2 recent studies found no correlation between the histopathological grade and ploidy of the prostate cancer.[14, 15] However, these studies were based on previously paraffin-embedded material, while the studies showing a correlation were made on fresh material. Large volume of the local tumor also has been correlated with aneuploidy of the prostate cancer.[2, 13] The same trend is seen in our study, which, however, did not reach significance probably due to the low number of stages T1 and T3 tumors. In metastasized prostate cancer the primary tumor has been shown previously to be aneuploid in the majority of cases.[8, 13] In our study the majority of the patients with metastases during the observation period had aneuploid tumors. Therefore, it seems as if the degree of aneuploidy of prostate cancer is correlated with at least the cytological grade and T category of the tumor, and presumably also with its metastatic potential.

Previous studies on the prognostic significance of the DNA pattern of prostate cancer have shown a correlation between nondiploidy and shorter survival of the patients.[2, 4, 14–18] The combination of Gleason grading and DNA content of the prostate cancer has been suggested to be superior to either of the parameters alone as a prognostic indicator.[17, 19] In all the aforementioned studies the patients had received some type of antitumoral treatment. In our investigation the number of deaths of prostate cancer was too low to allow any evaluation regarding the prognostic value of the DNA content in relation to the survival of the patients. However, with respect to locally progressive disease, we found a significant prognostic value of the modal DNA value at diagnosis as a single variable and also in combination with cytological grade. Only 10 patients had metastatic disease during the surveillance interval without treatment and 8 of them had a nondiploid tumor initially.

Prostate cancer of the diploid type apparently is less aggressive than nondiploid cancer. However, diploid tumors also are progressive, although more slowly. Whether progression of diploid tumors is combined with a change in the modal DNA values is not yet fully evaluated.

REFERENCES

1. Whitmore, W. F., Jr.: Natural history and staging of prostate cancer. Urol. Clin. N. Amer., **11:** 205, 1984.
2. Tribukait, B.: Flow cytometry in assessing the clinical aggressiveness of genitourinary neoplasms. World J. Urol., **5:** 108, 1987.
3. Tavares, A. S., Costa, J. and Costa Maia, J.: Correlation between ploidy and prognosis in prostatic carcinoma. J. Urol., **109:** 676, 1973.
4. Zetterberg, A. and Esposti, P.-L.: Prognostic significance of nuclear DNA levels in prostatic carcinoma. Scand. J. Urol. Nephrol., suppl., **55:** 53, 1980.
5. Ritchie, A. W. S., Dorey, F., Layfield, L. J., Hannah, J., Lovrekovich, H. and deKernion, J. B.: Relationship of DNA content to conventional prognostic factors in clinically localised carcinoma of the prostate. Brit. J. Urol., **62:** 254, 1988.
6. Esposti, P.-L.: Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. A five-year follow-up study of 469 hormone-treated patients. Scand. J. Urol. Nephrol., **5:** 199, 1971.
7. Tribukait, B., Rönström, L. and Esposti, P.-L.: Quantitative and qualitative aspects of flow DNA measurements related to the cytologic grade in prostatic carcinoma. Anal. Quant. Cytol., **5:** 107, 1983.
8. Tribukait, B.: Flow cytometry in surgical pathology and cytology of tumors of the genito-urinary tract. In: Advances in Clinical Cytology. Edited by L. G. Koss and D. V. Coleman. New York: Masson Publishers USA Inc., vol. 2, chapt. 8, p. 163, 1984.
9. Harmer, M. H.: TNM Classification of Malignant Tumours, 3rd ed. Geneva: International Union Against Cancer, 1978.
10. Cox, D. R.: Regression models and life tables. J. Roy. Stat. Soc., **34:** 187, 1972.
11. Berkson, J. and Gage, R. P.: Calculation of survival rates for cancer. Proc. Staff Meet. Mayo Clin., **25:** 270, 1950.
12. Zetterberg, A. and Esposti, P.-L.: Cytophotometric DNA-analysis of aspirated cells from prostatic carcinoma. Acta Cytol., **20:** 46, 1976.
13. Frankfurt, O. S., Chin, J. L., Englander, L. S., Greco, W. R., Pontes, J. E. and Rustum, Y. M.: Relationship between DNA ploidy, glandular differentiation, and tumor spread in human prostate cancer. Cancer Res., **45:** 1418, 1985.
14. Stephenson, R. A., James, B. C., Gay, H., Fair, W. R., Whitmore, W. F., Jr. and Melamed, M. R.: Flow cytometry of prostate cancer: relationship of DNA content to survival. Cancer Res., **47:** 2504, 1987.
15. Lundberg, S., Carstensen, J. and Rundquist, I.: DNA flow cytometry and histopathological grading of paraffin-embedded prostate biopsy specimens in a survival study. Cancer Res., **47:** 1973, 1987.
16. Winkler, H. Z., Rainwater, L. M., Myers, R. P., Farrow, G. M., Therneau, T. M., Zincke, H. and Lieber, M. M.: Stage D1 prostatic adenocarcinoma: significance of nuclear DNA ploidy patterns studied by flow cytometry. Mayo Clin. Proc., **63:** 103, 1988.
17. Fordham, M. V. P., Burdge, A. H., Matthews, J., Williams, G. and Cooke, T.: Prostatic carcinoma cell DNA content measured by flow cytometry and its relation to clinical outcome. Brit. J. Surg., **73:** 400, 1986.
18. Lee, S. E., Currin, S. M., Paulson, D. F. and Walther, P. J.: Flow cytometric determination of ploidy in prostatic adenocarcinoma: a comparison with seminal vesicle involvement and histopathological grading as a predictor of clinical recurrence. J. Urol., **140:** 769, 1988.
19. Pontes, J. E., Wajsman, Z., Huben, R. P., Wolf, R. M. and Englander, L. S.: Prognostic factors in localized prostatic carcinoma. J. Urol., **134:** 1137, 1985.

EDITORIAL COMMENTS

The authors ask whether active surveillance is an appropriate treatment option for patients with localized carcinoma of the prostate and whether information about DNA ploidy can be used to help make this decision. It should be emphasized that active surveillance does not mean that the patient remains untreated but, rather, that therapy is delayed until there is evidence of progression.

To put these questions into perspective, Johannson and associates recently reported that of 148 patients with grade 1 carcinoma of the prostate who were followed without treatment for 5 years 28 had progression and 4 (2.5%) died of carcinoma of the prostate.[1] In their study the local progression rate for grade 2 lesions was 47% and 10 patients (10.6%) died of carcinoma of the prostate.

This study differs from that of Johannson and associates in that the authors do not find differences in behavior between grades 1 and 2 tumors. However, they have confirmed previous suggestions in the literature regarding the prognostic advantage of having diploid localized prostate cancer. In this study 69 patients had diploid tumors, with 48% of these experiencing local progression. The mean interval to progression was 58 months. To date, only 2 patients (3%) have had metastatic disease and only 1 (1.5%) has died of carcinoma of the prostate. In contrast, of 77 patients with aneuploid tumors 57% experienced local progression and did so at a more rapid rate, with a mean interval of 37 months. To date 8 of the patients with aneuploid tumors (10.3%) have had metastatic disease and 4 (5%) have died of prostatic cancer.

If we consider the findings of both studies 290 patients have been reported on with localized prostatic cancer who were managed initially by active surveillance. For the combined group, at approximately 5 years of followup only 3.5% died of carcinoma of the prostate. Of these patients only 1.5% with diploid disease died. Therefore, I believe that it may be appropriate to compare active surveillance to immediate therapy for patients with low volume, low grade diploid prostatic cancer. It can be questioned whether a 3.4% cancer death rate seen in 290 patients within 5 years warrants that all such patients should undergo immediate radical prostatectomy or radiation therapy.

Ralph W. deVere White
Department of Urology
University of California, Davis
School of Medicine
Davis, California

1. Johannson, J.-E., Adami, H.-O., Andersson, S.-O., Bergstrom, R., Krusemo, U. B. and Kraaz, W.: Natural history of localised prostatic cancer. Lancet, **2:** 799, 1989.

In this most important report the authors have characterized, by flow cytometric analysis of fine needle aspiration specimens, the tumor DNA ploidy values of a large group of patients with low grade and clinically low stage prostatic cancer. The patients then were kept under close surveillance but received no active therapy until the tumor progressed. To my knowledge this is the only report in which DNA ploidy values are available for a large group of untreated patients with early prostate cancer. This design makes this study particularly valuable because it permits comparison with series of prostate cancer patients in which ploidy patterns have been investigated but in which the patients received some form of early active treatment, either radical prostatectomy or radiotherapy.

Of high significance for the future management of patients with low grade, low stage prostate cancer is the unique observation that patients with low grade, low stage, DNA diploid tumors did, in fact, have local progression and, occasionally, metastasis when left untreated. Indeed, slightly more than 50% of the DNA diploid tumor patients had tumor progression after 5 years of observation (see figure in article). Patients with diploid tumors did have more slowly growing tumors than those with DNA nondiploid disease but the DNA diploid tumors did eventually progress if left untreated.

By contrast, our research group recently reported analyses of the prognostic importance of DNA ploidy patterns for patients with apparently localized prostate cancer treated by radical prostatectomy.[1,2] In these series patients with histologically low grade, DNA diploid tumors have done remarkably well and have rarely had tumor progression with prolonged followup. For example, for a group of patients with low grade, DNA diploid, pathological stage C prostate cancer treated by radical prostatectomy only an 8% progression rate (local or systemic) was noted at 10 years postoperatively.[1] The current authors provide an untreated control arm. Together, such analyses suggest that radical prostatectomy does provide some therapeutic advantage for patients with low grade DNA diploid tumors treated by radical prostatectomy.

Finally, the multivariate analysis indicates that cytological grade and DNA ploidy status are independent and important prognostic variables for patients with early prostate cancer that is left untreated. We have observed similar results for patients with pathological stages B and C prostate cancer treated by radical prostatectomy.[1,2] The human pathologist and the flow cytometer provide synergistic, independent and highly important information about prognosis for patients with early prostate cancer. Tumor grade (or Gleason score) and DNA ploidy pattern currently must be considered in clinical research studies of patients with early prostate cancer and in the current routine clinical management of such patients as well.

Michael M. Lieber
Department of Urology
Mayo Clinic
Rochester, Minnesota

1. Nativ, O., Winkler, H. Z., Raz, Y., Therneau, T. M., Farrow, G. M., Myers, R. P., Zincke, H. and Lieber, M. M.: Stage C prostatic adenocarcinoma: flow cytometric nuclear DNA ploidy analysis. Mayo Clin. Proc., **64:** 911, 1989.
2. Montgomery, B. T., Nativ, O., Blute, M. L., Farrow, G. M., Myers, R. P., Zincke, H., Therneau, T. M. and Lieber, M. M.: Stage B prostate adenocarcinoma: flow cytometric nuclear DNA ploidy analysis. Arch. Surg., in press.