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Diagnostic DNA Cytometry of Prostatic Cancer

Key Words
DNA cytometry
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Prognosis
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Abstract
DNA cytometry is a powerful tool for grading the malignant potential of prostatic carcinomas, superior to histological or cytological evaluation. Independently of the clinical stage, the probability of tumor progression and death from cancer-specific causes can be assessed for individual patients. The mean nonprogression rate of 'DNA near-diploid' prostatic carcinomas seems to be 85–90% for 5 years. Near-diploid tumors are likely to respond to hormonal therapy. Changes in the DNA pattern towards the normal state under conservative therapy may serve as an early indicator of regression, whereas increasing DNA aneuploidy indicates tumor progression. The diagnostically relevant parameter is the degree of DNA aneuploidy. The parameters for prognostic interpretation of DNA data are not yet sufficiently standardized. DNA cytometry may help the urologist to predict the fate of an individual patient with prostatic cancer and to decide on a hormonal or adjuvant therapy. DNA cytometry is so far not useful for the detection of prostatic neoplasia or its precursors. DNA measurements may be performed by flow or static cytometry, the former method being more rapid, the latter more sensitive excluding the measurement of normal cells. Fine-needle aspirates are most suitable for performing DNA measurements with both methods.

Diagnostic Problems in Clinical Pathology

So far every cancer of the prostate has to be microscopically verified, classified and graded before any therapy can be scheduled. Today, core biopsy on which a histological diagnosis is made and fine-needle aspiration biopsy on which a cytological diagnosis is made compete with each other concerning their sensitivity, specificity and typing accuracy. The average sensitivity of the histological investigation (93.1%) is only slightly better than that of the cytological investigation (92.7%), if data from the literature are compared [1]. The specificity of the cytological diagnosis is 97.2% on an average, but that of histology is not 100%. According to our experience we have to face about 2% false-positive histological diagnoses on core biopsies of the prostate in daily practice [1]. Yet, the false-positive rate of histological tumor diagnosis has never been thoroughly investigated. Likewise, the typing accuracy of the cytological diagnosis of the tumor has never been analyzed in the prostate, but we suppose that urothelial cancers and squamous cell carcinomas are not always classified correctly but diagnosed and mistreated as adenocarcinomas. Furthermore, grading of tumor malignancy is not sufficiently reproducible to be reliable for an

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individual patient. Svanholm and Mygind [2] reported a 36% interobserver reproducibility of the histological grading according to Gleason [3] and 69% of that according to Böcking et al. [4]. The reproducibility of cytological tumor grading was reported to be only 60.0% [5]. The prognostic significance of the morphological grading systems is not precise enough to take it as a basis for therapeutic decisions in individual patients. The probability of tumor progression cannot be ascertained from morphological criteria alone. Finally, the effect of tumor therapy cannot be established reliably from microscopic investigations despite several proposals for histological or cytological grading of tumor regression [6, 7].

Morphometric parameters have failed to contribute significantly to more precise diagnoses in routine pathology [8–10]. Altogether the diagnostic sensitivity, specificity, typing accuracy and monitoring of therapy performed on biopsy material from the prostate have to be improved to achieve more precise and reliable diagnoses as a solid basis for individual therapy of prostatic cancers.

Since the work of Tavares et al. [11] many attempts have been made to improve the diagnostic accuracy of histological and cytological investigations of prostatic tissue using DNA cytometry. Nowadays this method may be used routinely to assist in grading the malignant potential of prostatic cancer and in monitoring the effect of conservative therapy. Many scientific publications reflect the prognostic validity of various DNA parameters. Modern knowledge acquired from tumor cytogenetics represents the biological basis on which the diagnostic interpretation of DNA-cytometric data may be performed. New technical equipment allows precise and rapid DNA measurements and assistance in data interpretation in daily practice.

Methodology of DNA Cytometry

Cytogenetic Basis

Nuclei of normal somatic cells contain two sets of 23 chromosomes (=2c). During the G_2 and M phases of the cell cycle four sets of chromosomes are present in each nucleus (=4c). In some tissues a regular multiplicity of this normal chromosomal set can be observed according to the integer valued exponents of 2c (4c, 8c, 16c, 32c) called euploid polyploidization. Polyploid cells are regularly encountered, for example in mesothelial, urothelial, seminal-vesicle epithelial cells, in hepatocytes, thyrocytes and others. During the S phase of the cell cycle every nuclear DNA content between 2c and 4c may be found. Numerical and structural aberrations of these normal chromosomal patterns are called chromosomal aneuploidy. Chromosomal aneuploidy distinguishes neoplastic cells from the normal somatic cells of an individual, since aneuploidy has so far only been observed in neoplasms [12, 13].

Chromosomal aneuploidy occurs very early in carcinogenesis and precedes the onset of clinical manifestations. Most evidence indicates that the tumour-specific primary chromosomal abnormalities are a conditio sine qua non in the multistage process of carcinogenesis [13]. Ionizing radiation and cytostatic agents may produce aneuploidy in single benign cells, as well.

Two types of chromosomal abnormalities can be distinguished. Primary, specific, nonrandom, mostly discrete changes occur, preferentially involving specific chromosomes, often as characteristic numerical or structural aberrations. The specificity of these aberrations may allow the diagnosis of certain tumor types. The detection of cells with aneuploid chromosomal sets is thus equivalent to the detection of neoplastic cells. Chromosomal abnormalities have so far been reported in fewer than 10 prostatic carcinomas. A deleted chromosome 10, del(10)q 24 was found in all 5 carcinomas studied by Atkin and Baker [14] and could represent a specific, primary rearrangement. Three of the carcinomas in the same series also had a deleted chromosome 7, del(7)q22. Further studies will clarify the significance of these interesting findings.

Secondary, additional, more massive, random or background abnormalities affecting all chromosomes occur as epiphenomena. They are observed in addition to the primary aberrations and frequently dominate the karyotypic picture in late stages of disease. They are associated with tumor progression, and their heterogeneity (range) is correlated with the malignant potential of the tumor [13]. The severity of a cancer based on such parameters as invasion (stage), pathology (grade), metastasis and response to therapy may be related to the number of chromosome changes present. Thus, Oshimura and Sandberg [15] reported on a mode of 70 chromosomes with considerable scatter in a metastasis to bone marrow of a prostatic cancer. This means that the quantitation of chromosomal aneuploidy in a given tumor may serve for grading its malignant potential [16].

The only setting in which cytogenetic analysis today plays a diagnostic role in clinical practice is in the examination of effusions of unknown cause. Especially in pleural effusions have numerous studies proven the value of chromosome analysis in differentiating between neoplas-

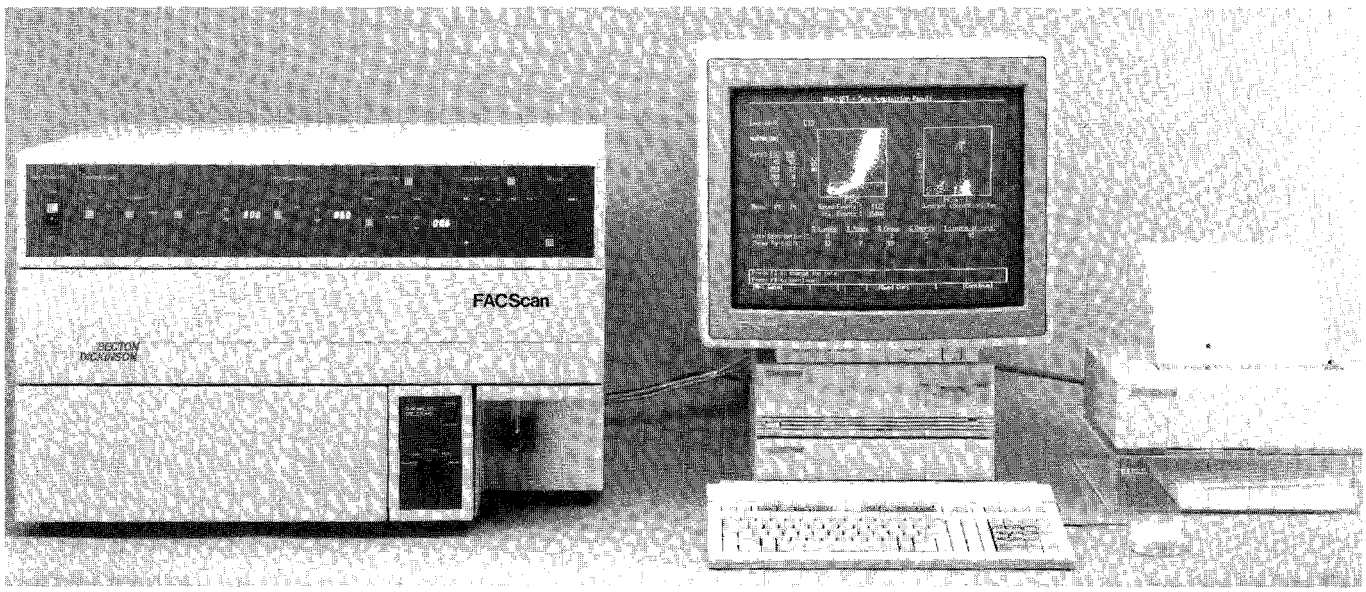

Fig. 1. Flow cytometer FACScan (Becton Dickinson, Mountain View, Calif., USA) for DNA flow cytometry.

tic and other diseases. DNA cytometry and not the discipline of tumor cytogenetics itself has used aneuploidy as a marker for neoplastic cell transformation and its extent for assessing the malignant potential of various tumors. Whereas primary chromosomal aneuploidy was often too discrete to be detected by DNA single-cell or flow cytometry, as occurs with cancers of the prostate, at least the additional secondary abnormalities lead to quantitative changes in nuclear DNA, which might be demonstrated by cytometry. The interpretation mode of DNA-cytometric data for diagnostic purposes should be consistent with the cytogenetic findings of each particular tumor entity.

Preparation and Staining

Fine-needle aspirations represent the most suitable specimens for DNA single-cell or flow cytometry. If core biopsies are performed, direct smears may be prepared from the tissue cylinder rolling it between two glass slides under slight pressure. Material for flow cytometry has to be subjected to special cell separation techniques [17, 18] and stained with fluorescent dyes such as propidium iodide or 4,6-diamidino-2-phenylindole [19]. Smears should be fixed in buffered 4% formaldehyde solution before Feulgen staining with parafuchsin [20]. Differently prestained smears may be cleared from their cover glasses in xylene, postfixed in formaldehyde and restained according to Feulgen, even after many years. Destaining will automatically be performed during acid hydrolysis with HCl. Cells may also be released for DNA measurements from old, formalin-fixed and paraffin-embedded tissue blocks using cell separation techniques [17, 21]. DNA measurements on sections are allowed only under certain conditions: an individual, mathematical correction of integrated optical density values has to be performed, taking the thickness of the section and the size of each individual nucleus into account. A minimum of about 300 nuclei has to be measured, and the results cannot be compared directly with those from whole nuclear measurements [22]. Epstein et al. [23] demonstrated that in 22% discordant DNA histograms resulted when comparing measurements on smears and tissue sections. Thus diagnostic DNA measurements on tissue sections are not recommended.

Measuring Devices

Measurements of nuclear DNA for diagnostic purposes are currently performed using either flow cytometers or televised-image analysis systems. Conventional microscope photometers are out of use, because measurements for that purpose are too time-consuming, circumstantial and often not precise enough [24]. Flow cytometers allow a rapid measurement of huge amounts of cells within a few minutes (fig. 1). For DNA measurements in solid tumors, cell suspensions have to be prepared by mechanical and/or chemical cell separation. Measurements in cytological or histological routine specimens are

not possible. If the DNA stemline ploidy has to be determined exactly or if a mere distinction between 'diploid' and 'aneuploid' is desired, the good representativity of the large cell number under investigation is an advantage. Another advantage of flow cytometry is the possibility of simultaneous determination of different parameters, such as nuclear DNA, nuclear size, cellular protein content and various cellular antigens, e.g. keratin or Ki 67, in the same material. Yet, the systems do not allow a morphological identification and classification of cells under investigation. This means that the tumor cells cannot be differentiated from nuclear aggregates, connective tissue cells, histiocytes or normal epithelial cells. Further, the lowest resolution of these instruments is about 1 %, which means that cells representing less than 1 % of the total population cannot be identified. Dysplastic cells in a smear cannot be measured selectively, or a few malignant cells cannot be detected. Few but diagnostically relevant cells in a mixed population will be overlooked. Finally, a repeat measurement of the same cells for quality control is not possible.

Televised-image analysis systems allow DNA measurements in cytological and histological routine specimens, which may be many years old (fig. 2, 3). Thus, no additional material for measurements must be obtained from the patient. Individual- and tissue-specific reference cells may be selected which are suitable for every case. Individual cells of interest can be selected by the user and classified morphologically; artifacts can be excluded. This offers the possibility to select and measure rare, dysplastic, abnormal or special cell types in mixed populations, Thus, grading of malignancy is also possible in a mixture of neoplastic and nonneoplastic cells. Different cell types can be measured simultaneously. In addition, these systems determine morphometric nuclear features such as size, form or chromatin pattern. Cells can be relocated and remeasured for quality control. Moreover, immunohistochemical reactions can be quantitatively investigated. A disadvantage, as compared with flow cytometry, is the more time-consuming measurement (about 20 min for 20 reference and 200 tumor cells) and the restricted number of cells that can be measured practically (some 100).

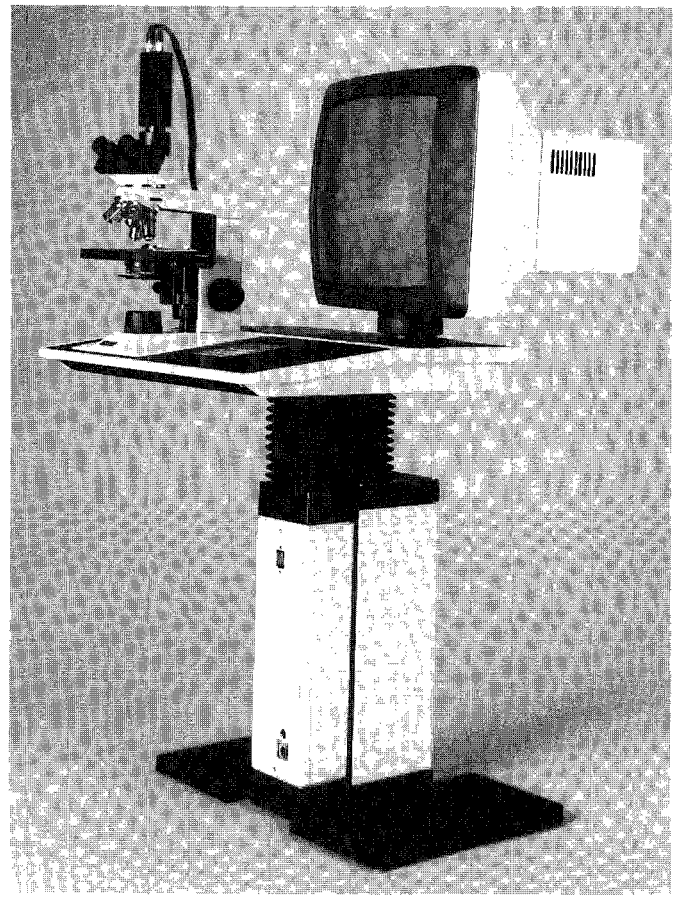

Fig. 2. Televised-image analysis system Cytometer CM1 (Hund, Wetzlar, FRG) for static DNA cytometry.

Data Interpretation

Strategies for the diagnostic interpretation of DNA-cytometric data of the given tumor should be consistent with cytogenetic findings. This implies that apart from very early stages in carcinogenesis, which mostly reveal only discrete, primary chromosomal abnormalities, cytogenetically based, fixed DNA distribution patterns do not exist during tumor progression. The reason is that the relevant secondary chromosomal aberrations are random effects. Objective, algorithmic data interpretation should be preferred instead of subjective descriptions of histograms [25].

Slight chromosomal aneuploidies in single cells cannot be detected cytometrically because of the limited resolution of the method. Yet, such small increases as 2% of the total DNA can be identified by measuring hundreds of cells, statistically comparing their DNA values with those of tissue- and individual-specific reference cells. If a statistically significant difference can be detected between both populations, DNA aneuploidy can be assumed. This procedure is known as 'stemline interpretation' of DNA aneuploidy [26, 27]. Using that interpretation mode the quantitative effect on nuclear DNA resulting from small numerical chromosomal aberrations can be detected cytometrically. Yet, this mode is rather insensitive, as many cells containing the relevant aneuploidies must be available for measurement. Rare cells revealing chromosomal

aneuploidy characteristic of neoplasia may be missed by this strategy of interpretation. Single cells from tissues without polyploidization may be identified as aneuploid, if their DNA content amounts to more than 4c plus the error of the method. In practice, a DNA content > 5c has been proven empirically as diagnostically relevant [1, 28]. Not the percentage of cells with a DNA content > 5c which can be subjectively influenced, but their absolute occurrence is of diagnostic relevance (5c-exceeding events). We call this procedure 'single-cell interpretation' of DNA aneuploidy. With this mode, quantitatively greater chromosomal aneuploidies can be detected in a few cells by DNA cytometry. This procedure is a very sensitive one for the detection of neoplastic cells, as most malignant tumors reveal single cells with an abnormally high DNA content [29]. Both modes of diagnostic DNA data interpretation for the detection of aneuploidy are correct and should be used in combination as they supplement each other.

Some authors use an increased percentage of cells in the G_2/M phase, e.g. 7%, as a marker for neoplastic transformation [30, 31].

For prognostic interpretation of DNA data the simple differentiation into 'diploid' and 'aneuploid' tumors has to be abandoned, as it lacks a cytogenetic basis [25]. Nearly all tumors are cytogenetically aneuploid [12], but this may not be detectable by DNA cytometry. Instead, DNA aneuploidy should be quantified either by defining the DNA content of the tumor stemline, especially the modal value, or by parameters reflecting the prognostically relevant DNA distribution, such as mean ploidy [32], 5c-exceeding rate [29], DNA grade of malignancy [33], coefficient of variation of DNA ploidy [34], standard deviation of ploidy or entropy [35].

Diagnostic Results

Diagnosis of Malignancy

On average, the sensitivity of DNA cytometry to detect cancer in specimens from the prostate amounts to only 67.9%, using DNA aneuploidy as a marker for neoplasia (table 1). The sensitivity becomes even worse when only low-grade carcinomas are taken into account. Whereas 90% of poorly differentiated carcinomas (n = 13) revealed DNA aneuploidy, this was found in only 42% of the well-differentiated tumors (n = 199) [48]. The sensitivity using single-cell cytometry seems to be higher (80.7%) as compared with flow cytometry (62.5%; table 1). The reason may be that in single-cell cytometry the relevant population can be measured selectively, whereas in flow cytometry a small population of grade 1 prostatic carcinoma cells may not be differentiated from benign hyperplastic cells.

It is a well-known fact that aneuploidy tends to increase during tumor progresssion [49]. As a result higher aneuploidy rates are found more often in advanced clinical stages. Adolfsson and Tribukait [49] reported on 20% in T_1 (n = 27), 66% in T_2 (n = 51) and 88% in T_3 (n = 33). The fact that the aneuploidy rate in prostatic cancer is on average significantly lower than in squamous cell carcinoma of the uterine cervix for example, where it is near 100% [29], corresponds to the lower malignant potential of the former tumor as compared with the latter. The reason for the reported low DNA aneuploidy rate may be on a cytogenetic basis in that the primary chromosomal aberrations in prostatic cancer are rather slight (see above) or that secondary changes might occur late during tumor progression.

The average specificity of DNA cytometry to detect normal, nonmalignant cells in prostatic specimens is 93.7% (table 1). The specificity using single-cell cytometry is slightly higher (97.8%) as compared with flow cytometry (92.6%). The reason may be that artifacts can more easily be excluded using interactive as compared with automatic measurements.

The reason for the high rate of 'false-positive' DNA aneuploidies in benign prostatic tissue is unknown. Possible explanations are that a correct internal standard with tissue- and individual-specific reference cells has not always been used or that a partial or complete euploid polyploidization may occur in benign prostate cells.

In summary, the reported sensitivities and specificities are too low to recommend DNA cytometry as a diagnostic acid in the detection of prostate cancer or its precursors.

Grading of Malignancy

Whereas the diagnosis of malignancy is routinely performed with an acceptable sensitivity and specificity using cytological or histological material, the prognostic significance of cytological or histological grading of tumor malignancy is neither sufficiently reproducible nor prognostically valid enough. As early as 1966 Tavares et al. [11] reported on quite different survival times of patients with prostatic cancer which revealed DNA stemlines around 2c or 4c as compared with 3c or 6c. Even the response to estrogen therapy was better in the 2c/4c group. About 20 years later these results were confirmed by several independent investigators (table 2). It could be demonstrated that there exists a statistically significant correlation of the DNA distribution pattern with

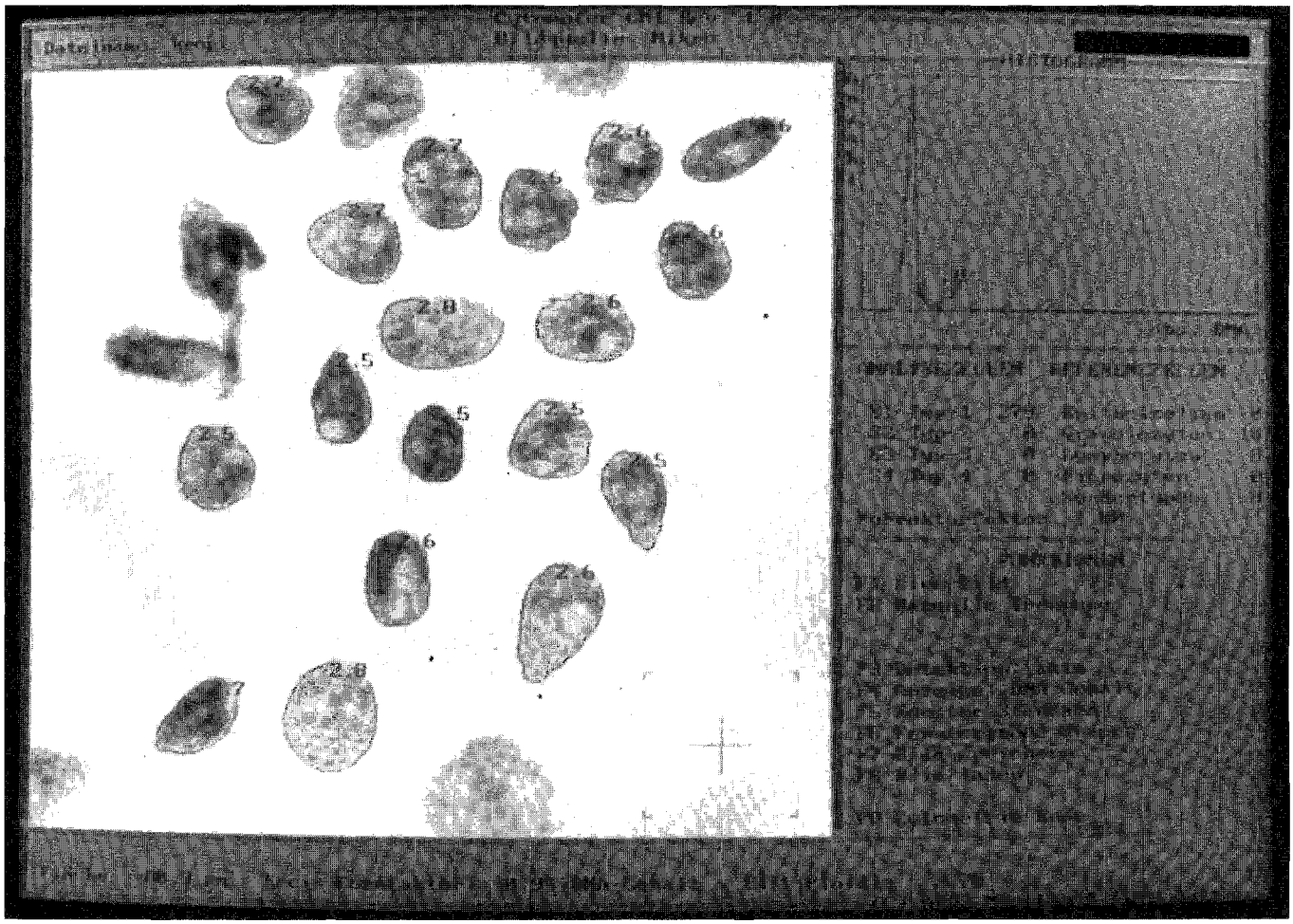

Fig. 3. Interactive monitor of the Cytometer CM1. Measured nuclei are marked by their individual DNA content in numbers of sets of chromosomes (c). Right above: on-line DNA histogram.

the survival time of the patients [44, 48, 54, 55, 58–62] (fig. 4).

It has furthermore been demonstrated that DNA aneuploidy correlates significantly with death from cancer [44, 48, 60, 62] and with nonprogression of the tumor [59, 60, 62] (fig. 5)

The follow-up of these studies was between 5 and 20 years. The DNA parameter used was mostly the differentiation between 'diploid' and 'aneuploid' distribution patterns, although this is not precisely defined in the literature. It seems obvious that a second distinct group with a rather good prognosis, slightly worse than that of patients with 'diploid' tumors, is represented by those with stemlines around 4c [44, 50, 60]. A third prognostically separate group seems to have a nontetraploid aneuploid distribution with one stemline, while in a fourth, aneuploid distributions with several stemlines seem to represent the prognostically worst category [56]. Böcking et al. [57] used the variance of the tumor cell DNA values around the normal 2c value (2c deviation index) as a prognostic parameter which also revealed a significant correlation with the survival time. Bibbo et al. [59] used the percentage of cells $> 5c$ as a prognostic marker. Auer and Zetterberg [52] showed that the percentage of cells $> 2.5c$ ($> 60\%$) correlated well with the occurrence of death from cancer with 1–3 years.

Single-cell cytometry seems to have an advantage over flow cytometry for DNA grading of prostatic carcinomas because preexisting routine smears on which the cytological diagnosis has been made can be used for measurements and even a few tumor cells can be specifically measured separating them from coexisting benign cells.

Table 1. Diagnostic significance of DNA-cytometry in prostatic cancer: data from the literature

Authors	Benign/ malignant cases	Type of material	Static (S) or flow (F) cytometry	Sensitivity %	Specificity %	Mode of discrimination
Sprenger et al. [36]	105/52	Fine-needle aspiration	F	42.1	96.2	Discriminant analysis
Zetterberg and Esposti [37]	3/24	Fine-needle aspiration	S	95.8	–	5c-exceeding events
Frederiksen et al. [38]	29/30	Fine-needle aspiration	F	60.0	100	Second significant peak
Zimmermann and Truss [39]	17/81	Fine-needle aspiration	F	76.5	93.8	Second peak outside 4, 8, 16c; 2c peak > 7%
Rönström et al. [30]	301/166	Fine-needle aspiration	F	73.0	92.0	Cells in G_2 + M phase or second aneuploid peak
Lämmel et al. [40]	24/32	Core biopsy	F	75.0	96.0	G_1 fraction
Zimmermann et al. [41]	102/73	Fine-needle aspiration	F	57.5	85.3	Second aneuploid peak or hypertetraploid cells
Tribukait et al. [31]	–/300	Fine-needle aspiration	F	69.0	–	G_2/M phase cells > 7%
Böcking et al. [29]	18/29	Fine-needle aspiration	S	69.0	100	5c-exceeding events
Seppelt et al. [42]	20/85	Fine-needle aspiration	S	89.4	95.7	Combination of 8 parameters
Willumsen et al. [43]	–/67	Cell suspension from paraffinized tissue blocks	F	43.7	–	Distinctive second peak
Winkler et al. [44]	–/91	Cell suspension from paraffinized tissue blocks	F	58.0	–	Distinctive second peak or > 13% in G_2/M phase
de Vere White and Deitch [45]	87/77	Fine-needle aspiration	F	55.0	87.2	> 20% hyperdiploid cells or additional peaks
Amberson and Koss [46]	–/47	Fine-needle aspiration and surgically removed tissue	F	78.7	–	DNA index = 1.00
Amberson and Koss [46]	–/68	Fine-needle aspiration	S	75.0	–	DNA index = 1.00
Tribukait [47]	531/866	Fine-needle aspiration	F	62.5	91.0	G_1 peak deviates more than 10% from internal diploid standard
Stenkvist and Olding-Stenkvist [35]	135	Fine-needle aspiration	S	74.3	–	More than 2% cells > 4.32c

Table 2. Prognostic significance of DNA cytometry in prostate cancer: data from the literature

Authors	Number of cancers	Type of material	Static (S) or flow-cytometric (F) measurements	Follow-up period years	Prognostic DNA parameter	Correlation with	Level of significance
Tavares et al. [11]	35	Paraffin sections	S	11	Stemline around 2c/4c vs. 3c/6c	Survival time, response to estrogen therapy	Descriptive
Tavares et al. [50]	76	Paraffin sections	S	12	Stemline around 2c/4c vs. 3c/6c	Survival time, response to estrogen therapy	Descriptive

Table 2 (continued)

Authors	Number of cancers	Type of material	Static (S) or flow-cytometric (F) measurements	Follow-up period years	Prognostic DNA parameter	Correlation with	Level of significance
Tribukait et al. [31]	300	Fine-needle aspiration	S	–	Diploid vs. aneuploid	Cytological grading	Descriptive
Seppelt and Sprenger [51]	80	Fine-needle aspiration	S	2.5	DNA maximum $< 3.3c$; $3.3–5.9c$; $> 5.9c$	30 months survival	Descriptive
Auer and Zetterberg [52]	143	Fine-needle aspiration	S	10	Percentage of cells $> 2.5c$ ($> 60\%$)	Death from cancer within 1–3 years	Descriptive
Frankfurt et al. [53]	45	Cell suspension from fresh tissue	F	–	Diploid vs. aneuploid	Staging, metastases, histological grading	Descriptive
Fordham et al. [48]	72	Cell suspension from fixed tissue	F	14	Diploid vs. aneuploid	Histological grading, survival time, death from cancer within 3 years	n.s., $p < 0.001$
Lundberg et al. [54]	50	Cell suspension from fixed tissue	F	> 5	Diploid vs. tetraploid vs. aneuploid	Histological grading, survival time	n.s., $p = 0.043$
Stephenson et al. [55]	82	Cell suspension from fixed lymph node tissue	F	> 5	Diploid vs. aneuploid	Survival time (only stage D_1 patients)	$p = 0.0109$
Tribukait [56]	125	Fine-needle aspiration	F	6	Diploid vs. tetraploid vs. aneuploid with 1 cell line vs. aneuploid with several cell lines	Survival time, distant metastases	$p < 0.05$
Böcking et al. [57]	52	Fine-needle aspiration	S	12	DNA malignancy grade	Survival time	$p = 0.009$
Winkler et al. [44]	91	Cell suspension from fixed tissue	F	> 5 up to 17	Diploid vs. tetraploid vs. aneuploid	Survival time, nonprogression, death from cancer (only stage D_1 patients)	$p = 0.001$
McIntire et al. [58]	39	Cell suspension from fixed tissue	F	18–20	Diploid vs. aneuploid	Survival time (only stage A patients)	$p = 0.001$
Bibbo et al. [59]	30	Sections	S	12	5c-exceeding rate, mean ploidy	Survival time	$p < 0.001$
Nativ et al. [60]	146	Cell suspension from fixed tissue	F	7.9	Diploid vs. tetraploid vs. aneuploid	Tumor progression, survival time, death from cancer (only stage C patients)	$p < 0.001$ $p < 0.06$ $p < 0.01$
Peters et al. [61]	44	Fine-needle aspiration	S	5.8	89% with aneuploid, 11% with normal-range DNA pattern progressed to stage D	Tumor progression from stages A and B	
Montgomery et al. [62]	283	Cell suspension from fixed tissue	F	> 10	Diploid vs. tetraploid vs. aneuploid	Tumor progression, survival time, death from cancer (only stage B patients)	$p < 0.001$ $p < 0.001$ $p < 0.001$

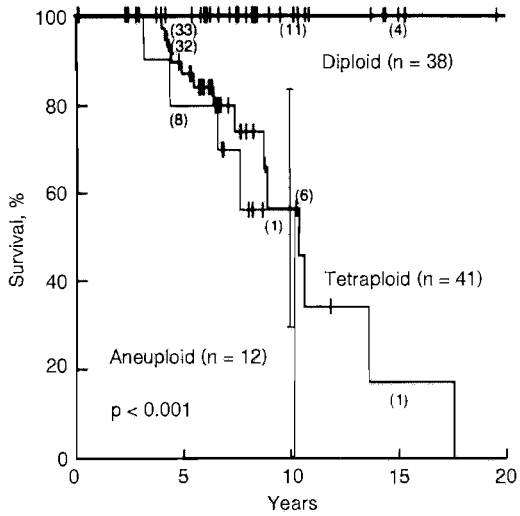

Fig. 4. Cause-specific survival after radical retropubic prostatectomy and lymphadenectomy for patients with stage D_1 prostatic adenocarcinoma for normal versus abnormal DNA patterns. From Winkler et al. [44].

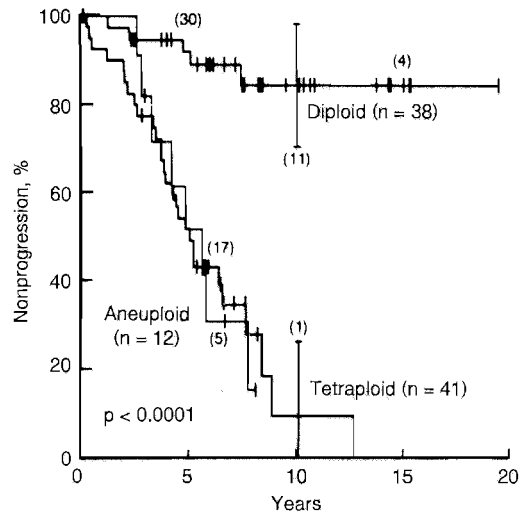

Fig.5. Postoperative probability of nonprogression of patients with stage D_1 prostatic adenocarcinoma treated with radical retropubic prostatectomy and lymphadenectomy. Normal versus abnormal DNA patterns. From Winkler et al. [44].

The question arises whether DNA cytometry is able to predict nonprogression in different stages of prostatic carcinoma. This could have a significant impact on adjuvant treatment in addition to androgen ablation. Older patients with low-stage and DNA low-grade tumors with a low risk of tumor progression could, besides androgen ablation, be submitted to a 'wait and see' strategy [51, 63]. Winkler et al. [44] could demonstrate that nonprogression for 91 patients with stage D_1 prostatic cancer who had undergone bilateral pelvic lymphadenectomy and radical retropubic prostatectomy correlated significantly with DNA 'diploidy' of the tumors. At 5 years the actual nonprogression rate was 92%, and 94% at 10 years. Patients with a tetraploid and aneuploid DNA pattern had a 5- and 10-year nonprogression rate of <15 and 10%, respectively (fig. 5). Remarkably, no patients with a 'DNA-diploid' tumor treated as mentioned above died of prostatic cancer, but 44% of those D_1 patients with an abnormal DNA pattern were dead of disease at 10 years ($p < 0.001$). The nonprogression rates within 5 years of identically treated patients with stage C prostatic cancer were 85% with a 'diploid', 64% with a tetraploid and 45% with an aneuploid DNA pattern [60]. In patients with stage B prostatic cancer also treated by prostatectomy and lymphadenectomy, only 15% with a 'diploid' tumor as compared to 31% with a nondiploid tumor had progressive disease [62]. A similar correlation was also reported by Stephenson et al. [55]. A significant impact of DNA aneuploidy on the survival time for patients with stage A_2 prostatic cancer was demonstrated by McIntire et al. [58]. For those patients, having 'diploid' lesions, the nonprogression rate was 85%, while for those whose disease was aneuploid it was 32%. Adolfson and Tribukait [49] could demonstrate that 90% of 'diploid' tumors in stage A_1 did not progress, whereas 78% of the progressive tumors were aneuploid. No morphological parameter is able so far to predict nonprogression with a similar specificity.

Several authors have noted that DNA low-grade or 'diploid' prostatic cancers responded well to hormonal and cytostatic therapy, whereas DNA high-grade or aneuploid tumors did not respond [11, 44, 50, 64–66]. Patients with 'diploid' or DNA low-grade tumors benefit most from adjuvant treatment such as androgen ablation.

Early orchiectomy in patients with a DNA-diploid tumor was not associated with progression at all, whereas a significant increase in progression occurred in patients with the same ploidy pattern but no adjuvant treatment [44].

Table 3. Therapy monitoring of prostatic carcinoma using DNA cytometry: data from the literature

Authors	Number of observed cases	Static (S) or flow-cytometric (F) measurements	Type of material	Clinical-cytometric correlation
Leistenschneider and Nagel [67]	16	S	Fine-needle aspiration	Tumors (n = 6) with DNA values up to 8c clinically did not respond to cytostatic therapy (Estracyt or Endoxan or 5-fluorouracil)
Colins et al. [65]	35 rats with R3327 prostate carcinoma	F	Cell culture	Percent aneuploid cells > 2c reflected responsiveness and unresponsiveness under hormone therapy
Leistenschneider and Nagel [64]	20	S	Fine-needle aspiration	Significant decline from aneuploid to diploid within the first 12 weeks of conservative treatment of high-grade carcinomas marks response to therapy and a better prognosis; lack of DNA content alteration or a histogram change to 'the right' under therapy indicates rapid clinical progression
Seppelt and Sprenger [51]	80	S	Fine-needle aspiration	Increase in diploid and hypodiploid DNA values and disappearance of secondary peaks are characteristic of remission; increasing scattering of DNA values and appearance of secondary peaks are characteristic of progression under endocrine therapy
Böcking et al. [66]	19	S	Fine-needle aspiration	The prospective tumor response to hormone therapy could be predicted from the DNA grade of malignancy; high DNA grades > 1,5 did not respond; DNA regression index provided independent additional prognostic information to the DNA grade of malignancy concerning tumor regression and survival under conservative therapy

In conclusion, DNA 'diploidy' seems to be a valuable marker for tumor nonprogression in all stages of prostatic cancer. Furthermore, DNA 'diploidy' seems to be an indicator for the sensitivity of prostatic cancer for hormonal therapy.

As the DNA ploidy pattern has been identified as the prognostically most valuable parameter for patients with prostatic cancer, clinical trials should be controlled for the tumors' DNA content in future if the results are to be interpreted correctly [45].

Monitoring of Therapy

The ploidy status may be used to monitor tumor progression by investigating fine-needle aspiration samples repeatedly (table 3). With tumor progression the amount of peridiploid cells decreases and that of tetraploid and clearly aneuploid cells increases, including the appearance of new aneuploid stemlines. Accordingly, the disappearance of aneuploid stemlines and reappearance of peridiploid cells which only occurs under conservative therapy represents a decreasing malignant potential or a down-grading of the tumor. This 'improvement' of the DNA histogram under conservative therapy is generally associated with clinical signs of tumor regression and was therefore proposed to be a useful marker for monitoring of therapy [45, 51, 64]. The loss of aneuploidy certainly coincides with response to therapy. When patients fail to respond to hormonal therapy, aneuploidy reappears. As this loss appears early enough before tumor regression can be clinically detected, it seems to be of potential clinical usefulness. Böcking et al. [66] described a DNA regression index. This index is defined as the change of the DNA grade of malignancy [33] in the course of time (t) expressed in months. Increasing DNA grade of malignancy values are signed negative (–), decreasing values positive (+):

$$\text{DNA regression index} = \frac{\Delta\text{DNA grade of malignancy}}{\Delta t\ (\text{months})}.$$

The dimension of the DNA regression index is in months^{-1}. We could prove statistically that independently from the DNA malignancy grade, the DNA regression index provided additional prognostic information concerning tumor regression and survival of patients with prostatic cancer under conservative therapy. Therapy-resistant tumors of patients who died within a few years

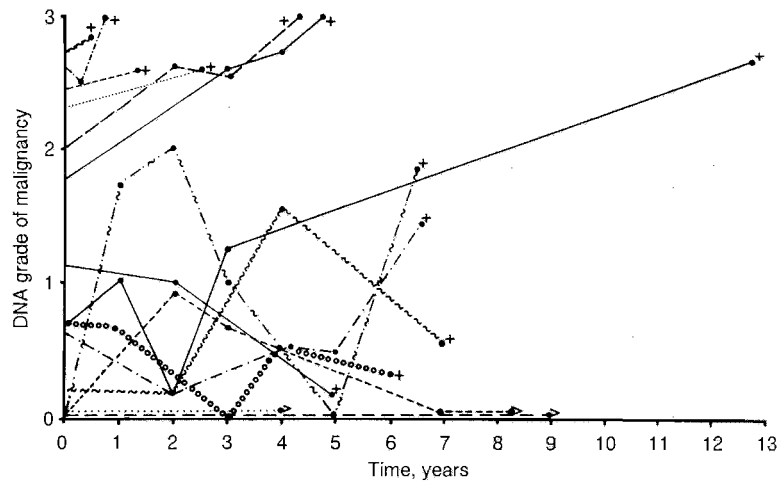

Fig. 6. Change in DNA grade of malignancy with time in patients with prostatic cancer under hormonal therapy. Each curve represents one patient. From Böcking et al. [66].

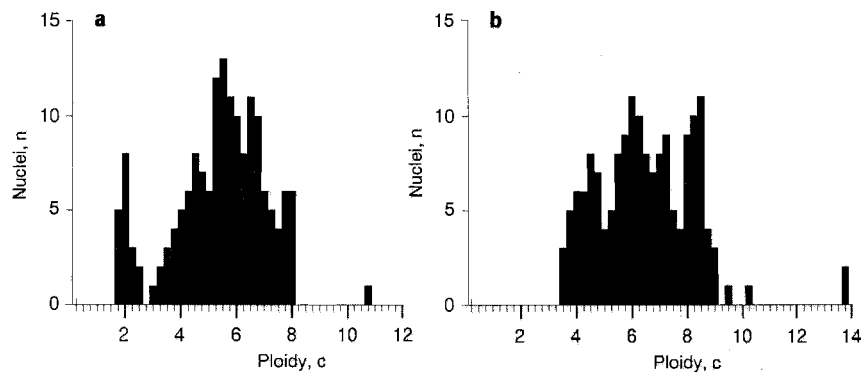

Fig. 7. DNA histograms of a 64-year-old patient with prostatic adenocarcinoma, stage T_1, grade 3, before (**a**) and after 6 weeks of estrogen therapy (**b**) without clinical response. Increasing amount of cells with high and variable DNA contents $>8c$. **a** DNA grade of malignancy = 2.07. **b** DNA grade of malignancy = 2.56; DNA regression index = −0.33. Survival time 54 months.

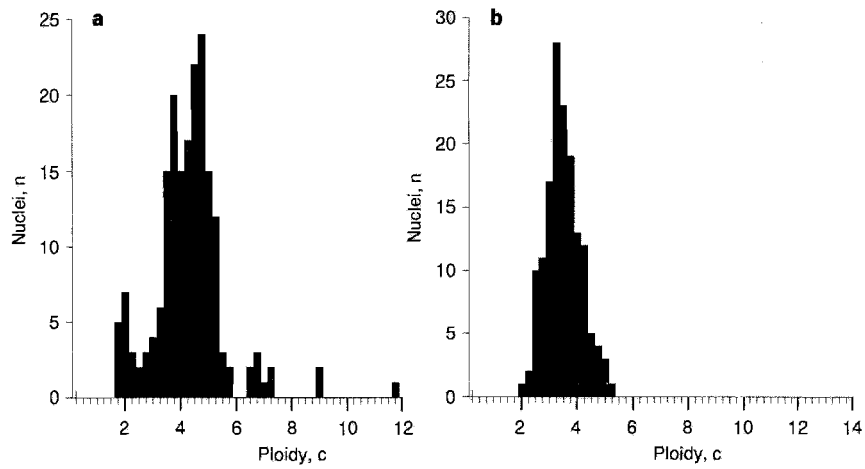

Fig. 8. DNA-histograms of a 62-year-old patient with prostatic adenocarcinoma stage T_2, grade 2, before (**a**) and after 6 weeks of estrogen therapy (**b**) with clinically obvious tumor regression decreasing the rate of aneuploid cells around 5c. **a** DNA grade of malignancy = 1.58. **b** DNA grade of malignancy = 0.98; DNA regression index = +0.4. Survival time 129 months.

following commencement of hormonal therapy showed a continuous increase in their DNA grade of malignancy (fig. 6, 7). On the contrary, patients with a long survival under hormonal therapy revealed a decrease in their DNA grade of malignancy (fig. 8). Thus we assume that the decrease in the DNA grade of malignancy during a certain period of time can be taken as an early indicator of the success or failure of conservative therapy.

The clinical application of these observations makes it necessary to have a pretreatment DNA histogram if we plan to monitor the response of the prostatic cancer to therapy. Judging response in terms of an improving histogram may not be possible unless the patient's initial tumor is clearly aneuploid, but a 'diploid' histogram which does not reveal a newly developing aneuploid stemline under conservative therapy may be taken as evidence for nonprogression of the tumor. Serial aspiration biopsies at about 6-month intervals are necessary for monitoring of therapy.

In conclusion, objective evidence for response and/or failure of conservative treatment of patients with prostatic cancer may be obtained from DNA cytometry by serially performed aspiration biopsies.

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