Prognostic Value of DNA Analysis of Prostate Adenocarcinoma: Correlation to Clinicopathologic Predictors

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The ability to accurately predict tumor behavior and patient survival is a problem in managing patients with prostate cancer. DNA ploidy provides important information for the evaluation of the prognosis of prostate cancer. The aim of this study was to investigate the DNA ploidy in imprints from prostate adenocarcinomas in a group of 70 patients in relation to Gleason score, tumor differentiation, stage and PSA serum levels. The DNA content was studied in Feulgen-stained imprint smears through the image analysis technique using a SAMBA 2005 Image analyzer. According to our measurements, a strong correlation was observed between DNA ploidy status and tumor differentiation ($p<0.001$). A statistically significant difference was found between DNA aneuploidy and increased pretreatment PSA serum levels (>4 ng/ml) ($p<0.001$), as well as between ploidy pattern and stage of the disease ($p<0.001$). Our results conclude that DNA ploidy status appears to be an additional marker in the field of prognosis of prostatic adenocarcinoma and could provide useful information on the potential behavior of prostate cancer.

Key Words: Image Analysis, DNA ploidy, Prostate cancer, Prognostic factors

To predict the behavior of malignant neoplasms, the usefulness of tumor DNA content, by image analysis, has been demonstrated (1,2).

Additionally, in conjunction with conventional prognostic factors, the DNA pattern distribution (ploidy level) in prostate carcinomas has proved useful of this procedure in monitoring of the natural history of prostate cancer and patient outcome (3-5).

DNA aneuploidy has been associated to higher Gleason grade and PSA serum levels and clinically aggressive prostate carcinomas. According to the literature, the average incidence of aneuploidy in prostate carcinoma has been in the 30-40% range (6,7).

The purpose of this study was to investigate the DNA ploidy status in imprint smear cells of prostatic adenocarcinomas in relation to Gleason grade, preoperative serum PSA, stage and the clinical course.

Materials and Methods

A total of 70 imprint smears, obtained immediately after prostate removal in the operating theatre from patients who underwent radical prostatectomy for prostatic carcinoma, were studied. Patients' age ranged from 59 to 75 years (mean 67.11 years).

The histopathological diagnoses were performed using sections from the same samples that were used for the imprints. The TNM system (11) (based on the staging system of the American Joint Committee on Cancer) was used for pathological staging and Grading of the primary cancer and was evaluated according to the Gleason score system. Tumor stages of the patients were as follows: stage T2a,T2b in 61 (87.2%), stages T2c and T3a in 9 (12.8%). The Gleason score was: 2-6 in 49 (70%) and >7 in 21 (30%) of the cases. The PSA value of our patients was 0-9.9 ng/ml in 49 (70%) and >10 ng/ml in 21 (30%).

Serum PSA concentrations were measured by immunometric assay with kits (M.E.I.A Axsym Abbott, Chicago, USA).

Follow up included serum PSA at 6 months postoperatively and every six months (mean 60 months) thereafter. The data of the studied prostate carcinoma cases are shown in Table I.

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DNA analysis was performed in imprint smears stained by the Feulgen technique on a SAMBA 2005 image analyzer, according to the customary protocol, using a Zeiss Axioplan microscope with X40 plan achromatic lens, a Sony three color camera CCD and a Compaq computer. In each case, at least 200 randomly selected cells were measured. The DNA histograms were classified according to the Auer classification (4). Histograms type I and II were characterized as euploid (Fig.1) and histograms type III and IV as aneuploid (Fig.2)

Statistical analysis. The relationship of DNA ploidy status with all prognostic factors (preoperative PSA values, Gleason score, tumor differentiation and stage) was assessed by one way analysis of variance (ANOVA), followed by tests of multiple comparisons, since DNA ploidy did not deviate from normality (Kolmogorov-Smirnov test p=0.237). The simultaneous effect of all variables to DNA ploidy was investigated by multiple linear regression model. Disease free survival was assessed by Cox's proportional hazard regression model. Survival rates were calculated using the Kaplan-Meier method.

Table I - Clinical characteristics of 70 patients with prostate adenocarcinoma treated with radical prostatectomy

	No	(%)
Age (yrs)		
<65	14	20.0
65-69	35	50.0
>70	21	30.0
Stage		
T2a	41	58.6
T2b	20	28.6
T2c	6	8.6
T3a	3	4.2
Gleason Score (grade)		
2-4	19	27.1
5-6	30	42.9
>7	21	30.0
Pretreatment PSA levels		
0-4	13	18.6
5-9	36	51.4
>10	21	30.0

(T2a: tumor involves 50% of a lobe or less, T2b: tumor involves more than 50% of a lobe, T2c: tumor involves both lobes, T3a: unilateral extracapsular extension of the tumor).

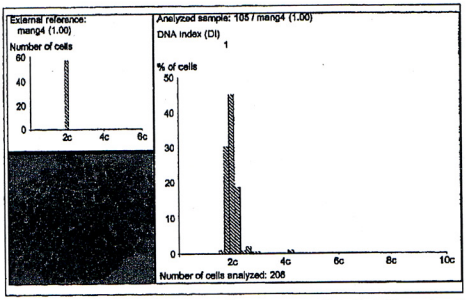

Fig. 1 - An euploid histogram from a case of well differentiated prostate adenocarcinoma with low Gleason score and PSA value. Cluster of prostate adenocarcinoma cells from a case with low Gleason score and PSA value stained with Feulgen method (X500).

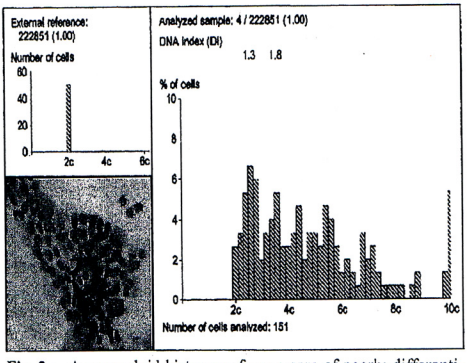

Fig. 2 - An aneuploid histogram from a case of poorly differentiated prostate adenocarcinoma with high Gleason score and PSA value. Prostate adenocarcinoma cells from a case with high Gleason score and PSA value: nuclear staining with Feulgen method (X500).

Results

The distribution of DNA ploidy patterns for the entire group was as follows: 67.1% were euploid and 32.9% were aneuploid (hypodiploid 4.3%, hyper-

diploid 12.9%, triploid 7.1% and hypertriploid 8.6%) (Fig.3).

A statistically significant difference was observed concerning the ploidy status (euploid versus aneuploid tumors) among Gleason score 2-4, 5-6, >7 ($p<0.001$). It can be seen that patients with Gleason score >7 had aneuploid tumors (32.9%) (Fig.4).

DNA ploidy provided additional prognostic information in patients with increased preoperative PSA serum levels (>10ng/ml). PSA concentrations were significantly higher in hyperdiploid / aneuploid tumors than in diploid tumors ($p<0.001$) (Fig.5).

Tumor DNA ploidy status (diploid versus aneuploid) showed a good correlation with tumor stage. Stages T2a, T2b were diploid, while T2c and T3a were hyperdiploid / aneuploid ($p<0.001$), respectively (Fig.6).

The multiple linear regression model shows that the most significant variables associated with DNA ploidy are Gleason score ($p<0.0001$) and preoperative PSA serum values ($p=0.0110$) (Table II). A higher incidence of recurrence was noted in those patients who had DNA index DI<0.90 (hypodiploid) or DI>1.16 (diploid, triploid, hypertriploid), than in those who had DNA index between 0.91 and 1.15 (euploid). Long rank test and Cox's model demonstrated that DNA ploidy had significant prognostic value for the disease free survival (Table III,IV).

During the follow up period (60 months) 8 (11.42%) patients died from their disease, 2 at 12 months, 3 at 24, 2 at 36 and 1 patient at 60 months (Fig.7). The remaining patients were alive and well at the last follow up.

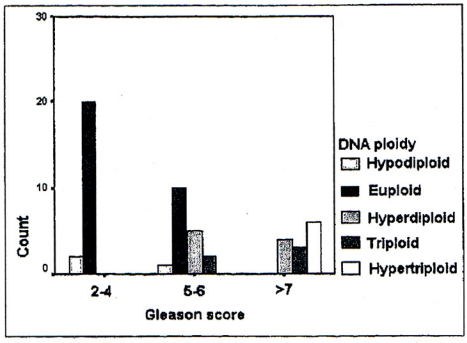

Fig. 4 - Correlation of DNA ploidy in smears of prostate carcinomas with the degree of Gleason score ($p<0.001$).

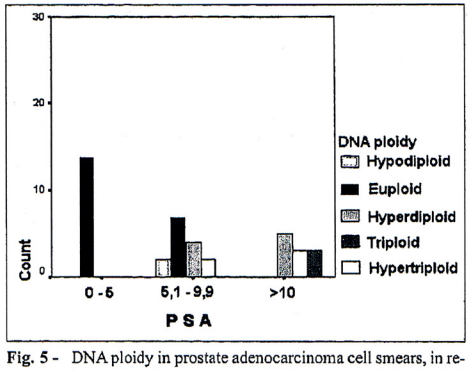

Fig. 5 - DNA ploidy in prostate adenocarcinoma cell smears, in relationship with pretreatment PSA serum levels ($p<0.001$).

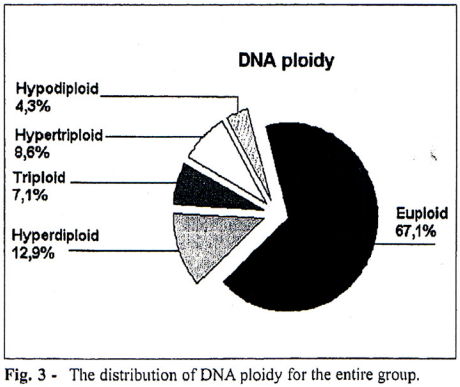

Fig. 3 - The distribution of DNA ploidy for the entire group.

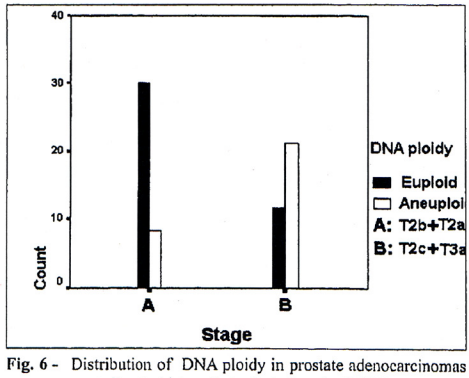

Fig. 6 - Distribution of DNA ploidy in prostate adenocarcinomas according to histopathological stage ($p<0.001$).

Table II - Contribution of various clinicopathological parameters to DNA ploidy status

	Clinicopathological parameters (Dependent variable DNA ploidy)				95% Confidence Interval for B	
	B	Std error	t	Sign.	Lower Bound	Upper Bound
Gleason score	7.20	1.10	5.47	<0.0001	4.62	9.74
Pretreatment PSA	0.61	0.22	2.17	0.0110	0.13	1.10
Stage	0.074	0.750	0.290			

Discussion:

Histological grade, clinical staging and PSA values are the standard prognostic parameters used in prostate cancer, although the significance of these has been challenged (1-4).

In recent years flow cytometry, as well as image analysis techniques have been used to predict the biological behavior of malignant neoplasms. DNA ploidy analysis appears to be particularly promising in predicting the clinical course of patients with prostate carcinoma. The presence of aneuploidy is associated with a poor prognosis, as already indicated in several other studies (5-11).

In the present study we attempted to compare DNA ploidy with the risk factors based on Gleason score, PSA values and histopathological stage. The material consisted of fresh samples that were obtained immediately after prostate removal in the operating theatre. Intratumoral heterogeneity of DNA content can result in discrepancies of ploidy determination. For this reason we used the most representative cancerous cell material for all our analyses.

There have been reports showing a direct correlation among increasing Gleason score, PSA level, tumor differentiation and DNA content (5). Some investigators have found that the use of DNA ploidy increases the prognostic value. Others have reported that DNA ploidy in association with tumor stage and grade is questionable (12-14). Many of these studies were retrospective image analyses of disaggregated paraffin-embedded and formalin-fixed specimens (5,7,12-15).

In comparison to fresh imprint smears, the tissue sections present a lot of difficulties in estimating the DNA ploidy. Depending on the thickness of the section there will always be a number of nuclei that are either sliced or overlapped, the first leading to false low and the latter to false high ploidy values. Consequently, researchers who study DNA ploidy in archive tissue sections should use internal control analysis such as the one recommended by Green et al. (16).

In the present study, the DNA ploidy correlated significantly with Gleason score ($p<0.001$). With regard to DNA ploidy in higher grade tumors aneuploidy rate, as expected, increased (32.9%). In our series, a significant difference was found in the clinical behavior of diploid (Low Gleason score:2-4) and aneuploid tumors (High Gleason score ≥ 7) (p:0.001)

When combining the DNA ploidy with the tumor stage, a significant difference between stages T2a,

Table III - Assessed by multiple linear regression model (Dependent variable: DNA ploidy, long rank test)

	Long rank test					95% CI for HR		
	B	SE	Wald	df	Sign.	HR	Lower	Upper
DNA ploidy	0.131	0.047	5.745	1	0.007	1.142	1.027	1.270

Table IV - Cox's model Variable not in the Equation

Cox's model: Variable not in the Equation	Score	df	Sign
Gleason score	0.897	1	0.201
Stage	0.042	1	0.005
PSA	0.249	1	0.47

Residual X^2=13,833 with 7df Sign=0,054

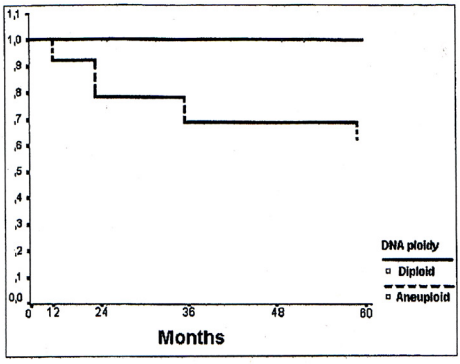

Fig. 7 - Kaplan-Maier survival curves of 70 patients.

T2b and T2c, T3a was observed ($p<0.001$). Similar observations were made by other investigators (8,14,15).

Prostate specific antigen (PSA) is currently the most sensitive and clinically important tumor marker available for the diagnosis and management of prostate cancer. Several large scale studies have demonstrated that serum PSA correlates well with advancing clinical stage, tumor volume and pathological stage (13,14,17-19).

In the present study high levels of serum PSA have proved to be associated with aneuploidy ($p<0.001$). This observation indicates the importance of this correlation in determining prognosis and confirms similar results obtained by other authors (8,13,14,20,21). It has also been reported by Di Silvero et al (9), that the preoperative PSA levels are an important and significant predictor of recurrence ($p<0.005$).

Previous studies showed that in localized prostate cancer, nuclear ploidy status is a significant predictor of disease outcome after radical prostatectomy (1,2,5-8,13,15,16,20,21). With regard to the disease-free survival after radical prostatectomy, in our study, DNA ploidy status correlated with recurrence by Cox's proportional hazard regression model. This analysis demonstrated that the most significant prognostic marker was the DNA ploidy ($p=0.007$).

In conclusion, these results showed that DNA ploidy could be an additional marker indicating the malignant potential of prostate cancer and an indicator for poor prognosis in patients with prostate cancer.

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