MEASUREMENTS OF DNA AS A PROGNOSTIC FACTOR IN PROSTATIC CARCINOMA*

James B. Amberson[1], Leopold G. Koss
Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, New York and [1]Department of Pathology, The New York Hospital-Cornell University Medical Center, New York, New York

INTRODUCTION

Adenocarcinoma of the prostate is the second most common form of clinically diagnosed cancer in American men, being exceeded only by lung cancer [1]. Numerous studies have documented the wide range in biologic behavior displayed by these neoplasms: some are indolent growths of no consequence to the patient, while others are highly aggressive cancers that metastasize widely [2-5]. It is, therefore, of great clinical importance to be able to predict the likely natural history of the disease in the individual patient in order to insure that the morbidity attendant the various treatment options is outweighed by their curative or palliative potential. The value of radical prostatectomy or orchiectomy is uncertain in those cases in which the probability of progression is exceeded by the probability of death from an unrelated disorder.

The most useful guide to patient treatment is clinical stage. Tumors confined to the prostate (Stages A and B) may be cured by radical prostatectomy. For more advanced lesions, radiation and/or hormonal therapy, and occasionally surgery are the main therapeutic options. Despite the importance of clinical staging, considerable variability in aggressiveness occurs even among tumors of the same stage. No doubt some of this variability reflects inaccuracies in clinical staging; however, it also appears to reflect innate differences in biologic behavior. In an effort to measure these differences, histologic and cytologic grading have been proposed. The most widely used histologic grading system is that of Gleason [6]. It has been shown to be of prognostic value, particularly when combined with stage. Cytologic grading, as advocated by Esposti [7] is taking on increasing importance as fine needle aspiration becomes more widely employed as a diagnostic modality. There are as yet no studies comparing the prognostic utility of cytologic versus histologic grading. Both types of grading suffer from their inherently subjective nature and relatively poor reproducibility by multiple observers [8].

Various objective methods of assessing aggressive potential in prostate cancer have been proposed, most notably the measurement of nuclear DNA content using cytophotometry and flow cytometry. Cytophotometric studies by Zetterberg and Esposti [9,10] have shown a correlation between aneuploid DNA content and aggressive behavior. Using flow cytometry Tribukait [11] was able to show a strong correlation between stage and DNA ploidy: 80% of low stage (T1) tumors were diploid, while over 90% of advanced tumors (T3 or T4) were tetraploid or aneuploid. More recent retrospective flow cytometric studies using archival paraffin-embedded tissue provide additional evidence for the prognostic significance of DNA content in prostate cancer [12,13].

---

*Supported by Grant CA1-34790 from the National Cancer Institute of the National Institutes of Health

Published 1989 by Elsevier Science Publishing Co., Inc.
Prognostic Cytometry and Cytopathology of Prostate Cancer
James P. Karr, Donald S. Coffey, and William Gardner, Jr., Editors

In this paper we present the preliminary findings from two simultaneous studies: a prospective study, based mainly on flow cytometry of fresh prostatic tissue and prostatic aspirates with emphasis on low stage tumors, and a retrospective study based on image cytometry of prostatic aspirates, the emphasis here being on high stage tumors.

METHODS

In the prospective study, material for flow cytometry was obtained from 2 different sources: surgically removed prostate tissue and aspirates performed as a diagnostic procedure on patients who exhibited a palpatory prostatic abnormality. The diagnosis of cancer was confirmed by either tissue biopsy and/or cytologic examination of the fine needle aspirates. All material was processed according to methods described in detail elsewhere [14]. Briefly, tissue samples or aspirated material was placed in CASC buffer (0.09M citric acid, 0.01M sodium citrate) and in the case of the tissue samples, minced with a scalpel blade. The material was sedimented and the pellet incubated in CASC for 20 minutes at room temperature with periodic agitation at 5 minute intervals. The resulting nuclei were filtered through 30 micron nylon mesh, washed 3 times in phosphate buffered saline (PBS), and counted with a hemocytometer. Samples were stained with propidium iodide (0.05 mg/ml PI in 0.1% sodium citrate at pH 7.0) after preincubation with RNAse, and then analyzed on an Ortho ICP-22A flow cytometer. Calf thymocytes admixed with each specimen served as internal controls.

In the retrospective study, material for image cytometry was obtained from fine needle prostatic aspirates diagnostic of carcinoma and confirmed by subsequent tissue biopsy. The Papanicolaou stained aspirates were restained with the pararosaniline Feulgen method [15] and DNA measured using the Cell Analysis Systems image cytometer. Three sets of controls were processed with each staining run: trout red blood cells, rat hepatocytes, and benign prostatic epithelial cells.

All DNA histograms from either flow or image cytometry were classified as either diploid or nondiploid. Nondiploid histograms were defined as those with a distinct aneuploid peak (DNA index not equal to 1.00) and those with tetraploid peaks containing more than 7% of the total cell population.

Clinical information was obtained from the review of hospital records and contact with the patient's physician. Staging was based on the Whitmore-Jewett system [16].

RESULTS

A total of 306 samples were successfully analyzed by flow cytometry in the prospective study (Table 1). Of these 306, 64 were from patients with adenocarcinoma, 47 of which have been fully evaluated in terms of clinical stage. As shown in Table 2, 38/47 (81%) of these tumors were confined to the prostate at the time of diagnosis (Stages A and B). Most (37/47 or 79%) were also diploid. Of the Stage A and B tumors alone, 32/38 (86%) were diploid. Only 7 Stage D tumors were encountered in this study; however, 4 of these were nondiploid.

Seventy-five cases have been analyzed in the retrospective image cytometry study, 68 of which have been evaluated regarding clinical stage (Table 3). In contrast to the prospective study, the majority (40/68 or 59%) of these cases were Stage D at the time of diagnosis (Table 4). Most (51/68 or 75%) were nondiploid. Of the tumors confined to the prostate (1 Stage A and 19 Stage B), 7/20 (35%) were diploid compared with 10/48 (21%) of the Stage C and D tumors.

Table 1. Prospective Flow Cytometric Study

Total Adequate Samples Studied	306
Carcinomas	64
Fully Evaluated	47

Table 2. Prospective Flow Cytometric Study

	DNA Ploidy		
Stage	Diploid	Nondiploid	Total
A	18	4	22
B	14	2	16
C	2	--	2
D	3	4	7
Total	37	10	47

Table 3. Retrospective Image Cytometric Study

Total Carcinomas Studied	75
Fully Evaluated	68

Table 5 is a composite from both the retrospective and prospective studies. The tumors are nearly equally divided between those with diploid versus nondiploid DNA content. There is a strong correlation between stage and DNA ploidy: 83% of Stage A tumors are diploid compared with only 26% of Stage D tumors.

DISCUSSION

Our results are in general agreement with the work of others, most notably that of Tribukait [11], in that there is a strong correlation between DNA ploidy and tumor stage. Of note is the difference in the 2 patient populations on which we are reporting. The retrospective study represents a relatively unselected group of patients with clinical disease encountered over a period of several years. All of the carcinomas were diagnosed by fine needle aspiration performed as a routine diagnostic procedure. As is the general experience in this country, many of these patients had advanced disease at the time of diagnosis [17]. The cases in the prospective study represent a separate group of patients, many of whom had clinically unsuspected cancers. A large proportion of these tumors were discovered incidentally in prostate tissue removed from presumed benign hyperplasia. It is not surprising, therefore, that the relative numbers of nondiploid tumors should

Table 4. Retrospective Image Cytometric Study

	DNA Ploidy		
Stage	Diploid	Nondiploid	Total
A	1	--	1
B	6	13	19
C	1	7	8
D	9	31	40
Total	17	51	68

Table 5. Combined Data From Prospective Flow Cytometric and Retrospective Image Cytometric Studies

	DNA Ploidy		
Stage	Diploid(%)	Nondiploid (%)	Total (%)
A	19 (83)	4 (17)	23 (20)
B	20 (57)	15 (43)	35 (30)
C	3 (30)	7 (70)	10 (9)
D	12 (26)	35 (74)	47 (41)
Total (%)	54 (47)	61 (53)	115

differ so dramatically in these two very different patient populations, one characterized by a large number of advanced tumors and the other consisting in large part of small, incidentally discovered lesions.

A number of autopsy studies have shown that the incidence of small clinically undetected prostate cancers such as were found in our prospective study is extraordinarily common. Fully half (and in all likelihood even more) or all men past age 80 will have at least one focus of adenocarcinoma in their prostate (Table 6) [2]. Clinically diagnosed carcinoma of the prostate, ie. Stage B or greater, represents only a small fraction of all prostate cancers, the majority going undetected during the patient's lifetime. Since the majority of our Stage A cancers were diploid, it is reasonable to assume that the same also holds true for most undiagnosed Stage A tumors. From this assumption it follows that taking all prostate cancers as a whole, that is, both the clinically detected and the clinically undetected, most prostate cancers are diploid and remain confined to the gland during the patient's lifetime. It also follows that, since most high stage tumors are nondiploid while very few Stage A tumors are, nondiploid tumors are less likely to remain confined to the prostate than their diploid counterparts.

Nondiploid tumors may be more aggressive than diploid tumors, but this distinction takes on less significance with advancing stage. As stage increases, there are fewer diploid tumors and more nondiploid ones. Presumably, these

Table 6. Mean Frequency of Prostatic Carcinoma at Autopsy: Relationship to Age (Step-section Microscopic Examination

Age	Mean % of Autopsies
40-49	8
50-69	22
70-79	37
80+	53

After Peterson [2]

changing frequencies reflect the larger fraction of nondiploid tumors, as compared with diploid tumors, that are capable of progression. Those diploid tumors encountered in more advanced stages represent the selected few whose aggressive potential is comparable to that of nondiploid tumors. There may still be some differences in the rate of progression between advanced diploid and nondiploid prostate cancers, but they are not likely to be as striking as in the lower stages where the population of diploid cancers is less selected. One recent retrospective study of Stage D1 tumors has, in fact, documented somewhat more rapid progression of nondiploid tumors at even this advanced stage [13]. Similar studies of low stage tumors are urgently needed.

The data reported in this paper suggests that nondiploid prostate cancers are more aggressive than diploid tumors and are more likely to present in advanced stages. Some diploid tumors, however, are capable of aggressive behavior. One major task for the future will be to identify those low stage diploid tumors which are likely to progress.

REFERENCES

1. Seidman, H., M.H. Mushinski, S.K. Gelb, and E. Silverberg. Probabilities of eventually developing or dying of cancer-United States. Cancer 35:36-56, 1985.

2. Peterson, R.O. (ed.). Urologic Pathology. Philadelphia: J.B. Lippincott Company, pp. 615, 1986.

3. Catalona, W. and W.W. Scott. Carcinoma of the Prostate. In: J. H. Harrison, R.F. Gittes, A.D. Perlmutter, T.A. Stamey, and P.C. Walsh (ed.). Campbell's Urology, Vol. 2, 4th Ed., Philadelphia: W.B. Saunders Company, pp. 1085-1124, 1979.

4. Grayhack, J.T. and D.G. Assimos. Prognostic significance of tumor grade and stage in the patient with carcinoma of the prostate. The Prostate 4:13-31, 1983.

5. Gleason, D.F. and the Veterans Administration Cooperative Urologic Research Group. Histologic Grading and Clinical Staging of Prostatic Carcinoma. In: Myron Tannenbaum (ed.), Urologic Pathology: The Prostate, Philadelphia: Lea & Febiger, pp. 171-197, 1979.

6. Gleason, D.F., G.T. Mellinger, and the Veterans Administration Cooperative Urological Research Group. Prediction of prognosis for prostatic adenocarcinoma combined histologic grading and clinical staging. J. Urol. 111:58-64, 1974.

7. Esposti, P.L. Cytologic malignancy grading of prostatic carcinoma by transrectal aspiration biopsy. Scand. J. Urol. Nephrol. 5:199-209, 1971.

8. Murphy, G.P. and W.F. Whitmore. A report of the workshops on the current status of the histologic grading of prostate cancer. Cancer 44:1490-1494, 1979.

9. Zetterberg, A. and P.L. Esposti. Cytophotometric DNA-analysis of aspirated cells from prostatic carcinoma. Acta. Cytol. 20:46-57, 1976.

10. Zetterberg, A. and P.L. Esposti. Prognostic significance of nuclear DNA levels in prostatic carcinoma. Scand. J. Urol. Nephrol. (Suppl.) 55:53-58, 1980.

11. Tribukait, B. Flow cytometry in assessing the clinical aggressiveness of genitourinary neoplasms. World J. Urol. 5:108-122, 1987.

12. Lundberg, S., J. Carstensen, and I. Rundquist. DNA flow cytometry and histopathologic grading of paraffin-embedded prostate biopsy specimens in a survival study. Cancer Res. 47:1973-1977, 1987.

13. Stephenson, R.A., B. James, H. Gay, W. Fair, W. Whitmore, Jr. and M. Melamed. Flow cytometry of prostate cancer: Relationship of DNA content to survival. Cancer Res. 47:2504-2507, 1987.

14. Koss, L.G., R.C. Wolley, K. Schreiber, and J. Mendecki. Flow-microfluorometric analysis of nuclei isolated from various normal and malignant human epithelial tissues: A preliminary report. J. Histochem. Cytochem. 25:565-572, 1977.

15. Gaub, J., G. Auer, A. Zetterberg. Quantitative cytochemical aspects of a combined Feulgen-naphthol yellow S staining procedure for the simultaneous determination of nuclear and cytoplasmic proteins and DNA in mammalian cells. Exp. Cell Res. 92:323-332, 1975.

16. Murphy, G.P., J.F. Gaeta, J. Pickren, and Z. Wajsman. Current status of classification and staging of prostate cancer. Cancer 45:1889-1895, 1980.

17. Klein L. Prostatic carcinoma. N. Engl. J. Med. 300 No. 15:824-833, 1979.

ACKNOWLEDGEMENTS

We wish to acknowledge our coworkers who have contributed to various portions of these studies: Robert Wersto, Ph.D., Daniel Dietch, M.D., Klaus Schreiber, M.D., Selwyn Freed, M.D., June H. Koizumi, M.D., and E. Darracott Vaughan, Jr., M.D.