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Clinical utility of cellular DNA measurements in prostate carcinoma

Chairman: F. Schröder, Department of Urology and Nephrology, Erasmus University, Rotterdam, The Netherlands

Co-chairman: B. Tribukait, Department of Medical Radiobiology, Karolinska Institute, Stockholm, Sweden

Participants: A. Böcking, Institute of Pathology, Technical University of Aachen, Germany
R. deVere White, Dept. of Urology, Univ. of California, Sacramento, CA, USA
L. Koss, Dept. of Pathology, Montefiore Medical Center, Bronx NY, USA
M. Lieber, Dept. of Urology, Mayo Clinic, Rochester MN, USA
B. Stenkvist, Dept. of Pathology, Karolinska Hospital, Stockholm, Sweden
A. Zetterberg, Dept. of Tumour Pathology, Karolinska Hospital, Stockholm, Sweden

Abstract. At a WHO consensus conference on Early Diagnosis and Prognostic Parameters in Localized Prostate Cancer, a working group discussed the clinical utility of DNA measurements in stages T2 and T3 prostate carcinoma. Incidentally discovered prostate cancer of stage T1 was excluded. The members of the working group, representing various clinical and laboratory disciplines, discussed technical considerations of DNA measurements by flow and image analysis, pretreatment prediction of prognosis, and posttreatment clinical relevance. The group agreed to subdivide tumors into diploid, tetraploid and non-tetraploid aneuploid, expressing various degrees of aggressiveness and gave guidance for the definition of limits of these groups. The panel agreed that knowledge on DNA ploidy prior to treatment is of value in treatment decisions, particularly when surveillance is a treatment option. Aneuploid tumors can be expected to respond very poorly to either irradiation or endocrine therapy, and the presence of aneuploid tumor, either on pretreatment biopsies or in radical prostatectomy specimens, is an ominous sign. The identification of a group of patients with a uniformly poor prognosis should encourage medical oncologists and basic scientists to develop adequate treatment options for this particular group. The panel expressed a strong opinion that DNA ploidy should be uniformly studied in clinical trials, particularly in patients with localized prostate cancer.

INTRODUCTION

The first report on the relationship of DNA ploidy of prostate carcinoma with prognosis appeared in 1966 (Tavares *et al.*). Two important observations were described in this study. The survival of patients with diploid and tetraploid tumors was identical with survival of the general population of same age; the good response of such tumors to estrogen therapy was also noted. This was in contrast to the very bad outcome of patients with triploid or hexaploid tumors that were also resistant to estrogen therapy. Since that time a large number of studies of ploidy related to various clinical aspects of prostate cancer have been published, particularly since the introduction of flow cytometry for DNA measurements about twenty years ago. The published literature has recently been critically evaluated by Shanky *et al.*, (1993).

The statements in this paper relate exclusively to prostate cancer limited to the gland (categories T2 and T3). The working group did not deal with incidentally discovered prostate cancer (T1 disease).

The discussions of this working group were focused on the following subjects: Technical considerations of DNA measurements by flow and image analysis: pretreatment clinical relevance and prediction of prognosis, and posttreatment clinical relevance.

1. TECHNICAL ASPECTS

1.1. Samples

Specimens from fresh fixed or paraffin embedded material can be used for both image and flow cytometric measurements. The advantage in the use of fresh fixed material is the higher quality of measurements. The measurements on paraffin embedded material can be performed on selected areas of tumors and enables examination of archival specimens. However, in some cases, a loss of aneuploidy has been observed in nuclei isolated from paraffin embedded tissue (Koss, personal communication). The presence of representative tumor material in fresh material processed by flow cytometry should always be confirmed by histological or cytological inspection of adjacent tissue or an aliquot of material secured by an aspiration biopsy. The value of direct measurements of DNA content in histological sections was debated by the working group but the use of histological sections was not recommended.

The material for DNA ploidy determination may be obtained by a transurethral resection, fine-needle aspiration biopsy, punch biopsy or from prostatectomy specimens. The results depend on the skill of the clinician in obtaining proper material and on specimen processing. Flow cytometry is limited by the very small numbers of tumor cells that may be available in some specimens. There was agreement that image analysis is probably the most useful technique for such specimens.

1.2. Fixation, preparation and staining

The techniques for fixation, isolation and staining of nuclei have been described for image analysis and for flow cytometric measurements. Using flow cytometry it is now possible to obtain routinely from fresh samples DNA histograms of high quality without clumping and a minimum amount of debris. In image analysis under well controlled conditions of specimen preparation and optimization of the measuring system the coefficient of variation of reference cells should be about 3%. For flow cytometry, fresh ethanol or formalin fixed tissues can be stored for long periods of time and can therefore be mailed to specialized centers for analyses (Alanen *et al.*, 1989, Castro *et al.*, 1993). The use of paraffin embedded tissue which is critically dependent on proper formalin fixation may change the interpretation of histograms in some cases. Bouin's fluid is unsuitable as a fixative for DNA measurements.

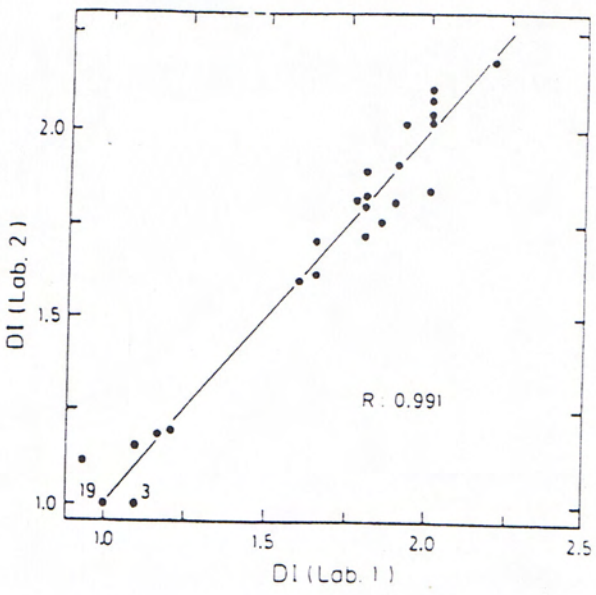

Fig. 1. Comparative determinations of the DNA-index of 45 paraffin-embedded specimens from prostate carcinomas made by two centres. The two laboratories applied their own techniques for preparation, staining and analyses of the cell-nuclei by flow-cytometry (Fossa *et al.*, 1992).

1.3. Quality control

The panel agreed that there is need for comparative studies among different laboratories to establish inter- and intra-observer agreement concerning DNA ploidy determination. The results of one of the few studies that are available in this respect are shown in Fig. 1 (Fosså *et al.*, 1992). Forty-five formalin fixed and paraffin embedded specimens from transurethral resections of prostate carcinoma and from lymph node metastases were analyzed by two laboratories using different techniques of cell preparation and staining and different flow cytometers. This figure shows that adequate agreement has been reached between the two laboratories.

1.4. Definitions

There was agreement that three groups of tumors with different prognostic significance can be identified: diploid, tetraploid and non-tetraploid aneuploid tumors.

The last group can be subdivided further into tumors with one or several aneuploid cell populations. The application of standard techniques allow the identification of diploid range tumors and of aneuploid tumors with fair reliability. The definition of tetraploid tumors is still somewhat uncertain. Tetraploid tumors in general appear to have a prognosis which is similar to diploid tumors. Commercially available machines may, however, not always be capable of differentiating between aneuploid and tetraploid tumors (Takai *et al.*, 1993).

The definition of diploid and tetraploid tumors as well as of the aneuploid group must be based on statistical analysis. Statistical cut off points presented during the discussion represent one possible approach to the solution of this problem. For high quality image analyses and flow cytometry using fresh cell material, a histogram with a single peak within limits of 1.9–2.1c (DNA index 0.95–1.05) and with <10% of cells at 4c is an acceptable definition of diploid tumors. The limits for a tetraploid tumor are DNA ploidy range of 3.9–4.1c (DNA index 1.95–2.05). A tetraploid tumor should have >10% of cells in tetraploid position; this corresponds to the 4c value +3 standard deviations above the mean for control benign tissue. An octaploid G2 peak may be observed in flow cytometry. In image analysis a hypertetraploid cell population may be observed.

1.5. Tumor heterogeneity

It is well known that a single prostate carcinoma specimen may contain several (up to 5) architecturally different tumor patterns. The multifocal origin of prostate cancer is also well documented. It is therefore not surprising the DNA heterogeneity has also been observed in such tumors. In one study 63 different architectural patterns of prostatic carcinomas removed by radical prostatectomy from 30 patients with disease confined to the prostate were investigated by image analysis (Green *et al.*, 1992). Within the same specimens, diploid and aneuploid tumors were found. The presence of diploid and aneuploid tumor cells within the same tumor has also been observed in material obtained by fine-needle aspiration or core biopsies of the prostate (Tribukait, 1991, van den Ouden *et al.*, 1993).

1.6. Accuracy of DNA ploidy in biopsy material

The use of biopsy material poses technical problems which may lead to serious limitations in the evaluation of DNA ploidy. Some of this is related to the difficulty of carrying out flow cytometric studies due to scarcity of material. Image analysis on tissue specimens is not accurate and may not allow the differentiation between tetraploid and aneuploid tumors.

It must also be considered that a biopsy may not be representative of the entire tumor. It has been shown that the grade of differentiation is often underestimated in prostate cancer on the basis of biopsy evidence. The same may be true for identifying aneuploid disease. In smaller tumors the aneuploid fraction may be small and may be missed on one or even several biopsies. One study (Böcking *et al.*, in preparation) reported an 11% failure in identifying aneuploid pattern of DNA on one biopsy. When two or three biopsies were taken that percentage decreased to 5% and 3%, respectively. The panel did recommend routine use of several DNA ploidy determinations per specimen.

There is agreement that the determination of DNA ploidy in core biopsy specimens is not an easy procedure. Care should be exercised in the interpretation of the data. Prospective studies comparing DNA ploidy in biopsies and radical prostatectomy specimens are recommended. Data from the Mayo Clinic issues suggest that the result of DNA ploidy studies comparing biopsies and radical prostatectomy specimens are not always identical. Many of the tumors identified as diploid by image analysis of the pre-treatment needle biopsy cores were found to be non-diploid when subsequent radical prostatectomy specimens were studied. (Takai *et al.*, 1993)

1.7 S-phase and growth fraction determination.

The determination of S-phase and growth fraction by means of flow cytometry is possible but has not been generally applied to prostatic carcinomas. The determination of the S-phase is very difficult in image analysis. At this time there is no agreement

Table I. *Information processing and data extraction in flow cytometry and image analysis*

	Flow cytometry	Image analysis cytophotometry
Sample preparation	Single particle suspension (complex)	Smear (simple)
Staining	Specific fluorochromes	Nonspecific or specific stains
Principal single parameter measurement	Histograms of fluorescence (i.e., DNA)	Histograms of digitized images based on light absorption or transmission (i.e., DNA, steroid receptors)
Two or more parameter measurements	Synchronous, limited (i.e., DNA vs. oncogene expression)	Synchronous or defined by stain
Morphologic features of cells	Very limited	Multiple feature extraction
Visual control	None (except by sorting)	Yes
Software	LIST mode histogram analysis. Cell cycle compartments (rapid)	Multifeature analysis
Cell sorting	Yes	No
Control samples	Multiple options	Multiple options with appropriate optics and software
Applicability to tissue	No	Yes

(Koss *et al.*, 1992)

on the usefulness of these parameters in spite of the fact that in some studies the determination of the S-phase had prognostic value (Visakorpi *et al.*,1991, Eskelinen *et al.*, 1991, van den Ouden *et al.*, 1993). New technology which makes use of immunofluorescence staining of cells in different phases of the cell cycle is becoming available and may replace other techniques in the near future.

Table II. *DNA ploidy in benign prostatic hyperplasia*

DNA histograms	n
Diploid	520
Non-diploid	11*
	531

* No cancer found in extensive studies of chips.
(Koss et al., 1992)

1.8. Flow cytometry versus image analysis.

Flow cytometry: The preparation techniques require technical excellence and accuracy. The machines are expensive. The performance and interpretation of flow data require a skilled and experienced technical team.

Image analysis: Preparatory techniques are less complicated and readily available to pathologists, who routinely deal with morphological assessment of cells and tissues. A standard of uniform interpretation of DNA histograms is, however, at present lacking which makes comparisons among studies in different centers difficult and limits a widespread clinical application of this technique.

In the hands of an experienced team, the result of flow cytometry and image analysis are comparable. Flow cytometry is superior at identifying the DNA index of aneuploid cell populations but may

Table III. *DNA ploidy in relation to cytology in 505 fine-needle biopsies of the non-malignant 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

Tribukait, 1992

Table IV. *Frequency of diploid, tetraploid and non-tetraploid aneuploid cell lines in fine-needle aspiration biopsies of untreated prostate carcinomas as related to cytologic grade*

Degree of differentiation	Diploid	Aneuploid		Multiple cell lines	Total
		Tetraploid	Non-tetraploid		
Well	199 (58%)	110 (32%)	25 (7%)	10 (3%)	344
Moderate	113 (29%)	144 (37%)	106 (28%)	23 (6%)	386
Poor	13 (10%)	31 (23%)	56 (41%)	36 (26%)	136

Tribukait, 1991

be seriously limited if the specimens contain only a small number of tumor cells. In such cases image analysis may offer a clear advantage. A summary of the advantages and disadvantages of image analysis versus flow cytometry of prostate carcinoma is given in Table I.

2. DNA PLOIDY IN RELATION TO TUMOR CHARACTERISTICS AT INITIAL DIAGNOSIS AND IN PROSTATECTOMY SPECIMENS

2.1. DNA ploidy of epithelial elements of benign prostatic hyperplasia

The epithelial elements of BPH may not always be diploid. Three studies (Deitch *et al.*, 1989, Koss *et al.*, 1992, Tribukait, 1993) show that non-diploid cells occur in 2–4% of cases of BPH. Representative data are shown in Tables II and III. The origin of these cells and their prognostic significance are unknown. In a study of 505 patients with apparent benign prostatic hyperplasia, Tribukait (1993) observed that after a mean follow-up of 7 years 38% of the patients with aneuploid cell populations developed clinically manifest prostate cancer.

2.2. Relationship of DNA ploidy to tumor grade and stage (T category)

There is a clear correlation between tumor grade and stage with respect to ploidy, which was reported in several publications. Most of the well differentiated tumors are in the diploid range, whereas most of the poorly differentiated tumors are in the aneuploid range. There is a broad range of intermediate grade tumors which may be either diploid, tetraploid or aneuploid. Table IV illustrates the relationship between tumor grade and DNA ploidy. The correlation between ploidy and stage (T-category) is shown in Table V. Information on DNA

Table V. *DNA ploidy in relation to tumor stage. Data from [A] Koss et al., (1992) and [B] Tribukait (1991)*

A. Proportion of diploid prostate carcinomas in stages A, B, C, and D*

Stage	n	%
A	35	100
B	30	62.5
C	7	53.8
D	9	19.1

*Prospective and retrospective study.

B. Frequency of diploid and aneuploid cell lines in fine-needle aspiration biopsies of untreated prostate carcinomas in stages T1, T2, T3 and T4

Tumor stage	Diploid	Aneuploid		Multiple cell lines	Total
		Tetraploid	Non-tetraploid		
T1	27 (80%)	5 (14%)	2 (6%)	0	34
T2	51 (34%)	72 (49%)	19 (13%)	6 (4%)	148
T3	33 (12%)	129 (48%)	68 (26%)	37 (14%)	267
T4	1 (2%)	18 (36%)	16 (32%)	16 (30%)	51

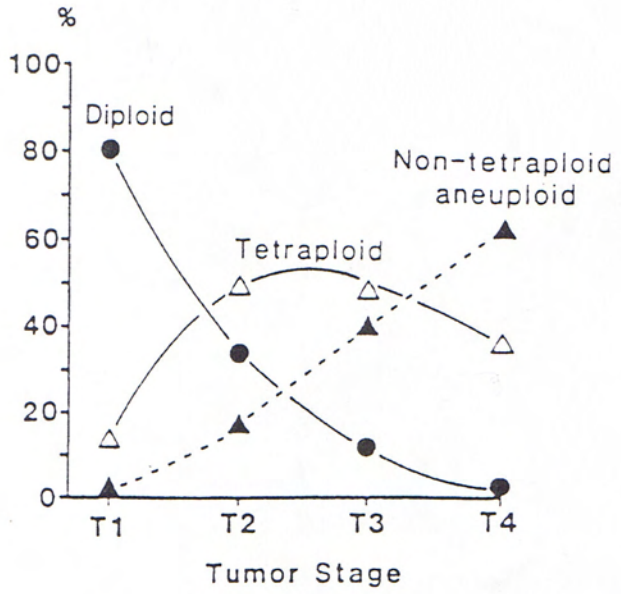

Fig. 2. Frequency of diploid, tetraploid and non-tetraploid aneuploid cell lines found in fine-needle aspiration biopsies of 500 untreated carcinomas of the prostate in relation to tumor stage. The curves are best fits calculated according to a three-compartment system (Tribukait, 1991).

ploidy in T2 disease is available and was reviewed. Tribukait (1991) reported on 148 patients with locally confined disease where he found diploid, tetraploid range and aneuploid tumors in 34%, 49% and 17% respectively. Koss *et al.* (1992), and Forsslund *et al.* (1992), dealing with 48 and 84 patients identified respectively 62% and 63% of diploid cases and 38% and 37% of aneuploid cases. Tetraploid cases were not identified in these series. In similar patients with lymph node metastases, van den Ouden *et al.* (1993), reported on 46 patients in whom DNA ploidy was studied in lymph nodes and in prostatic biopsies. There were 54% of diploid, 26% of tetraploid and 20% of aneuploid tumors. A study of a large number of prostatectomy specimens of a similar series of cases at the Mayo Clinic revealed diploid, tetraploid and aneuploid tumors in 68%, 28% and 4% of 261 cases (Montgomery *et al.*, 1990). The panel concluded that the distribution of ploidy among these sets of data was fairly similar.

The change of the ploidy pattern with increasing tumor stage observed in 500 untreated patients is shown in Fig. 2. Clearly, with increasing T-stage the proportion of diploid tumors decreases and non-tetraploid aneuploid tumors increases. Tetraploid tumors reach a maximum in T2–T3 stages. Similar relationships, which are typical for a three-compartment system, were observed within the same patient population for cytological grades 1–3 (Tribukait, 1991). These findings were the subject of an extensive discussion in the panel on the natural history of prostate carcinoma. Two options were considered: Aneuploid tumors may develop through stepwise development from diploid through tetraploid lesions. Alternately, a multifocal origin and subsequent selective clonal overgrowth were considered as a second option.

2.3. DNA Ploidy and prostate specific antigen (PSA)

Nativ *et al.* (1990), presented data on the relationship of nuclear DNA ploidy patterns and PSA levels. These data were obtained in 71 patients and show that all patients with a non-diploid tetraploid or aneuploid tumor had a PSA value above 4.0 ng/ml. All patients with PSA values below 4 ng/ml were seen in the DNA diploid group. Thus, normal PSA values below 4 ng/ml seem to have a high specificity of excluding non-diploid tumors. However, in a recent more extensive publication, also from the Mayo Clinic, comprising 687 patients, 26% and 6% of the patients with tetraploid and aneuploid tumors had PSA values below 4 ng/ml (Kleer *et al.*, 1993) Similar results have been reported by Badalament *et al.*, (1991).

2.4. Relationship of DNA ploidy to tumor volume, and other prognostic factors

As stated before when discussing the tumor staging, there is a correlation between DNA ploidy and tumor volume. Several studies are available which correlate in radical prostatectomy specimens tumor volume to other prognostic variables including DNA ploidy (Jones *et al.*, 1990, Greene *et al*, 1991, Deitch *et al.*, 1993). In these three studies, the tumors with the smallest volume were, with one single exception, diploid, irrespective of tumor grade. In one study of intraepithelial neoplasia (PIN) all foci of PIN, regardless of volume and grade displayed only diploid DNA profiles (de la Torre *et al.*, 1993). Seventy-three percent of large-volume tumors were non-diploid. Nearly all tumors of transition zone origin were diploid, irrespective size (Greene *et al.*, 1991). In about 25% of tumors confined to the prostate, non-diploid cell populations were found. In tumors with extracapsular

extension and/or seminal vesicle invasion the percentage of non-diploid tumors were 43% and 84% as reported by Jones *et al.* 1990, and Green *et al.*, 1991. In the large study of PT3 tumors reported by Nativ *et al.* (1989), 54% were non-diploid. Similarly, in the study by Lee *et al.* (1988), 58% of tumors with seminal vesicle invasion were non-diploid.

2.5. DNA ploidy and metastases

The metastatic potential of prostate carcinoma is obviously related to DNA ploidy. In a group of 419 patients with prostate carcinoma and bone metastases on admission, there were 7% with diploid tumors, 17% with tetraploid tumors, 28% with aneuploid tumors and 52% with tumors containing multiple aneuploid cell lines (Tribukait, 1987).

Although extensive data are lacking, there seems to be agreement on the similarity of DNA ploidy between the primary tumor and lymph node or other metastases. In 46 patients with local lymph node metastases there was an 85% agreement for the diploid and aneuploid tumors. In tetraploid tumors, however, the metastases were aneuploid or diploid in about 80% of the cases (van den Ouden *et al.*, 1993). It is still not clear how and why the change of DNA ploidy within the same lesion occurs and how often this event can be observed. It has been observed that metastatic deposits in local lymph nodes are more frequently diploid than in distant metastases. In combining three studies (van den Ouden *et al.*, 1993, Jones *et al.*, 1990, Stephenson *et al.*, 1987), 47% of 187 lymph node metastases were diploid. Tribukait (unpublished) reported that about 95% of bone metastases are aneuploid. However, the majority of these patients have been treated by endocrinologic manipulation and thus, selection of aneuploid cell lines can not be excluded.

3. PREDICTION OF OUTCOME

3.1. Parameters for tumor progression and treatment failure

A considerable number of parameters is used to describe the clinical course of patients with prostate cancer. Parameters concerning the response to treatment are not the subject of this discussion. Tumor progression and death can be analyzed by various statistically well defined terms. These include the non-progression rate, progressive disease, progression-free survival, disease-free survival, death due to cancer, survival corrected for intercurrent death and over-all survival. All these parameters are adequate if statistically evaluated. Readers of papers using these terms must be aware of the statistical meaning of these parameters. Only then is an adequate scientific comparison possible.

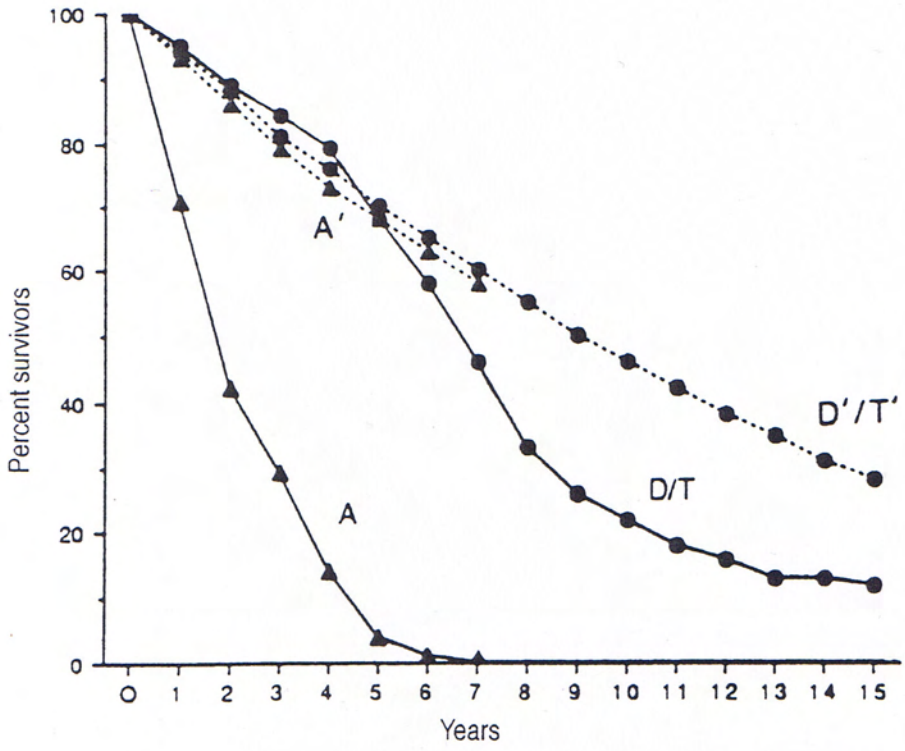

Fig. 3. Life table crude survival for patients with aneuploid (triangles) and diploid or tetraploid (circles) tumors of the prostate. The dotted lines represent expected survival and the solid lines the crude survival (Forsslund *et al.*, 1992).

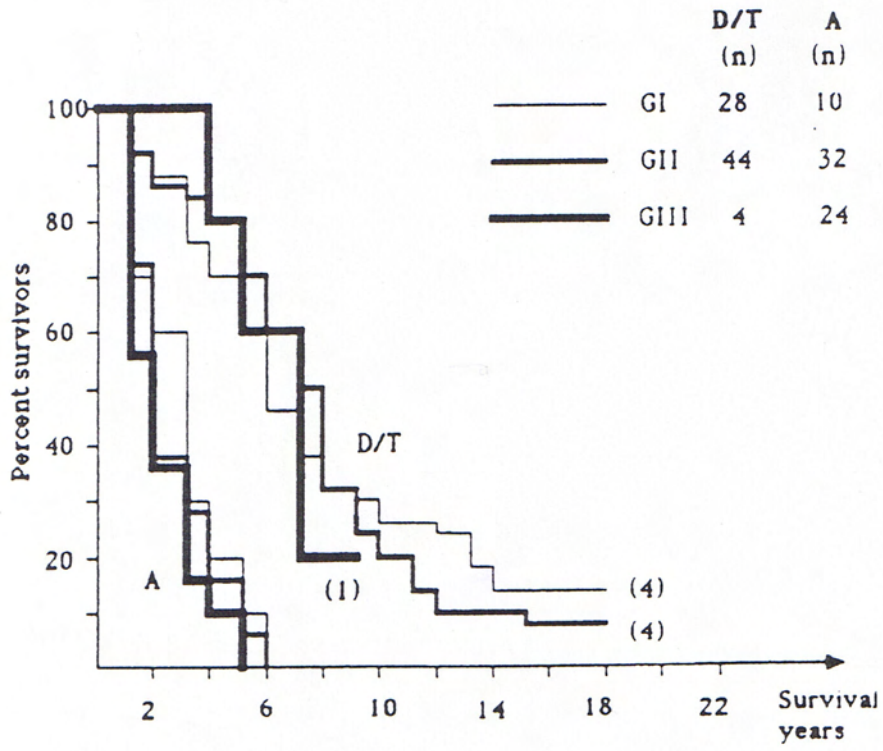

Fig. 4. Life table crude survival for patients with diploid or tetraploid (D/T) and aneuploid (A) tumors with respect to grade. Numbers within brackets are patients at risk (Forsslund *et al.*, 1992).

3.2. Observations in untreated patients

In a series of 287 initially untreated patients reported by Tribukait (1993) pronounced differences in the Kaplan-Meier curves of corrected survival were seen between groups with diploid and aneuploid tumors. Patients with tetraploid tumors had an intermediate survival statistics. The difference between patients with diploid and tetraploid tumors was, however, not significant. The series contained patients of all stages but mainly with locally confined disease and a low grade of malignancy.

3.3. Observations in patients after prostatectomy and hormone treatment

Similar data were shown in large groups of patients treated by radical prostatectomy at the Mayo Clinic

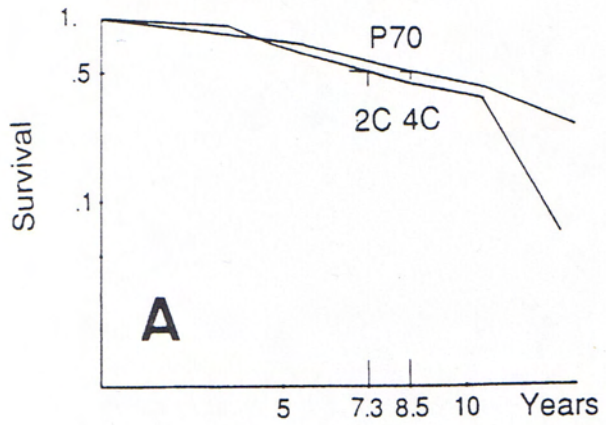

Fig. 5 a. Survival rate of patients with diploid/tetraploid (2/4C) prostatic carcinomas compared to that of Portuguese male population aged 70 (P70). Median life expectancy is indicated for both groups.

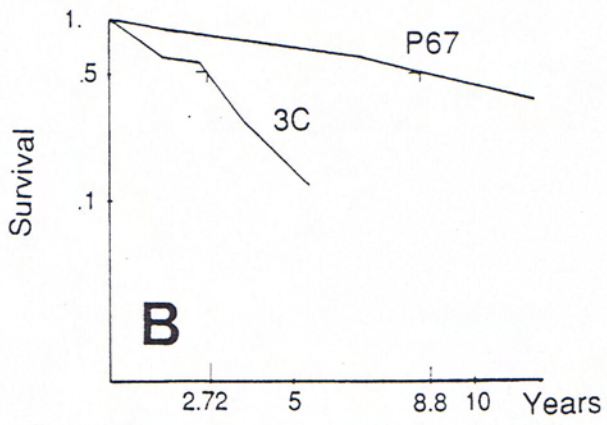

Fig. 5 b. Survival rate of patients with triploid (3C) prostatic carcinomas compared to that of Portuguese male population aged 67 (P67). Median life expectancy is indicated for both groups (Tavares *et al.*, 1973).

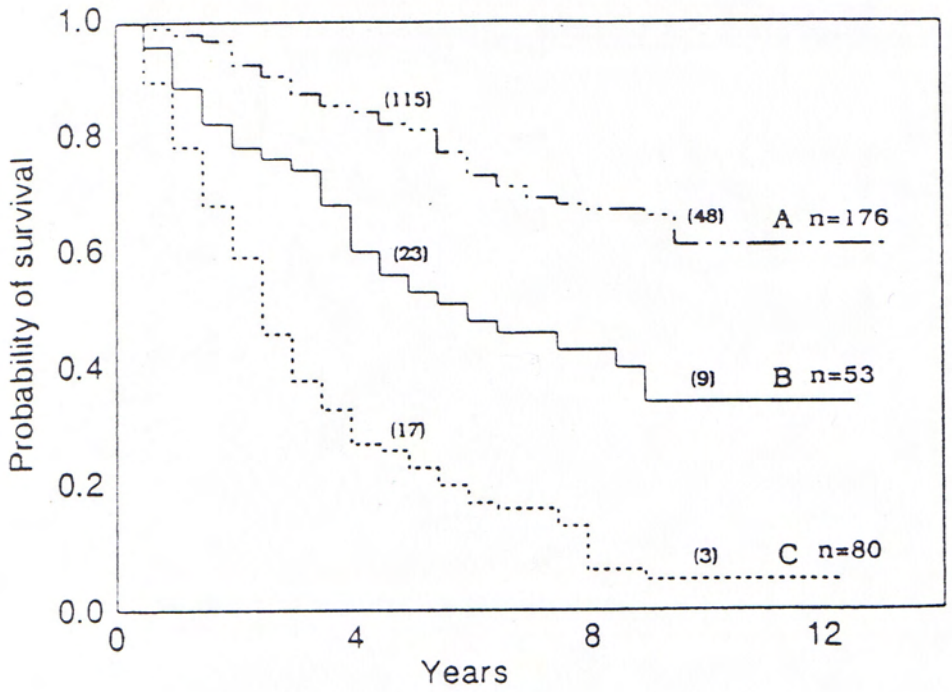

Fig 6. Corrected survival of 309 hormonally treated patients with prostate carcinoma in relation to tumor ploidy. A=diploid, B=tetraploid, C=aneuploid. Numbers within brackets are patients at risk (Tribukait, 1993).

(Montgomery *et al.*, 1990) and also by Zetterberg *et al.* in hormonally treated patients (Forsslund *et al.*, 1992). Forsslund's data are shown in Figs. 3 and 4. Patients with aneuploid tumors died of prostate cancer irrespective grade within five to eight years. Data shown by Tribukait (1993) were similar. It was of interest that these newer data were remarkably similar to the first results published on this subject by Tavares (1966, 1973).

These data as well as the data resulting from the series of Tribukait are shown as Figs. 5 and 6. In this context the effect of poorly differentiated tumors (grade 3) on survival was discussed. The data reported by Elder *et al.* (1982), suggest that fifteen years were required until all patients with grade 3 tumor had either progressed or died of their disease.

3.4. Observations in patients after radical radiotherapy.

Data are available from the Mayo Clinic from 65 patients with clinically localized prostate carcinoma treated by external beam radiotherapy (Song *et al.*, 1992). Preirradiation needle biopsy specimens were analyzed using static cytometry. Ten-year survival of patients with non-diploid tumors (DNA-index >1.5) was 20% compared to survival of patients with near-diploid tumors (DNA index <1.5) of 75%. In another study from the Memorial Sloan-Kettering Cancer Center on 82 lymph node positive patients, treated by ^{125}I seed implants and pelvic lymph node dissection, median survival time of the aneuploid and diploid groups was 5.0 and 8.8 years respectively. The differences in survival were highly significant and in the Cox' multivariate analyses, DNA ploidy stood out as the most significant independent variable (Stephenson *et al.*, 1987).

3.5. Can DNA ploidy be used as a time dependent prognostic factor?

Böcking *et al.*, (1985) suggested that in conservatively treated patients repeated prostatic biopsies and the repeated determination of DNA ploidy over time may be of prognostic value. Changes in DNA ploidy can be detected by such procedures. The data may provide information on progression of the primary tumor. Tribukait (1993) provided supporting data based on repeated aspiration biopsies in a group of 146 untreated patients. He showed that after a mean observation time of two years, about 1/3 of the tumors may change from diploid and tetraploid to aneuploid at an annual rate of about 15%.

Forsslund *et al.*, (1993) report a long term follow-up study of 30 patients with localized prostate cancer who had undergone repeated aspiration biopsies at diagnosis and after relapse. All patients

were treated by endocrinological means or by external irradiation. The follow-up period was up to a maximum of 25 years. The patients were carefully selected long term survivors with 20 grade 1, 9 grade 2 and 1 grade 3 tumors at diagnosis. A shift in grade of differentiation from grade 1 to grade 2 or to grade 3 with time was seen in 26 of 30 patients. In the same aspiration biopsies DNA ploidy was studied. Initially all patients had diploid tumors. Only 4 patients showed a shift to either tetraploidy or aneuploidy. Because of scarcity of data and because of a selection bias in this series, there was agreement that more long term follow-up studies are necessary to evaluate the value of DNA ploidy determinations as a time dependent prognostic factor.

3.6. Relation of DNA ploidy with other prognostic factors.

There is agreement that DNA ploidy can not be predicted from tumor grade. Aneuploid tumors, however, are more frequently seen in patients with grade 3 lesions. The panel agreed that in cases of discrepancies between grade and DNA ploidy, the latter is the more valid prognostic factor. In other words, a high grade tumor with a favourable (i.e. diploid) DNA ploidy pattern will most likely behave as a diploid tumor. A well or moderately differentiated tumor with aneuploid DNA pattern is likely to have a worse outcome than a similar diploid tumor. However, the extensive data on several hundred patients with long term follow-up from the Mayo Clinic show that grade and DNA ploidy as well as the pathologic stage are independent prognostic variables in a Cox´ multivariate analysis (Montgomery *et al.*, 1990, Nativ *et al.*, 1989). For tumors with identical grading and staging, the difference between diploid and non-diploid tumors varies about three-fold. These data also show that the analysis of DNA ploidy in tumors of the intermediate Gleason score between 5 and 7 allows a further subclassification of this inhomogenous group of patients.

Tribukait (1993) referred to his series of 309 patients of various disease stages who were all treated by endocrinologic manipulation and in whom DNA ploidy was determined in aspiration biopsies of the primary tumor. In this series DNA ploidy was shown to be an independent prognostic factor with a superior prognostic significance in comparison with other variables. The data are shown in Table VI. In this analysis DNA ploidy even overruled the presence and absence of metastases as a prognostic factor.

Tribukait (1993) also referred to his analysis of grade, age, stage (T) and metastases (M) in 287 initially untreated patients. After exclusion of metastatic, high stage (T4), and grade 3 tumors, there were 254 patients with grade 1 and grade 2, stage 1–3 tumors. In this selected group the ploidy was the only significant prognostic factor (P=0.014).

The significance of ploidy in 65 prostatectomized patients with seminal vesicle involvement as a predictor of recurrence was studied by Lee *et al.*, (1988). Aneuploidy, Gleason grade and seminal vesicle involvement were independent variables and all correlated significantly with recurrent disease. The probability of disease free interval of 60 months in this group of patients was 73% for diploid tumors compared with 8% for patients with aneuploid tumors. In patients without seminal vesicle involvement the likelihood of disease free survival for 60 months was 100% for diploid tumors and 65% for aneuploid tumors.

Proliferation related markers, such as Ki67, MIB1, PCNA and BUDR are now widely available. No studies have been carried out which compare DNA ploidy to these proliferation markers.

Long term studies of levels of PSA compared with DNA ploidy are still lacking.

The relationship among these parameters requires further study, preferably in a prospective fashion.

Table VI. *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

Tribukait, 1993

4. CAN TREATMENT DECISIONS IN INDIVIDUAL PATIENTS BE BASED ON THE RESULTS OF DNA PLOIDY STUDIES?

Information on DNA ploidy in prostatic carcinoma may be considered for use in some specific treatment decisions. Patients with aneuploid tumors who have a high probability of progression and of dying of the disease may be candidates for potentially curative aggressive forms of management, in spite of the fact that the treatment results in such patients have not been very encouraging so far. Patients with aneuploid tumors should not be considered for long term surveillance. Still in a number of patients with diploid tumors the disease will progress (Adolfsson & Tribukait, 1990) and may develop diploid metastases. Additional prognostic factors are needed to identify those diploid tumors which are likely to remain stable and which may be suitable for long term follow-up without treatment. The identification of these factors should be a top research priority. Another priority is a reliable identification of tetraploid tumors (within the group of non-diploid tumors) because they appear to have a more favourable prognosis. It is possible that some additional prognostic factors are available at this time. Candidate prognostic factors in this respect are PSA, the evaluation of proliferation related markers in the primary tumor and in the metastatic deposits, and the proteins expressed by oncogenes and tumor suppressor genes, such as M23. The studies which are available to date, however, are not conclusive as to the value of these markers. Also, it is recognized that flow cytometric or static image analysis of ploidy may lack the sensitivity to detect aneuploidy if only the loss or gain of one chromosome is present. Thus, new techniques such as FISH studies of certain tumors classified by cytometry as diploid may show them to actually be aneusomic, for example, chromosome 7 or 8 (Persons *et al.*, 1993).

5. SELECTION OF PATIENTS FOR SPECIFIC TREATMENT OPTIONS

The panel felt strongly that the outcome of radiotherapy in patients with aneuploid tumor is extremely poor. The same may be true for the results of radical prostatectomy. The Mayo Clinic data show that of 248 patients with pathological stage B disease, 10 patients had aneuploid tumors. All these cases progressed within 12 years. However, half of these patients are still alive after ten years. A similar observation can be made with relation to D1 patients. Also here the progression and death rates from prostate cancer were strongly adversely influenced, by aneuploidy, however, the median survival times were in the range of ten years. This was independent of early or delayed endocrine treatment (Myers *et al.*, 1992).

It can be concluded from the available data that patients with diploid and tetraploid tumors in general have much more favourable outcome than patients with aneuploid tumors. The data, however, seem to suggest that survival after radical prostatectomy is longer than after radiotherapy. The uncertainties about correct identification of DNA ploidy in biopsy specimens does not allow at this moment to reach any conclusions as to cure and survival based on biopsy evidence only.

The Mayo Clinic experience on immediate or delayed endocrine treatment is quite extensive and has been repeatedly reported. It shows that the outcome of patients with DNA aneuploid and tetraploid tumors is poor in D1 patients. In another study from the same institution (Myers *et al.*, 1992) which reports on 62 D1 patients with long term follow-up, there was a significant difference in the rate of death from prostate cancer in patients with diploid tumors subjected to early versus delayed endocrine treatment. There was, however, no difference in overall survival. Prior reports from the same institution failed to show this difference.

6. ASPECTS OF QUALITY OF LIFE

It was pointed out that much of the value of DNA ploidy is related to the posttreatment prognostic situation of the individual patient. The important question how the information on DNA ploidy may adversely influence the quality of life of patients who obtain this information can not be answered at present. Will a patient benefit from knowing the truth about having a non-diploid tumor confirmed on radical prostatectomy? Will a patient benefit from knowing the DNA ploidy of his tumor prior to radiotherapy or endocrine therapy? These decisions are presently left to the individual clinician. However, studies to evaluate quality of life in these particular situations should be carried out.

Considerations on the proper management of prostate cancer patients should also address the issue of the possibility of no treatment or delayed treatment of patients with DNA diploid disease. The outcome of conservative management of such patients has not been studied in statistically valid prospective randomized studies which must include a non-treatment control group. The outcome of such studies would be decisive for establishing further treatment guidelines. For the time being it can be assumed that differences between treatment and non-treatment groups of diploid tumors will be small.

So far as aneuploid tumors are concerned, the outcome seems to be uniformly poor, irrespective of treatment. However, even in this group of patients prolonged survival times are seen after radical prostatectomy. No control population is available to confirm that these results are not due to treatment. In counselling patients with low stage, low grade and low ploidy tumors, the available data should be used. Since quality of life aspects also play a role after whatever treatment option is applied, patients who are capable of understanding this situation should be involved in decision making, which may be determined even by factors which are beyond the domain of the treating surgeon.

CONCLUSIONS

7. IS THE ROUTINE USE OF PRE- OR POSTTREATMENT DNA PLOIDY DETERMINATION CLINICALLY JUSTIFIED?

Considering the technical limitations of DNA analysis that have been previously discussed in this positive paper the panel agrees that knowledge of DNA ploidy prior to treatment is of value in treatment decisions, particularly when surveillance is a treatment option. Aneuploid tumors can be expected to respond very poorly to either irradiation or endocrine treatment. The issue of benefit of early, versus delayed, endocrine treatment in diploid tumors remains controversial.

The presence of aneuploid tumor either on pretreatment biopsies or in radical prostatectomy specimens is an ominous sign. The panel does, however, not feel that patients with aneuploid tumors should not undergo a radical prostatectomy if they otherwise qualify. The identification of a group of patients with a uniformly poor prognosis should encourage medical oncologists and basic scientists to develop adequate treatment options for this particular group. The issue of adjuvant treatment in this particular group of patients has been insufficiently explored.

The panel expressed a strong conviction that DNA ploidy should be uniformly studied in clinical trials particularly in patients with localized prostate cancer. The retrospective scientific analysis of clinical data will be seriously hampered by the absence of knowledge of this important prognostic factor.

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Correspondence to:

Bernhard Tribukait, M.D.
Department of Medical Radiobiology,
Karolinska Institute,
S-171 76 Stockholm,
Sweden