

DNA Ploidy and Survival of Patients With Clinically Localized Prostate Cancer Treated Without Intent to Cure

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BACKGROUND. The optimal approach to diagnosis and treatment of localized prostate cancer remains controversial. Deoxyribonucleic acid (DNA) ploidy has been suggested as an important predictor for outcome in prostate cancer. The purpose of this study was to correlate DNA ploidy with disease-specific survival in patients with clinically localized prostate cancer treated with no intent to cure.

METHODS. DNA ploidy was determined by flow cytometry in archival formalin fixed, paraffin embedded tumor tissue obtained at diagnosis in 120 patients with clinically localized prostate cancer with a nearly complete follow-up.

RESULTS. Ninety (75%) of the tumors were diploid, while only 11 (9%) tumors were categorized as tetraploid. Tumor DNA ploidy (diploid versus nondiploid) significantly associated with histopathological grade ($P = 0.002$) and disease-specific survival ($P = 0.011$), while there was no association with tumor stage ($P = 0.054$). In a multivariate Cox analysis, histopathological grade ($P = 0.005$) was the only significant predictor of disease-specific death, while analyzing the 96 low-grade tumors separately, DNA ploidy became significant ($P = 0.024$).

CONCLUSIONS. Flow cytometric determined nondiploidy was associated with disease-specific death in patients with clinically localized prostate cancer, but DNA ploidy provided additional prognostic information in patients with low-grade tumors only. *Prostate 36:244–249, 1998.* © 1998 Wiley-Liss, Inc.

KEY WORDS: prostatic adenocarcinoma; DNA ploidy; prognostic parameters; flow cytometry; histopathological grade

INTRODUCTION

Prostate cancer has become one of the most common malignant diseases in Western communities, exceeding lung cancer as the most commonly diagnosed cancer in American men [1]. It is the second most commonly diagnosed nonskin cancer disease in Danish males and the second leading cause of cancer death in the same population [2,3]. Although prostate cancer has been regarded as a slowly progressing tumor of which men died *with* and not *from*, it is well known that many prostate cancers have an aggressive feature [4,5]. The discrepancy between the high prevalence of this disease at autopsy and the much lower incidence of the disease clinically [6,7] has resulted in an urgent

need for useful biological prognostic markers. Optimal selection of patients for locally radical treatment is needed if prostate cancer mortality as well as morbidity are to be reduced.

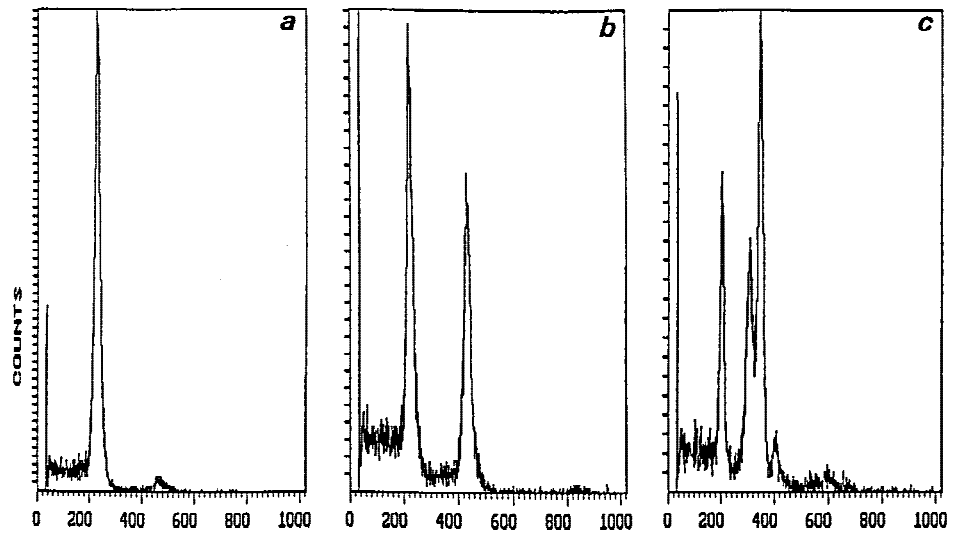
Several flow cytometric and image analysis studies have suggested that DNA ploidy provides significant

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Fig. 1. DNA histograms representing (a) a diploid tumor, (b) a tetraploid tumor, and (c) a nontetraploid nondiploid tumor.



prognostic information for patients with prostate cancer [8–18]. However, there have been conflicting results in the evaluation of the additional prognostic value offered by DNA ploidy in prostate cancer patients [19–25].

The aim of this study was to test the prognostic value of DNA ploidy determined by flow cytometry in clinically localized prostate cancer patients treated with no intent to cure.

MATERIALS AND METHODS

Patients

The study included 120 patients with clinically localized (T1–2,Nx,M0) prostate cancer. During the 5-year period, January 1, 1979–December 31, 1983, 719 inhabitants of Aarhus County were diagnosed with prostate cancer irrespective of clinical stage. The patients were identified by the Danish Cancer Registry and retrospectively followed from the time of diagnosis until death. From this previously described complete prostate cancer population [5], 221 patients had both available histologic tissue obtained at diagnosis, as well as complete clinical data [26]. The total of 125 of these patients had clinically localized disease. Flow cytometric determination of the DNA content failed in five cases, leaving 120 patients in the study. In 108 (90%) cases, the tumor specimen originated from transurethral resection of the prostate (TURP), while 12 (10%) patients were nonradical prostatectomized. The tumors have been retrospectively restaged according to the International Union Against Cancer [27], while the original histopathological grade according to the World Health Organization (WHO) [28] was employed.

Flow Cytometry

The specimens for the immunostaining procedures were retrieved from the archived formalin-fixed, paraffin-embedded tissue used for the original histopathological examination. Routine hematoxylin and eosin (H&E) sections were performed confirming the presence of cancer cells at that level of the block. In this study, 30- μ m sections were cut and left unmounted. The sections were prepared according to the principle previously described [29]. In brief, the sections were dewaxed in xylene, rehydrated in graded ethanol, and treated with 0.5% pepsin (Sigma No. P-7012; Sigma Chemical Co., St. Louis, MO) in 0.9% sodium chloride, adjusted to pH 1.5. The specimens were incubated at 37°C for 30 min and subjected to frequent intermittent vortex mixing. The nuclear suspension was centrifuged at 2,000 rpm at 4°C for 10 min, and the pepsin supernatant was removed. The suspension was stained with 5–8 ml of propidium iodide solution (10 μ g/ml) at 4°C for 20 hr. The cells were centrifuged at 1,200 rpm for 5 min at 4°C. The samples were filtered through a 50- μ m pore nylon mesh. The DNA index was determined by an Epics Profile flow cytometer as the channel of tumor G1 relative to normal cell G1. In the case of half-peak coefficient of variance greater than 8% and for unidentifiable DNA peaks, patients were excluded from analysis. Histogram analysis was performed by use of a computer software designed by Ib Jarle Christensen [30]. The tumors were subdivided into diploid and nondiploid. The diploid tumors had a normal amount of DNA (DNA index: 0.95–1.05), while tumors with any other amount of DNA were considered nondiploid. A tetraploid tumor subpopulation, with twice the normal amount of DNA (DNA index: 1.95–2.05), was analyzed separately (Fig. 1).

TABLE I. DNA Ploidy Correlated With T Classification and Histopathological Grade in 120 Clinically Localized Prostate Cancer Patients

	n	DNA ploidy			P
		Diploid	Nondiploid		
			Tetraploid	Non tetraploid	
Total	120 (100%)	90 (75%)	11 (9%)	19 (16%)	
T class					
T1a	18 (15%)	17 (19%)	0 (0%)	1 (5%)	0.05 ^a /0.18 ^b
T1b	80 (67%)	55 (61%)	9 (82%)	16 (84%)	
T2	22 (18%)	18 (20%)	2 (18%)	2 (11%)	
Grade					
Well	49 (41%)	45 (50%)	2 (18%)	2 (10%)	0.002 ^a /0.009 ^b
Moderate	47 (39%)	29 (32%)	7 (64%)	11 (58%)	
Poor	24 (20%)	16 (18%)	2 (18%)	6 (32%)	
Cause of death					
Prostate cancer	49 (41%)	30 (33%)	6 (55%)	13 (68%)	0.009 ^a /0.02 ^b
Other causes	65 (54%)	54 (60%)	5 (45%)	6 (32%)	
Alive ^c	6 (5%)	6 (7%)	0 (0%)	0 (0%)	

^aDNA divided into diploid and nondiploid.

^bDNA ploidy divided into diploid, tetraploid, and nondiploid nontetraploid.

^cExcluded from chi-square test.

Statistics

Statistical analysis was performed using SPSS 6.1 for Windows (SPSS Inc., Chicago, IL) program package. The two-sided chi-square test was used to test for an association between categorical data. The survival functions were calculated according to the Kaplan-Meier method and the differences between the survival curves were tested using the log-rank test. The Cox proportional hazards regression model was used analyzing the prognostic value of the different variables determined at the time of diagnosis. Overall death as well as disease-specific death (all deaths caused directly to prostate cancer exclusive deaths from coexisting disease, accidents and unknown causes) were used as endpoints. Values of two-sided $P < 0.05$ were considered statistically significant.

RESULTS

The median age at diagnosis was 76 (range 49–95) years. At the end of the registration period (May 15, 1996), only 6 (5%) patients were alive. According to the hospital charts and death certificates, 49 (41%) patients died from prostate cancer, while 65 (54%) patients died from other causes (Table I). Examples of DNA histograms from diploid, nondiploid, and nondiploid tetraploid tumors are shown in Figure 1. Ninety (75%) of the tumors were diploid and 30 (25%) were nondiploid, in contrast to 54% diploid and 46% nondiploid tumors in the 91 patients excluded from

the study suffering from advanced disease (T>2,Nx and/or M1). Eleven (37%) of the nondiploid tumors were tetraploid. Only a single (5%) T1a tumor and 4 (8%) well-differentiated tumors were nondiploid. Two out of three tumors were classified as T1b (67%), and only 20% of the tumors were poorly differentiated. DNA ploidy divided into diploid and nondiploid was significantly associated with histopathological grade ($P = 0.002$) and cause of death ($P = 0.009$), while there was no significant association between DNA ploidy and T classification ($P = 0.054$). All patients who survived the observation period had diploid tumors at diagnosis. One-third of those with diploid tumors eventually died from prostate cancer, while nearly two-thirds (63%) of the nondiploid tumor patients experienced disease-specific death. There existed a similar correlation between the variables when ploidy was divided into three categories: diploid, tetraploid, and nontetraploidy nondiploid (Table I). Figure 2 illustrates the statistically significant association ($P = 0.011$) between DNA ploidy (diploid vs. nondiploid) and disease-specific survival, while the association between DNA ploidy and overall survival was insignificant ($P = 0.4$).

A Cox multivariable regression analysis was carried out examining DNA ploidy, T classification, and histopathological grade. Histopathological grade was the only significant variable ($P = 0.005$) including all 120 patients. However, when analyzing the 96 low-grade tumors separately, the predictive value of the

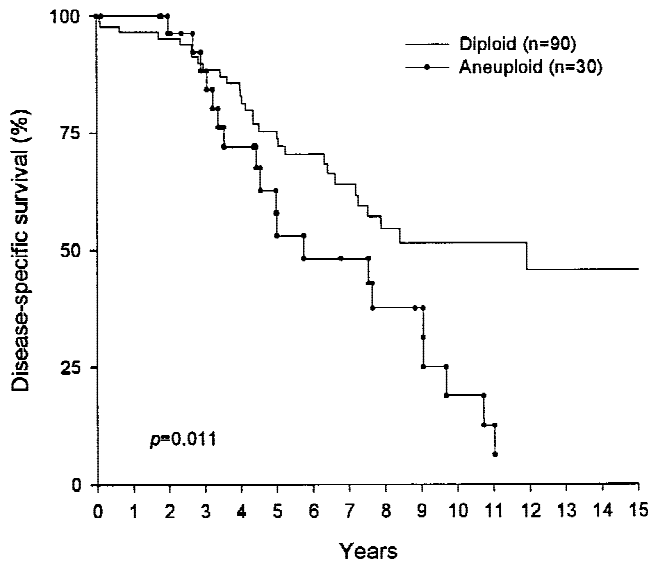


Fig. 2. DNA ploidy correlated with disease-specific survival in 120 patients with clinically localized prostate cancer.

histopathological grade was deprived by DNA ploidy, which then became the only significant ($P = 0.024$) predictor of disease-specific death (Table II). When using overall death as endpoint in this elderly population neither DNA ploidy ($P = 0.7/0.2$) nor grade ($P = 0.4/0.1$) turned out significant in any of the multivariate analyses.

DISCUSSION

The current retrospective study had a nearly complete long-term follow-up and was based on previously described population-based prostate cancer patients treated with no intent to cure [5,31]. The total of 120 of the 125 clinically localized prostate cancer patients with available archived tumor tissue and complete clinical data were included into the study. Despite the fact that incomplete clinical information and understaging are typical elements of risk in retrospective studies, the immediate available long-term follow-up makes this study design especially attractive in slowly progressing cancers. We found the natural course of clinically manifest prostate cancer to be very poor, and even clinically localized cancer patients appeared to be at great risk of dying from their cancer if they survived long enough [5]. Similar suggestions have previously been made from other population based, long-term follow-up studies [4,32]. Screening programs may tend to identify cases destined to have a benign course (“overdiagnosis”) [33], while variation in the length of follow-up may be another explanation of the immediately great difference in the clinical outcome of the patients in the present study and in those in whom curative treatment was offered.

TABLE II. Cox-Multivariate Regression Analyses, Including All 120 Patients (A) or 96 Low-Grade, Clinically Localized Prostate Cancer Patients (B), Using Disease-Specific Death as Endpoint*

	Disease-specific death		
	<i>P</i>	RR	CLI
A: (n = 120)			
DNA ploidy (diploid vs. nondiploid)	0.14		
Grade (well vs. moderate vs. poor)	0.005	1.67	1.17–2.37
T class (T1a vs. T1b vs. T2)	0.07		
B: (n = 96)			
DNA ploidy (diploid vs. nondiploid)	0.024	2.36	1.17–4.78
Grade (well vs. moderate)	0.08		
T class (T1a vs. T1b vs. T2)	0.40		

*RR, relative risk; CLI, 95% confidence limit.

Although the nature of prostate cancer has changed with prostate-specific antigen (PSA) screening, the spontaneous prognosis of patients with favorable marker status provides important information that cannot be achieved in treated patients.

In the present study, flow cytometric determined DNA content (diploid vs. nondiploid) was significantly associated with both histopathological grade and long-term survival in patients with clinically localized prostate cancer. This population was in contrast to patients in most other studies treated with no intent to cure. The result was based on flow cytometric determination of DNA ploidy in archived formalin-fixed, paraffin-embedded tissue, which was based primarily on TURP specimens. DNA ploidy analysis could to some extent be distorted by the fact that the TURP giving rise to the diagnosis may have rendered some of the patients free of disease. The multifocality and heterogeneity of prostate cancer [16,34–36], and a probably fundamental difference between cancers of transition zone origin and peripheral zone origin [16,24,35,37], combined with an unavoidable contamination by benign stromal and glandular cells may have resulted in an underestimation of nondiploid tumors in the current study. Pindur et al. [14], comparing the results of DNA ploidy measurements in prostate carcinoma, found that image analysis detected 54% nondiploid tumors compared to only 31% nondiploid tumors by flow cytometry. However, the time-consuming image analysis may assess the individual tumor focus on histology slides but, because of the multifocality and heterogeneity of prostate cancer, DNA ploidy values still depend on the degree to which the samples are representative of the cancers.

In a review of previous results of the prognostic value of DNA content in prostate cancer in 1994, Adolfsson [38] showed that DNA content was

strongly related to tumor grade and stage, and ploidy was proved to have prognostic value with respect to both overall and disease-specific survival in univariable analyses. However, in agreement with our results, the additional prognostic value of the DNA content was less convincing when analyzed with tumor grade and stage in multivariable analyses. The discrepancy between univariate and multivariate analyses was explained by a close covariance of the DNA content and tumor grade, which may reflect independent, but frequently concordant parameters. Conflicting results assessing whether DNA ploidy adds prognostic information to that already provided by histopathological grade have also been suggested to be a question of whether poorly differentiated tumors were included in the analyses or not [17]. As in the present study, Carmichael et al. [17] found that DNA ploidy only provided additional prognostic information to the histopathological grade (Gleason), when examining only the low-grade tumors. The poor differentiation seemed to overshadow the effect ploidy might have. Correspondingly, Adolfsson et al. [9] demonstrated an additional prognostic information of DNA ploidy in low-grade, low-stage untreated prostate cancer.

CONCLUSIONS

Flow cytometric determined DNA ploidy was significantly correlated with histopathological grade, using paraffin-embedded archival specimens from clinically localized prostate cancers. The association between DNA ploidy and disease-specific survival was significant, but additional prognostic value to that already provided by histopathological grade was only demonstrable among patients with low-grade tumors. Thereby, the prognostic importance of DNA ploidy seems to be attached to a limited, but in regard to future aggressive therapeutic strategies, very important group of patients.

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