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Nuclear Deoxyribonucleic Acid Determination in Patients with Prostate Carcinomas: Clinical Research and Application

Key Words
Prostate carcinoma
Treatment
DNA measurements
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Abstract
The objective of this prospective study of patients with prostate carcinoma was to assess the diagnostic and prognostic value of DNA measurements based on fine-needle aspirates of the prostate. Two hundred and eighty-seven untreated patients under active surveillance and 309 hormonally treated patients were followed for a minimum of 10 years. Five hundred and six patients with cytologic benign prostate lesions served as the control group. The subdivision of tumors into diploid, tetraploid and aneuploid enabled further characterization of cytologically defined tumors. By repeated biopsies of untreated patients an increasing dedifferentiation combined with a shift towards aneuploidy at an annual rate of about 16% was found, leading to tumor heterogeneity. Significantly better survival for untreated patients over hormonally treated patients was found when comparing patients of same stage, grade and tumor ploidy. The reason was the adverse effect of androgen deprivation on tetraploid and aneuploid tumors. This unexpected finding was considered due to the elimination of hormone-dependent diploid tumor parts, leading to growth advantage for hormone-independent tetraploid or aneuploid cell lines. Multivariate analysis confirmed the high prognostic value of tumor ploidy, particularly in low-grade, low-stage tumors in which other known variables did not provide any prognostic information.

Introduction

Adenocarcinoma of the prostate has become the most common malignancy diagnosed among Swedish men. According to the last data available the incidence in 1988 was 5,008 corresponding to 115 per 100,000 men [33]. This incidence is the highest among the Scandinavian countries and also one of the highest in the world [36]. It also exceeds the incidence of carcinomas in the female breast. In 1977, at which time the present study has started, the incidence was 3,192 or 77.9 per 100,000 and has thus increased by 57% between 1977 and 1988 [34].

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Latent carcinomas found at autopsy, which might be three or more times as frequent as clinical forms [6], were excluded from these figures.

The proportion of men older than 65 years of age increased only slightly, from 14 to 15.4%, between 1977 and 1988 at an almost unchanged total population of men of about 4.1 millions. Thus, a change in the longevity has has only marginal significance for the increase in the prostate cancer incidence.

Newer diagnostic techniques such as transrectal ultrasound imaging and serum prostate specific antigene assay were not generally used in Sweden in 1988 and cannot explain the increase in cancer incidence.

Specific for Sweden, and not commonly used in other countries, is the frequently applied technique of fine-needle aspiration biopsy for diagnosis. This technique accounted for 49 and 54.6% of all newly detected prostate carcinomas in 1977 and 1988, respectively. The ease by which cell material is obtained from the prostate by this method might permit an earlier detection of malignancy of the prostate and might indeed contribute to the high frequencies of tumors detected in Sweden provided that part of these tumors are only latent malignancies which do not progress further towards clinically manifest tumor forms. The 58% increase of the tumor incidence between 1977 and 1988 can, however, not be explained by an increase in the application of fine-needle biopsy technique.

The high and further increasing incidence of prostate carcinoma is a substantial problem for the health care system and necessitating clear strategies of therapy. However, the treatment of prostate cancer is controversial, particularly in localized tumor disease [8]. Radical prostatectomy or no therapy at all until disease progression are the two extremes among various possible therapeutic alternatives. Overtreatment by the first one and fatal outcome of the disease due to delayed treatment are the prizes entailed by the two alternatives.

In this situation, the development of predictive tests to assess the malignant potential of prostate carcinomas has high priority in urologic oncology. Measurement of the cellular DNA content by single or flow cytometry has gained increasing interest in this context during recent years. Most investigations are based on the study of archival cell material, particularly prostatectomy specimens in which decision for therapy has already been made. Clearly, these studies have substantially contributed to the knowledge of the biology of the prostate carcinoma. Still, the prognostic value of DNA measurements is debated [1, 3, 4, 7, 11, 12, 14–16, 22–28, 30–32, 35, 37–39, 42–46]. Most important, for such methods to contribute to therapeutic decision, they must be performed in cell material from the prostate in situ before therapy has been initiated, i.e. by biopsy or TUR.

The present study on DNA measurements of prostate carcinomas has mostly been performed in cell material obtained by fine-needle aspiration biopsy under routine clinical conditions. The aim was:

(1) To establish the diagnostic value of DNA measurements as an adjunct to common basic clinical and morphological tumor properties. The results of DNA measurements in a large number of patients with cytologic benign prostate lesions were included in this study to ascertain the reliability of the fine needle aspiration technique in the diagnoses of the prostate carcinoma.

(2) To assess, in a prospective study, the prognostic significance of such measurements by long-term follow-up of a large number of patients with various tumor stages and grades subjected also to various forms of therapy.

(3) To discuss some biological tumor properties of the prostate carcinoma of clinical relevance, based on the results of this and other studies using single cell and flow cytometry.

Material and Methods

Cells. DNA Analysis

Since the introduction of the fine-needle aspiration biopsy technique by Franzén et al. [17] for the diagnosis of prostate carcinoma in 1960, the validity of this technique is well established in Sweden but still debated in other countries [9, 18]. Aspiration of tumors of the prostate results in a high yield of tumor cells (table 1). The proportion of tumor cells increased from 65% in well differentiated to 85% in poorly differentiated tumors. The proportion of inflammatory cells was about 4%. Samples with higher proportions of inflammatory cells were excluded. Two biopsies were taken for cytology and one or two for flow cytometry. The smears were air dried, stained with May-Grünwald-Giesma solution and classified as well, moderately and poorly differentiated according to the criteria of Esposti [3]. Tumor stage was assessed by rectal examination and classified according to UICC [21]. Bone metastases were assumed to be present if bone scans followed by X-ray examination were positive for metastases. If only bone scans were done, the tumors were categorised MX. For flow cytometry, the cells were fixed in ice-cold 96% ethanol. After pepsin and RNase treatment, the cell nuclei were stained and analyzed in a flow cytometer as previously described in detail [43]. The tumors were classified as diploid, tetraploid, or nontetraploid aneuploid. Tetraploidy was assumed if the proportion of tetraploid cells exceeded the mean value of tetraploid cells found in benign lesions by +2 SD and, if, in addition, a peak at the octoploid position was present (see below).

Surgical biopsies were mechanically dispersed, fixed in ethanol, and further treated as cells from fine needle aspirates. A part of the tissue specimen was prepared for comparative histopathological evaluation.

Table 1. Composition of cell material (%) obtained by fine-needle aspiration biopsy from hyperplastic and malignant prostates: mean values ± SD

	Number of cases	Tumor cells	Benign epithelium	Poly- and mononuclear cells	Unidentified cells
Hyperplasia	10	–	89.9 ± 6.5	9.3 ± 5.7	0.8 ± 1.6
Well differentiated	28	65.3 ± 19.9	23.2 ± 18.7	2.1 ± 2.6	9.4 ± 7.4
Moderately differentiated	26	72.8 ± 21.8	15.2 ± 20.1	3.9 ± 6.8	8.1 ± 2.5
Poorly differentiated	16	85.2 ± 13.4	2.5 ± 7.3	5.1 ± 8.8	7.2 ± 8.6

Statistical Evaluation

The difference between the groups was calculated with the t test or χ^2 test. Life-table method was used for survival analysis and the curves were compared by the log-rank test. The prognostic strength of different patient, tumor, and treatment parameters was assessed by Cox' regression for multivariate analysis.

Results

Cytologic Benign Prostate Lesions

DNA Ploidy and Morphology. The detection rate of prostate carcinomas in a population depends on the number of patients submitted for examination and the diagnostic accuracy by which lesions can be separated into benign and malignant. During a period of about 3 years, 505 patients with cytologic benign prostate lesions were studied by DNA flow cytometry. The aim was to establish the DNA characteristics of benign prostate lesions to be used as the basis for the assessment of malignant lesions. Furthermore, by follow-up of the patients we wanted to establish the reliability of the diagnostic procedure. Finally, since the age distribution of the patients was about the same as in patients with malignant prostate lesions, these patients with benign lesions served as controls in survival studies of prostate cancer patients.

Of 505 patients with benign lesions, the DNA histograms from 8 (1.6%) were clearly aneuploid. All histograms with one peak at the diploid position and with the same position as standard lymphocytes had also a second peak at the tetraploid position comprising between 0.1 and 33% of the cell material. The proportion of tetraploid cells were Gaussian distributed when depicted in a logarithmic scale. The mean value was 3.1 ± 2.2 (1 SD). As the upper borderline for diploid tumors, +2 SD (8%) was chosen. Cell populations exceeding this border were called tetraploid. All these tetraploid cell populations also contained a small octoploid peak.

The results of the measurements from these 505 cytologic benign lesions are summarized in table 2. 41 samples with slight cytologic atypia but without any suspicion of malignancy are shown separately. 96.2% of all samples were diploid. The frequencies of tetraploid and aneuploid histograms were 2.2 and 1.6%, respectively; most of the tetraploid histograms were at the border of 8% cells. A relatively high number of tetraploid histograms were found among the samples with slight atypia. These figures of abnormal DNA histograms are much lower than described by others in benign prostate lesions, with gross abnormal DNA histograms in about 25% [10]. The quality of the cell material of the fine-needle aspirates obtained, preparation and analysis techniques in flow cytometry as well as the cytological interpretation of the cell material can be discussed as possible reasons for these differences.

Follow-Up. The median age of the 505 patients studied was 69 years (range 26–89 years). The mean follow-up time of all patients was 7 years and that of patients still alive 10 years. The probability of 5 and 10 years survival was 70 and 50%, respectively. The probability of 10 years' survival decreased with increasing age from 90% for men <55 years old to less than 15% for men >75 years of age.

The causes of death were obtained from the Regional Swedish Causes of Death and Cancer Registries in which all patients are registered. Of the 505 patients 46% were alive, 51% died of other diseases than prostate carcinoma and 3% died of prostate carcinoma.

Of particular interest was the frequency of occurrence of prostate carcinomas during follow-up. The diagnosis of prostate carcinoma was based on histological or cytological examinations and excludes carcinomas detected postmortem by autopsy. Fifty prostate carcinomas (10%) among the 505 patients were registered. In table 3, the newly detected prostate carcinomas were subdivided according to the results of the initial cytological judgement

and DNA measurement. The incidence rate among patients with atypia was 22% and significantly higher than the 9% among those with benign lesion ($p < 0.001$). The incidence rate of 38% in aneuploid biopsies differed significantly from those found in diploid and tetraploid biopsies, 9.5 and 9.1, respectively ($p < 0.001$).

The importance of atypia for the subsequent diagnosis of cancer in the fine-needle aspiration cytology of the prostate confirms the experiences of others [40, 41]. The observation of subsequent malignancy in 37% of aneuploid DNA histograms stresses the importance of careful further evaluation of patients with such findings.

In order to discuss the reliability of the cytologic diagnosis, the time at which the prostate carcinoma was detected was related to the time of the primary cytological evaluation. Thirteen (26%) of the cancers were detected around the time of the fine-needle aspiration biopsy while the remaining were found 2.5–12 years later. Of the 13 patients diagnosed early, histopathology of resected cell material revealed carcinomas in 6 patients. One of these patients died early of a poorly differentiated prostate carcinoma. The remaining 5 are, however, still living and without further signs of clinical or morphological prostate carcinomas and the significance of the histopathologic diagnosis in these patients is more doubtful. In the other 7 patients the diagnosis was changed from benign to malignant after repeated fine-needle aspiration biopsy. Thus, 8 of 505 biopsies (1.6%) were clearly false negative. False-negative results between 0 and 34% have been reported in the literature [19].

Untreated Prostate Carcinomas

In the consideration of therapy of the localized prostate carcinoma active surveillance, i.e. delayed therapy, until tumor progression is one of the therapeutic options. Two recent Swedish studies [1, 24] try to answer the question whether delayed therapy is an appropriate treatment alternative. Two hundred and twenty-three and 146 patients, respectively, with mostly low-grade, low-stage tumors were followed by active surveillance. At approximately 5 years follow-up, only 15 patients had died of prostate cancer. The rate of local progression was, however, high and progression-free survival rates varied between 72 and 20% depending on tumor characteristics. Metastases developed in 35 patients.

In one of the studies, DNA ploidy of the tumor was included, which gave significant and independent prognostic information in favor of diploid tumors but showed, nevertheless, that half of the diploid tumors progressed locally during the 5-year follow-up [1].

Table 2. Relationship between cytology and DNA ploidy in 505 fine-needle biopsies of the nonmalignant prostate

	Diploid		Tetraploid		Aneuploid		Total
	n	%	n	%	n	%	
Benign	450	97.0	7	1.5	7	1.5	464
Atypia	36	87.8	4	9.8	1	2.4	41
Total	486	96.2	11	2.2	8	1.6	505

Table 3. Frequency of prostate carcinomas detected during follow-up of 505 patients with cytologic benign prostate lesions in relation to cytologic findings and results of flow cytometric DNA measurements

		Tumors	Patients	Percentage
Cytology	Benign	41	464	8.8
	Atypia	9	41	22.0
DNA analysis	Diploid	46	486	9.5
	Tetraploid	1	11	9.1
	Aneuploid	3	8	37.5

In the present extended study, a large number of patients were followed by active surveillance for more than 10 years. This study on the natural history of the untreated prostate carcinoma may contribute to the discussion about surveillance without therapy.

Patients and Tumor Characteristics. Two hundred and eighty-seven patients with a median age of 71.7 years (range 48–93 years) were included in the study. As can be seen from table 4 in which grade, stage and the DNA characteristics of the tumors are summarized, part of the patients had high-grade, high-stage tumors. These patients, who were untreated for various reasons and not under active surveillance were included to further illustrate the natural course of the disease. The selected group of patients under active surveillance is also discussed separately. Ninety percent of the tumors were well or moderately differentiated and 64% were confined to the prostate. Distant metastases were present in 4.6% of the patients, but reports of both bone scans and X-ray studies were available in only two thirds of the patients, mostly with low-grade, low-stage tumors.

52.6% of the tumors were diploid, 24.7% tetraploid and 22.7% aneuploid. There was a clear correlation

Table 4. Relationship between clinical stage, cytological grade and DNA ploidy in 287 untreated patientes with prostate carcinoma

	Total		Ploidy					
			diploid		tetraploid		aneuploid	
	n	%	n	%	n	%	n	%
Grade								
1	139	48.4	102	73.4	26	18.7	11	7.9
2	121	42.2	46	38	40	33.1	35	28.9
3	27	9.4	3	11.1	5	18.5	19	70.4
			151	52.6	71	24.7	65	22.7
Stage								
1	48	16.7	38	79.2	7	14.6	3	6.2
2	137	47.7	82	59.8	36	26.3	19	13.9
3	90	31.4	30	33.3	26	28.9	34	37.8
4	12	4.2	1	8.3	2	16.7	9	75.0

between ploidy and grade: 73% of grade 1 tumors were diploid and 70% of grade 3 tumors were aneuploid. Of grade 2 tumors, 1/3 were diploid, 1/3 tetraploid and 1/3 aneuploid. Thus, particularly this tumor type could be further subdivided according to ploidy.

There was also a clear correlation between tumor stage and ploidy: 79% of stage 1 tumors were diploid and 75% of stage 4 tumors were aneuploid. Again, tumors of stages 2 and 3 could be subdivided into three fairly equal groups of diploid, tetraploid and aneuploid tumors. These findings are in good agreement with the experiences from a large material of newly detected prostate carcinomas [43].

Follow-Up. Mean follow-up time of all 287 patients and of the living patients only were 7 and 11 years, respectively. Eighty-seven (30%) were alive, 80 (28%) had died of cancer and 120 (42%) had died of intercurrent diseases. Crude survival and corrected survival of all 287 patients are shown in figure 1. Five and 10 years' survival rates were 83 and 67%, respectively. An annual death rate of cancer of about 3% was unchanged up to 12 years, but increased after this time.

The patients were subdivided into three age groups of patients: <65, 65–75, and >75 years of age. The corrected survival of men below 65 and 65–75 years of age was equal but decreased significantly in the higher age group. Age appears, thus, as a significant prognostic factor.

Grade, stage and ploidy were all significantly related to survival. This is shown in figures 2–4. The difference within the large group of patients with well or moderately differentiated tumors mostly confined to the prostate was at the margin of significance. In fact, when the 13 patients with distant metastases with a known poor prognosis were excluded, the small difference between well and moderately differentiated tumors became insignificant.

The marginal difference in survival of patients with grade 1 and grade 2 tumors was unexpected and has not been found in the studies of others [24]. This illustrates the known difficulties in subdividing well and moderately differentiated tumors in an objective and reproducible fashion [20].

In spite of the well-known limitations of staging the prostate carcinoma by rectal examination, tumor stage was highly prognostic. There was, however, no difference between stages 1 and 2 and it should also be recognized that the difference in survival between stage 1 + 2 and stage 3 tumors, as shown in figure 3, only means that death is delayed by about 4 years.

While the difference between diploid and tetraploid tumors was not significant aneuploidy had a highly significant impact on survival (fig. 4).

Cox' multivariate analysis includes age, stage, grade and ploidy and the presence or absence of metastases. All these variables were prognostically significant when all 287 untreated patients were included in the analysis. When the 21 patients with M1 and T4 tumors were excluded, age lost significance and the strongest prognostic variable was grade ($p = 0.008$) followed by ploidy ($p = 0.0269$) and stage ($p = 0.028$). When grade 3 tumors were also excluded, leaving 254 patients with grade 1 and 2, stage 1–3 tumors, ploidy was the only significant prognostic factor ($p = 0.014$). When stage was excluded, the significance of ploidy further increased to $p = 0.0010$. Considering only patients with grade 1 and 2 tumors confined to the prostate, the only prognostic significant factor was ploidy ($p = 0.0003$).

The conclusion from this comparison of various properties of the untreated prostate carcinomas was that all assessed clinical, morphological, and DNA characteristics provided prognostic information but ploidy was the most powerful to predict prognosis in low-grade, low-stage tumors.

Changes of Ploidy during Follow-Up. The prognostic value of any tumor characteristic depends on its stability. If the genome remains unchanged after the initial transformation, the prognostic value of certain tumor properties such as grade and ploidy is high but becomes more

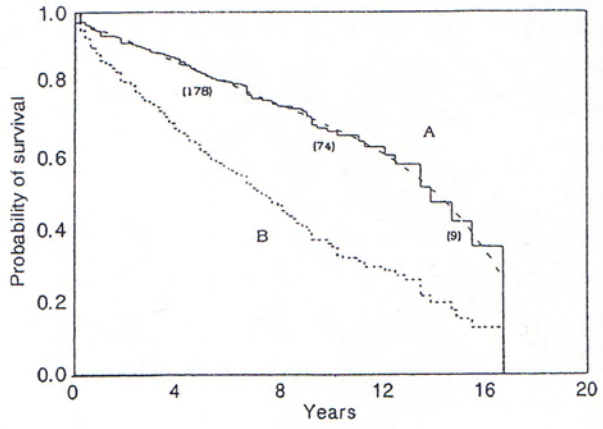

Fig. 1. Crude survival (B) and corrected survival (A) of 287 patients with untreated prostate carcinomas.

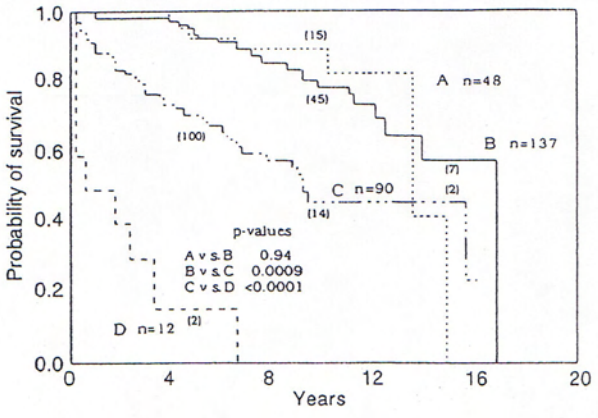

Fig. 3. Corrected survival of 287 patients with untreated prostate carcinomas in relation to tumor stage. A = Stage 1; B = stage 2; C = stage 3; D = stage 4.

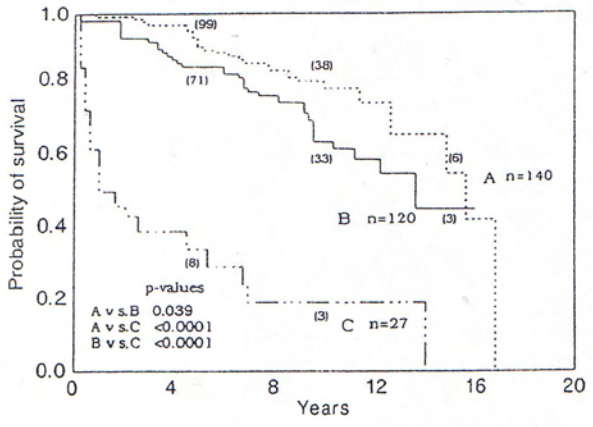

Fig. 2. Corrected survival of 287 patients with untreated prostate carcinomas in relation to tumor grade. A = Grade 1; B = grade 2; C = grade 3.

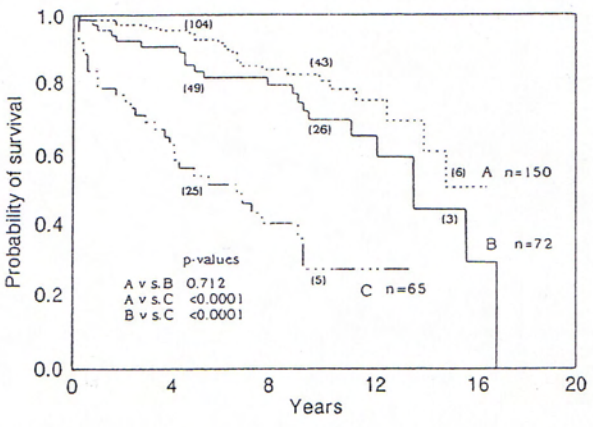

Fig. 4. Corrected survival of 287 patients with untreated prostate carcinomas in relation to tumor ploidy. A = Diploid; B = tetraploid; C = aneuploid.

uncertain if the genome is unstable and changes with time.

The results of repeated biopsies in untreated patients are of obvious interest in this context. Altogether 146 patients were studied by DNA measurements during a mean observation time of 2.0 years. The mean number of biopsies/patient was 3.25. Of the 87 patients with diploid tumors 59 (68%) remained unchanged diploid and 28(32%) changed. Of these 28 tumors, 18 (64%) changed to tetraploid and 11 (36%) to aneuploid. Of the 38 patients with tetraploid tumors 26 (68%) remained tetraploid, 11 (29%) changed to aneuploid and 1 (3%) to diploid. Among the 21 aneuploid tumors, ploidy of 12 (57%) remained unchanged but changed in 9 (43%). The mean annual rate of ploidy changes of all tumors was 16.6% and was nearly the same in diploid, tetraploid and aneuploid tumors.

Table 5. Tumor grade and stage in 309 hormonally treated patients with prostate carcinoma

Grade	Stage				Total	
	1	2	3	4	n	%
1	3	56	15	2	76	24.6
2	4	110	59	9	182	58.9
3	–	11	28	12	51	16.5
Total (%)	7 (2.3)	177 (57.3)	102 (33.0)	23 (7.4)	309	100

Table 6. Tumor grade, stage and ploidy in 111 patients with DNA analysis of the tumor before hormonal treatment (A) and in 198 patients with DNA analysis after initiation of hormone treatment (B)

		A		B	
		n	%	n	%
Grade	1	26	23.4	50	25.3
	2	59	53.2	123	62.1
	3	26	23.4	25	12.6
Stage	1	1	0.9	6	3.0
	2	45	40.5	132	66.7
	3	54	48.7	48	24.2
	4	11	9.9	12	6.1
Ploidy	Diploid	45	40.5	131	66.2
	Tetraploid	28	25.2	25	12.6
	Aneuploid	38	34.2	32	21.2

Table 7. Cox' multivariate analysis on corrected survival in 309 hormonally treated patients with prostate carcinoma

Factor	Coefficient	χ^2	p
Ploidy	0.65	8.56	<0.0001
Metastases	1.12	4.88	<0.0001
Age	0.04	3.61	0.0003
Stage	0.36	2.66	0.0077
Grade	0.31	2.15	0.0315

These results are in conformity with changes to higher tumor grade observed in repeated transuretral resections of the prostate [5] and to higher cytologic grade in repeated fine-needle aspiration biopsies [2].

The consequences of changes in the chromosomal composition of grossly aneuploid tumors may only increase their degree of malignancy but hardly the outcome. In diploid tumors with minor abnormalities and in tetraploid tumor, changes of the genome can be expected to have more serious consequences and may be a prerequisite for the change from low to high grade malignant behavior.

Therapy during Follow-Up. During follow-up of the 287 patients 89 (31%) received some kind of hormonal treatment after a mean observation time of 5.2 years (range 9 months to 15 years). There was no difference between the mean time for initiation of therapy of patients with diploid and tetraploid tumors: 5.3 and 5.9 years, respectively. However, the mean time between diagnosis and initiation of therapy in aneuploid tumors was 3.6 years after diagnosis and was significantly shorter than for diploid or tetraploid tumors ($p = 0.05$ and $p = 0.007$, respectively). This further demonstrates the high aggressiveness of the aneuploid tumors. Altogether, 23% of patients with diploid tumors, 52% with tetraploid and 18% of patients with aneuploid tumors received hormonal therapy. The low proportion of treated patients with aneuploid tumors depends on the high death rate of these patients with a 50% mortality already after 3 years. The significant difference in the proportion of treated patients with tetraploid tumors compared to those with diploid tumors is also a clear sign of the higher aggressiveness of tetraploid compared to diploid tumors, though the survival of patients with tetraploid tumors was not significantly different from patients with diploid tumors.

Hormonally Treated Prostate Carcinomas

Endocrine therapy is the cornerstone in the management of patients with advanced prostate carcinoma. Still, there is no general agreement whether T3, T4, M0 or asymptotic M1 patients should be treated immediately or first at the time of symptoms. In locally confined tumors, a rational discussion on hormone therapy will only be possible when the malignant potential of the tumors and the exact mechanisms of endocrine response on the cellular level are better understood.

To approach an answer on the malignant potential of the prostate carcinoma and the response of the tumor under hormone therapy, the results of nuclear DNA measurements in addition to conventional tumor character-

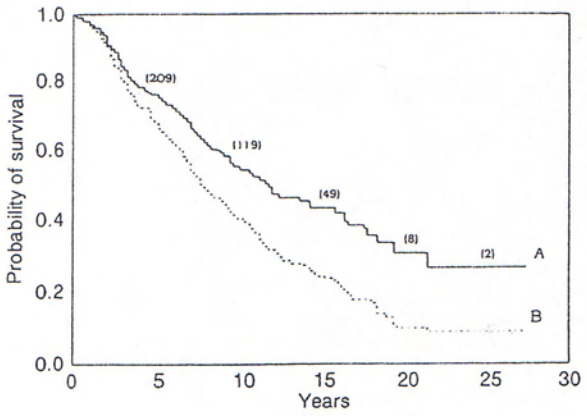

Fig. 5. Crude survival (B) and corrected survival (A) of 309 hormonally treated patients with prostate carcinoma.

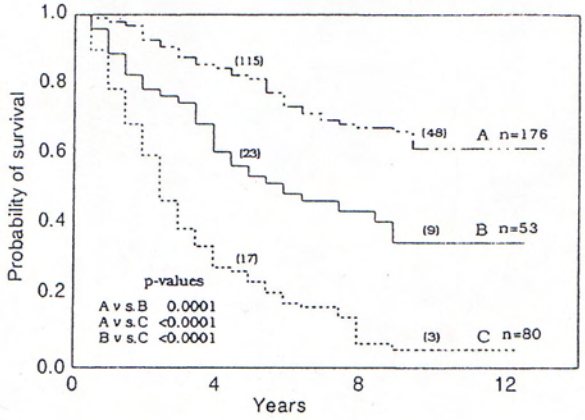

Fig. 6. Corrected survival of 309 hormonally treated patients with prostage carcinoma in relation to tumor ploidy. A = Diploid; B = tetraploid; C = aneuploid.

izations will be discussed. Patients given various forms of therapy: surgical castration, estrogen alone or in combination with nitrogen mustard (Estracyt) or antiandrogens were not assessed separately.

Patient and Tumor Characteristics A total of 309 patients with a median age of 69 years (range 47–86 years) were studied. There were two groups of patients: (A) one group of patients with newly detected prostate carcinomas in whom DNA measurements were done before treatment, and (B) another group also treated at diagnosis but in whom DNA measurements were first done 4.7 years (range 1 month to 20 years) after initiation of therapy. Thus, in the latter group the nuclear DNA content may have changed due to endocrine therapy, while the initial stage and grade of the tumor were known. Grade and stage of the tumors at diagnosis can be seen in table 5. The majority of the tumors were of grade 2 or 1 and in 60% confined to the prostate.

58% of the patients had M0 tumors, 15% M1 tumors but in 27% both bone scans and X-ray examination were not available (MX).

Ploidy characteristics of the tumors in relation to grade and stage at diagnosis are shown separately in table 6 for patients with DNA measurements at diagnosis (group A) and for those treated hormonally for 4.7 years (group B).

Some significant differences between the two groups can be noticed: the generally higher proportion of patients with high-grade, high-stage tumors and particularly the high proportion of DNA-abnormal tumors in group A in which DNA measurements were obtained at diagnosis. One reasonable explanation is selection of less-aggressive tumors after the death of patients during therapy in group B. Another explanation is the more frequent selection of low-grade, low-stage tumor patients for active surveillance among patients admitted and selected either for hormone treatment (group A) or active surveillance.

Follow-Up. Mean follow-up time of all 309 patients and of the living patients only was 8.6 and 14.7 years, respectively. Sixty-four (21%) were alive, 151 (49%) had died of cancer and 94 (30%) had died of intercurrent disease. Crude survival and corrected survival are shown in figure 5. Crude survival after 5, 10 and 15 years was 68, 40 and 25% and corrected survival 76, 56 and 44%, respectively.

Age has a prognostic impact supporting the experience made in untreated patients. The cancer specific survival of men <75 years of age was significantly better than for men >75 years ($p < 0.001$).

Stage and grade of the tumor were also significantly related to death in tumor. There were, however, no significant differences between stage 1 and 2 tumors, stage 3 and 4 tumors or between grade 1 and 2 tumors.

By classification of the tumors into diploid, tetraploid and aneuploid three significantly different subgroups of patients were obtained (fig. 6). Entirely the same results were achieved when the patients with bone metastases were excluded.

Cox' multivariate analysis of the various tumor characteristics including age and metastases showed significant impact of all variables (table 7). Dominating was

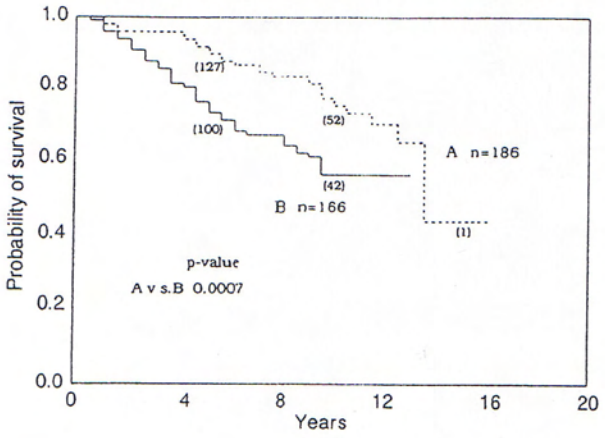

Fig. 7. Comparison between corrected survival of 186 untreated (A) and 166 hormonally treated (B) patients with prostate carcinoma grade 1 and 2, stage 1 and 2.

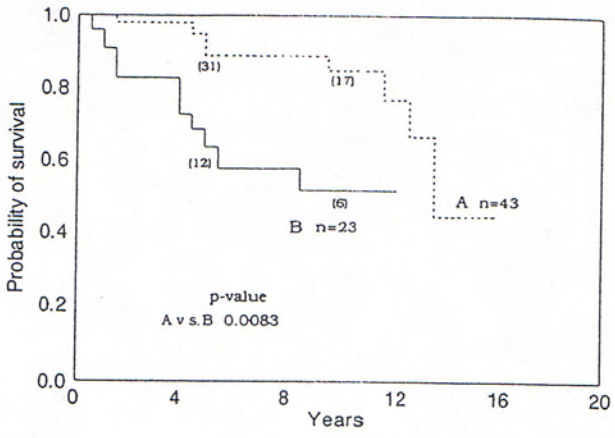

Fig. 9. Comparison between corrected survival of 43 untreated (A) and 23 hormonally treated (B) patients with tetraploid prostate carcinoma grade 1 and 2, stage 1 and 2.

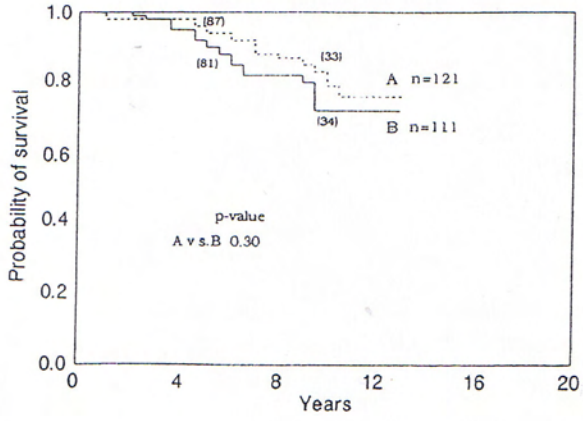

Fig. 8. Comparison between corrected survival of 121 untreated (A) and 111 hormonally treated (B) patients with diploid prostate carcinoma grade 1 and 2, stage 1 and 2.

ploidy, followed by metastases after exclusion of ploidy. After exclusion of metastases, stage, grade and age were still significant.

Comparison between Untreated and Hormonally Treated Patients

To decide whether a certain therapy is superior to another, randomised clinical studies are needed. Thus, the aim of this comparison between hormonally treated and untreated patients was only to get essential information for future investigations and for strategies in the designing of clinical studies.

Crude survival and corrected survival of all untreated and all hormonally treated patients showed no significant or only marginally significant differences. Both the untreated and treated groups were, however, highly heterogeneous in respect to grade, stage, ploidy of the tumors and presence of metastases (see tables 4, 6). The proportion of high-grade, high-stage tumors among the treated patients was, however, only slightly higher than in the untreated patients (46 and 37%, respectively).

Considering only patients with stages 1 and 2 and grade 1 and 2 tumors without metastasis 186 untreated patients and 166 hormonally treated patients were identified. As already shown, there were no or only marginal differences in survival between patients with grade 1 and 2 tumors or tumors of stage 1 and 2 in both groups. The age of both groups was the same, 71.5 and 71.3 years, and also the proportion of diploid, tetraploid and aneuploid tumors: in the untreated group 65, 23 and 12% and in the hormonally treated patients 67, 14 and 19%, respectively.

Corrected survival of the untreated patients was significantly better compared with the hormonally treated patients (fig. 7). A highly significant difference remained when only the 78 and 108 grade 2 tumors of the both groups were selected ($p = 0.014$) [data not shown].

For the further analysis, the possible influence of ploidy was studied in these low-grade, low-stage tumors.

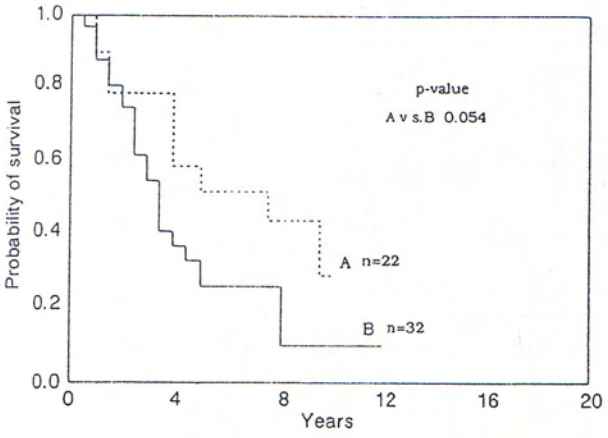

Fig. 10. Comparison between corrected survival of 22 untreated (A) and 32 hormonally (B) treated patients with aneuploid prostate carcinoma grade 1 and 2, stage 1 and 2.

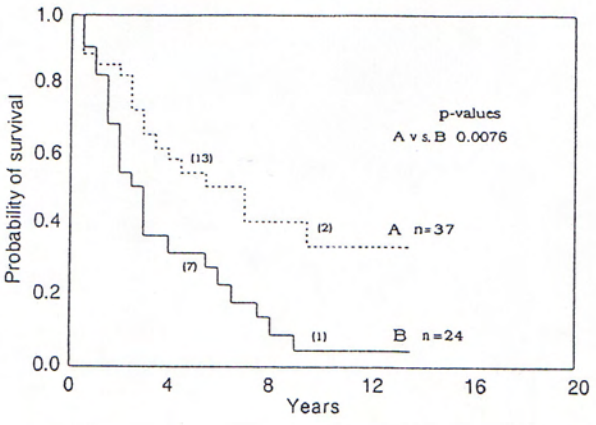

Fig. 11. Comparison between 37 untreated (A) and 24 hormonally treated (B) patietns with aneuploid prostate carcinoma grade 3, stage T3 and T4.

The results are depicted in figures 8–10. There was no significant difference between untreated and hormonally treated patients with diploid tumors. However, untreated patients with either tetraploid or aneuploid tumors had significantly better survival.

Among the high-grade, high-stage tumors of the untreated and hormonally treated patients without bone metastases 97 patients in each group were identified. The difference in survival between these groups was not significant. There was no difference in survival between patients with diploid tumors and the difference between patients with tetraploid tumors did not reach significance either. However, survival of patients with aneuploid tumors was significantly lower in hormonally treated patients (fig. 11).

When survival of all untreated patients was compared with survival of all hormonally treated patients excluding those with bone metastases, survival of patients with diploid tumors did not differ. The differences in survival of patients with tetraploid and aneuploid tumors were, however, highly significant [data not shown].

Comparison between Untreated Patients and Patients with Benign Lesions

Crude survival of untreated patients was significantly lower than survival of patients with benign prostate lesions. When, however, only the untreated patients with low-grade, low-stage tumors were considered, no difference in crude survival was found (not shown).

Discussion

One of the aims of this study was to evaluate the value of nuclear DNA measurements in cell material from fine-needle aspirates in the assessment of prostate carcinomas. Tumors were subdivided into diploid, tetraploid and aneuploid. The subdivision into diploid tumors with small chromosomal aberrations, not detectable by quantitative nuclear DNA measurements, and into gross chromosomal aberrant aneuploid tumors is not debatable.

A subject of general discussion is, however, the border between diploid and tetraploid tumors. In this study, for the border between diploid and tetraploid tumors, 8% cells at the tetraploid position were chosen. Obviously, this border is not strict and depends on the proportion of admixed diploid cells to the tetraploid cell population. According to the quantitative morphologic assessment of the cell material, about 75% of the cells in the aspirates were tumor cells.

The reproducibility of tetraploidy or aneuploidy was, in fact, quite high. In repeated biopsies of tetraploid or aneuploid tumors from 59 untreated patients, a change from tetraploid to diploid was found in only 1 patient.

The biological relevance of the subdivision into diploid and tetraploid tumors can also be discussed. Both by random measurements of a large number of Feulgen-stained and morphologically identified tumor cells, and by the comparison of the proportion of tetraploid cells as measured by flow cytometry and the proportion of microscopically identified tumor cell nuclei in the cell suspen-

sions, all tetraploid tumors with few exceptions contained diploid tumor cells. This is also true for aneuploid tumors. Thus, tumors defined as tetraploid but also aneuploid tumors are mixed with diploid tumor cells. By the follow-up of untreated patients with prostate carcinoma, no significant differences in the behavior of diploid and tetraploid tumors were observed. However, in hormonally treated patients, significant differences between these two tumor categories appeared justifying this subdivision.

The finding of changes in the gross chromosomal composition of the tumors with time as found by repeated biopsies during follow-up supports the concept of progressive dedifferentiation of the prostate carcinoma [43]. This concept was based on the observation of the relationship of the tumor ploidy with stage of the tumors. In this relationship, the proportion of diploid tumors decreased and the proportion of aneuploid tumors increased exponentially with increasing tumor stage. Tetraploid tumors increased with a maximum at stages 2 to 3 but decreased at stage 4. This correlation is typical for the flow through a three-compartment system and appears to be valid for the prostate carcinoma.

In such a system, after the initial transformation the near diploid genome doubles to tetraploid but changes further to nontetraploid aneuploid due to loss of chromosomal material.

An important consequence of the assumption of such a compartment system for prostate carcinoma is the coexistence of several tumor cell populations. Coexistence of diploid with tetraploid or aneuploid tumor cells is also the rule in prostate carcinomas according to results on fine-needle aspirates as already discussed. Tumor heterogeneity was further confirmed by the study of eight different parts from each of 27 T0 prostate carcinomas. Surgical biopsies were obtained by fractionated transuretral resection. In 75% of tetraploid or aneuploid tumors additional diploid tumor parts were found [unpubl.].

From the view point of therapy, tumor heterogeneity has been discussed as one of the major difficulties and the reason for the failures of chemotherapy. In prostate carcinoma, coexistence of androgen-dependent and independent cell populations can be expected to be a consequence of the tumor heterogeneity and the reason for real therapeutic problems: as far as a tumor contains a single, hormone-dependent cell population, androgen deprivation will result in excellent clinical response with shrinking of the tumor, release of pain, etc. In a tumor composed of hormone-dependent and -independent parts, androgen deprivation will initially also result in good clinical response, corresponding to the size of the hormone-dependent part of the tumor. Elimination of the hormone-dependent part of the tumor may, however, have the adverse effect that the hormone-independent part of the tumor, now without competition by its brother, gains advantages in growth conditions.

In the light of such a view, the differences in survival of untreated and hormonally treated patients can be interpreted in the following way: Diploid, mostly well or moderately differentiated tumors, are hormone dependent and may remain silent for a long time upon androgen deprivation. The further progress into tetraploid or aneuploid variants may also be suppressed resulting in long term survival of a part of these patients. Follow-up of patients with diploid tumors up to 14 years in this study was, however, not long enough to decide whether hormonally treated patients really have an advantage over untreated patients.

Grossly aneuploid, moderately or poorly differentiated tumors are hormone independent. Androgen deprivation eliminates hormone-dependent parts of the tumor. The result is more rapid progression to disseminated disease and significantly shorter survival of these patients compared to untreated patients.

Most interesting is the reaction of tetraploid tumors, which usually remain stable for many years in the presence of androgens but progress rather rapidly upon androgen deprivation.

Considering active surveillance of patients with the aim of androgen deprivation when tumor progression appears, the obvious problem is to define the most appropriate time for initiation of therapy. In this context, tumor size and growth outside the prostate are clinically important parameters. As described by McNeal et al. [29], loss of differentiation and capacity to give rise to metastases was strongly correlated with tumor volume. The critical size was 4 cm^3. The increase of abnormal DNA patterns with tumor size was confirmed by the correlation of tumor size as measured by sonography with the results of fine-needle biopsies. In 45 tumors <2 cm^3, tetraploidy was found in 10 (22%) and aneuploidy in 1 (2%), in 17 tumors >2 cm^3 tetraploidy was found in 7 (41%) and aneuploidy in 3 (10%) [unpubl.]. According to these results, the critical tumor size for initiation of hormone therapy is rather less than 4 cm^3.

In conclusion, tumor heterogeneity and continuous change towards increasing malignancy and heterogeneity of the tumor are serious problems in the handling of prostate carcinomas. In the choice of therapy, life expectancy of the patient, stage of development of the tumor reached,

and the expected further development of the tumor have to be considered. Fine-needle aspiration biopsy combined with measurements of the nuclear DNA content obviously provide significant information on the state of tumor development. To identify the factors that govern the speed of development of prostate carcinoma is one of the important subjects of further research and one of the prerequisites for individual patient management.

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