Brazilian J Med Biol Res (1994) 27: 25-32 ISSN 0100-879X

Ploidy status correlates with outcome in stage B prostate adenocarcinoma

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1. While there are extensive data showing that aneuploidy is associated with adverse outcome in stage D prostate cancer, the utility of ploidy analysis in stage B disease is unclear. We determined ploidy in radical prostatectomy specimens from 28 patients with clinical stage B prostate cancer, and with a mean follow-up of 4.1 years (2-10 years). Patients who had no recurrences had a minimum 5 years of follow-up. Patients who had only 2 years of follow-up were included if they had developed bone metastases during this period.

2. Ploidy determinations were done on Feulgen-stained 5-μm paraffin-embedded sections using a CAS 200 image analyzer. At least 400 tumor cells were counted in every case. Tumors with at least 70% diploid cells were classified as diploid, while those with less than 70% diploid cells were classified as aneuploid. The mean percentage of diploid cells in tumors classified as diploid was 90.6 ± 7.4, while the mean percentage of diploid cells in tumors classified as aneuploid was 36 ± 21.9.

3. Ploidy status correlated with disease progression: seven of the 10 patients (70%) with disease recurrence had aneuploid tumors, while 13 of 18 patients (72%) who remained disease-free had diploid tumors ($P = 0.03$, Chi-square test).

4. These data show that patients with stage B disease with aneuploid tumors at the time of prostatectomy are more likely to have recurrent disease within a mean of 3 years (2-6 years) compared to patients with diploid tumors. Ploidy determination done at the time of surgery may offer useful prognostic information.

Key words: prostate cancer, ploidy, image analysis.

Research carried out in the Department of Urology and Pathology, Veterans Administration Medical Center, 4150 Clement Street, San Francisco CA, 94121. S.T.Jr. was supported by a grant from FAPESP (No. 90/3758-8).
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Bestellnr.: **1711654**
Eingangsdatum: **20.08.09 - 11:32** **TAN:** **FM6I8G**

Bestellende Bibl.: Düsseldorf UuLB <61>

Benutzer: Martin Schramm/N0055320

Bem. des Best.:

Kostenübernahme:

Signatur: **Sigel: 38 M - Bestand: 14.1981 - 40.2007# Standort: >Zs.B 1359< -**

Zeitschrift: **Brazilian journal of medical and biological research = Revista brasileira de p**
Körperschaft:
Ort: São Paulo
Autor Tucci JS, Blumenfeld W, Narayan P
Aufsatztitel: Ploidy status correlates with outcome in stage B prostate adenocarcinoma

Jahrgang: 1994
Band/Heft: 27, (1)
Seiten: 25-32

Lieferant: **Deutsche Zentralbibliothek für Medizin**
Dokumentenlieferung - medea3@zbmed.de

Leitweg: [1.] GBV
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Introduction

One of the problems in the management of patients with prostate cancer is the ability to accurately predict the prognosis. As a general rule tumor aggressiveness and prognosis are assessed by clinical stage and histologic grade. The Gleason histopathologic grade provides a reasonable prediction of outcome since patients with a Gleason sum of 8 or more may have a worse prognosis than patients with a Gleason sum of 7 or less (Gleason, 1977).

After radical retropubic prostatectomy for localized adenocarcinoma, aside from tumor differentiation, capsular penetration and large tumor bulk have also been associated with poor prognosis (Byar and Mostofi, 1972; Zincke et al., 1981; Benson et al., 1984). However, these parameters do not tell the whole story, since patients with stage D1 or D2 disease may have long survivals (Winkler et al., 1988; Miller et al., 1991).

In the search for another feature of the tumor that would define a more accurate potential for disease progression, many studies have been done to determine if the nuclear DNA content of prostatic cancer cells is useful in assessing the biological potential of each individual tumor. Most of these studies have been done by flow cytometry (FCM) and investigators usually agree that the determination of the ploidy may be an important prognostic factor in prostate cancer (Frankfurt et al., 1985; Dejter et al., 1989; Al-Abadi and Nagel, 1990; Badalament et al., 1991; Lieber and Cheng, 1991; Paulson, 1991; Zetterberg and Forsslund, 1991). However, there is some controversy since some investigators have shown that the DNA status fails to significantly enhance the ability of standard histopathological grading to predict disease recurrence in patients having clinically localized prostate cancer (Ritchie et al., 1988; Ring et al., 1990).

In this investigation we studied the DNA ploidy by image cytometry and histological grading in a group of patients with stage B prostate cancer and related the results with clinical outcome.

Patients and Methods

Patients

Twenty-eight patients with stage B adenocarcinoma of the prostate who underwent radical retropubic prostatectomy were studied. Their disease was staged at the time of diagnosis by rectal examination and bone scintigraphy. After surgery they were followed-up at regular intervals over a period of 2 to 10 years (mean 4.1

years). Patients who had no recurrences had a minimum of 5 years of follow-up. Patients who had only two years of follow-up were included if they had developed bone metastases during this period. Development of metastases was assessed by bone scintigraphy and serum prostatic specific antigen level (PSA).

Histologic grading

The surgical specimen was fixed in buffered 10% neutral formalin solution and later embedded in paraffin wax. Sections were cut and stained with hematoxylin-eosin and graded histologically according to the Gleason classification (Gleason, 1977). Two numbers between 1 and 5 were ascribed to each tumor, one number for the main histologic pattern and the other for the secondary pattern. The sum of these two numbers is the Gleason score.

Image cytometry (ICM)

Ploidy determination was done for each patient on Feulgen-stained 5-µm paraffin-embedded sections using a CAS 200 image analyzer. At least 400 tumor cells were counted for each patient. Tumors with at least 70% diploid cells were classified as diploid, while those with less than 70% diploid cells were classified as aneuploid.

Statistical analysis

Correlation of DNA ploidy and Gleason score with progression of disease was analyzed by the chi-square and Student *t*-tests.

Results

Patients

The average age at diagnosis was 65.1 years (range 44-74 years). No patients had evidence of metastases at the time of surgery. During the follow-up period of 2 to 10 years, 10 patients (35.7%) developed bone metastases as evidenced by bone scan and serum PSA. The mean time for appearance of metastases was 3 years after surgery (range 2-6 years). The remaining 18 patients (64.3%) did not have progression of the disease as assessed by the same methods.

Correlation between Gleason score and clinical outcome

In these patients, the Gleason score ranged from 4 to 8 (mean 6.1). There was no statistical difference in the mean Gleason score when patients with recurrence were compared with the non-recurrence group. The former presented a mean Gleason score of 6.3 (range 5 to 8), while those without progression had a mean score of 6.0, with a range from 4 to 7 ($P = 0.4$, t-test).

Ploidy analysis

Two patterns were recognized from ploidy analysis by image cytometry. The first one was similar to a benign tissue pattern (Figure 1). These were called diploid tumors and the DNA content of their cells was in the same range as benign cells. The second pattern was characterized by the presence of an additional peak produced by cells with higher DNA content, and was called aneuploid (Figure 2). The mean percentage of diploid cells in tumors classified as diploid was 90.6 ± 7.4, while the mean percentage of diploid cells in tumors classified as aneuploid was 36 ± 21.9.

Correlation with ploidy and clinical outcome

Ploidy status presented a strong correlation with disease progression. Seven of 10 patients (70%) with disease recurrence had aneuploid tumors, while 13

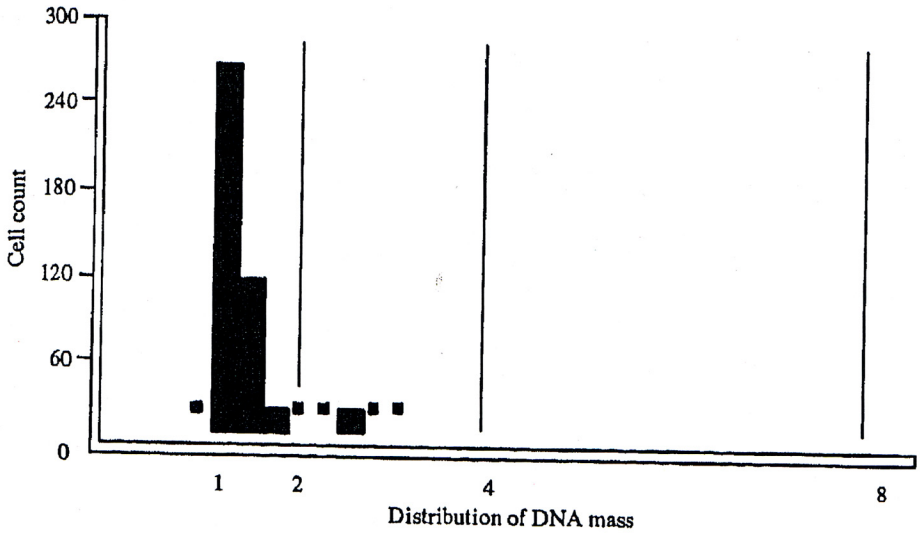

Figure 1 - Image analysis histogram of a prostate carcinoma classified as a diploid pattern.

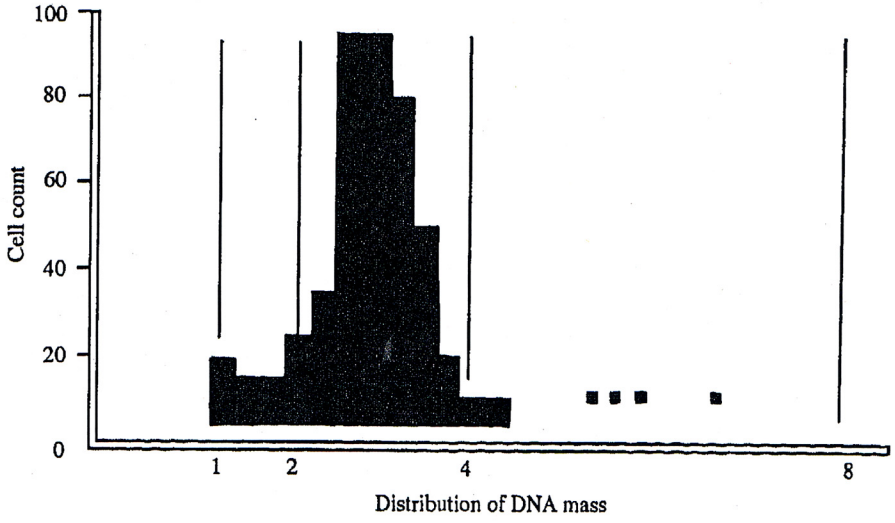

Figure 2 - Image analysis histogram of a prostate carcinoma classified as an aneuploid pattern.

of the 18 patients (72%) who remained disease-free had diploid tumors (P = 0.03, chi-square test).

It was not possible to do a correlation between ploidy analysis and Gleason score since there was no statistical difference in the Gleason sum between patients with and without recurrence.

Discussion

Prostate cancer has a great variation in biological behavior, ranging from latent to highly aggressive disease. Since this clinical range is partly reflected in the morphological features, the histologic classification has a predictive value that is not perfect, and patients with apparently similar disease status may have different clinical outcomes. As this clinical evolution has great implications for the patient, from observation to radical surgery, new criteria for prognostic significance are necessary to achieve a better understanding of the disease and for patient management. In this regard, the nuclear DNA distribution pattern of neoplastic cells done by flow or image cytometry has been studied as an objective parameter for diagnosis of premalignant and malignant lesions (Auer et al., 1989; Stage et al., 1990), and as a prognostic tool in a large number of human tumors, including prostate cancer (Friedlander et al., 1984; Koss et al., 1989; Merkel and McGuire, 1990). Several authors have suggested that aneuploidy is related to disease

progression in prostate cancer (Blute et al., 1989; Dejter et al., 1989; Adolfsson et al., 1990; Robertson and Paulson, 1991). Even in stage D1 and D2, patients with a diploid tumor have a better prognosis (Winkler et al., 1988; Miller et al., 1991). This difference can be explained, at least in part, by the higher response of diploid tumors to estrogen treatment (Tavares et al., 1966; Zetterberg and Esposti, 1980).

Most of the studies on DNA ploidy were done by FCM. Flow and image are well-established procedures and each has advantages and disadvantages. While FCM can rapidly analyze a large number of cells, with ICM it is possible to do a cytodiagnostic cell identification and to repeat the assessment (Falkmer, 1991). Using ICM in this series of stage B prostate cancer, we observed that 70% of the patients with recurrence had aneuploid tumors. On the other hand, 72% of the non-recurrence group had diploid tumors. These results agree with the statement that diploid tumors have a better prognosis.

The relationship between Gleason score and progression of the disease has been documented (Grayhack and Assimos, 1983; Ring et al., 1990). In our cases we did not detect a correlation since the Gleason score was the same for the groups with and without recurrence. In the group of patients studied, only the DNA status showed a correlation with clinical outcome. In the same way as Lundberg et al. (1987) and Ritchie et al. (1988), with our results it was not possible to do a correlation between DNA ploidy and histopathologic grade even though other authors have reported a positive correlation between these two parameters (Frankfurt et al., 1985; Montgomery et al., 1990).

This present study is limited by the relative small number of patients studied. We did not evaluate tumor volume or zone of origin, which may influence DNA status (Greene et al., 1991). Our results show that DNA ploidy analysis may be of prognostic benefit over routine histopathologic grading in stage B prostate adenocarcinoma. We believe that a prospective study may define the place of DNA ploidy analysis in the evaluation of prostate cancer.

We conclude that image cytometry is a suitable and useful method in the evaluation of nuclear DNA status in prostate cancer and that patients with stage B disease with aneuploid tumors at the time of prostatectomy are more likely to have recurrent disease within a mean of 3 years compared to patients with diploid tumors.

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Received June 25, 1993
Accepted November 3, 1993