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CLINICAL RELEVANCE OF DNA PLOIDY AND CELL CYCLE PHASES IN TRANSITIONAL CELL CARCINOMA OF THE RENAL PELVIS AND URETER: A STUDY BY MEANS OF STATIC DNA-CYTOPHOTOMETRY

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ABSTRACT

In 72 patients with urothelial carcinoma of the renal pelvis or ureter the ploidy and counts of cell cycle phases in the tumor were analyzed by means of single cell DNA cytophotometry with the intention of finding new prognostic factors in addition to those already known (stage and grade). Follow-up ranged from 1 to 10 years. The results of the DNA analyses were related to the tumor categories, histopathological grading and clinical course. Malignancy grade 1 tumors showed DNA frequency peaks in the diploid range, while tumors assessed as malignancy grade 2 showed heterogeneous DNA distribution patterns. Malignancy grade 3 tumors exhibited 71% aneuploid and 29% tetraploid DNA values.

DNA histograms also show the distribution of the cell cycle phases in a cell population measured. The individual tumor cell nuclei are assigned to the different phases of the cell cycle (G0/G1, S phase and G2/M phases) according to the DNA content. Our results show that the prognosis for patients with more than 50% diploid cells in G0/G1 phase is better than for those with a lower percentage of cells. As a rule, a high percentage of G0/G1 cells is a sign of a slow growth rate of the tumor. The differences in survival times associated with the rates of G0/G1 cells and S and G2/M cells are not only evident in the patients with malignancy grade 1 but also in those with malignancy grades 2 and 3.

There was also a positive correlation between pT category and DNA ploidy. The cell lines were aneuploid in 38% of the patients with stage pT1 tumors, 56% with stage pT2 tumors and almost 85% with stage pT3, N+ tumors. A significant correlation was found between the results of DNA cytophotometry and the clinical course of the disease. Patients with diploid tumor cell nuclei had no metastases and no local tumor progression for up to 10 years, whereas patients with aneuploid tumor cell nuclei suffered metastasis and no local

The Cell Cycle: Regulators, Targets, and Clinical Applications
Edited by V.W. Hu, Plenum Press, New York, 1994

tumor progression within 24 to 36 months. The patients died of the tumor 36 months after primary diagnosis on the average.

The determination of tumor ploidy and tumor cell cycle phases in urothelial carcinoma of the renal pelvis and ureter by means of DNA cytophotometry yields valuable prognostic information.

INTRODUCTION

The histomorphological classification of tumors of the urothelium is a difficult task even today, mainly because there is no grading system which affords reliably reproducible results (1 - 3). Urothelial tumors that are not highly differentiated constitute a heterogeneous group with overlapping histological characteristics, clinical courses and biological behaviors (1, 2). Far more accurately than is possible with visual morphology, the different malignancy potentials of the tumor cell nuclei can be evaluated with the aid of DNA cytophotometry of the actual tumor cell (4 - 8), and the progression of these tumors can thus be predicted more exactly. The combined information obtained by the two methods - morphology and DNA cytophotometry - provides valuable prognostic indicators which have an influence on treatment and follow-up (4 - 9).

The heterogeneity of tumor cells has recently received great interest by clinicians because of its significant interrelation with the course of the tumor before and during treatment and because of its value as a prognostic indicator. This fact has also been pointed out by several authors of studies on malignant tumors in a wide variety of organs (4 - 8, 10 - 13).

In the present prospective study, the ploidy, DNA heterogeneity and the counts of cell-cycle phases in the individual malignancy grades were investigated by single-cell DNA absorption cytophotometry with the aim of finding new cell-related prognostic factors to complement those conventionally employed (stage and grade).

PATIENTS AND METHODS

Between February 1982 and December 1991, 72 patients underwent radical nephroureterectomy with a bladder cuff and lymphadenectomy for carcinoma of the renal pelvis and ureter. The average age of these patients (38 women and 34 men) was 67 years, with a range of 43 to 85 years. Patients with some other malignant tumor were excluded from the study. Of the patients 52 (72%) were diagnosed as having a tumor of the renal pelvis and 20 (28%) as having a tumor of the ureter (see table 1). Abuse of analgesics was reported by 15% of the patients during a 10 to 25-year period and 97% reported recurrent macroscopic hematuria (for an average of 5 to 34 months) before hospitalization. Follow-up examinations included exfoliative urine cytology every 6 weeks; sonography, cystoscopy. and lavage cytology and, if necessary, also DNA cytophotometry every three months, chest radiography, voiding urogramm and, if necessary, also computer tomography every six months.

Immediately after removing the tumor-bearing kidney, the renal pelvis was dissected and one or several wedge-shaped segments were excised from the tumor, the number depending on the size of the tumor. Several cytological preparations were made of the sagittal cut surface of these sections and of several macroscopically normal areas of the renal pelvis or ureter and fixed with Merckofix Spray (Merck). Some of the preparations were stained with Papanicolaou's stain for cytomorphological evaluation. These specimens were later compared with the final histological results. For the determination of DNA the preparations were stained by Feulgen's reaction (30 minutes' hydrolysis in 5 N HCl at room temperature). Leukocytes from peripheral blood from healthy donors treated in the same

manner as the test material were used as reference preparations for the determination of DNA diploidy.

As a rule, approximately 100 tumor cell nuclei were measured, occasionally more. The DNA content of these nuclei was determined by means of single-cell absorption photometry.

The fine-scanning procedure with a Leitz MPV-2 cytophotometer was used to measure the cell nuclei. The scanning stage, measuring procedure and recording of the absorption were controlled by a digital pdp 8a process computer. The respective total extinction values

Table 1. Clinical, pathological and DNA cytophotometric parameters in 72 patients with transitional cell carcinoma of the renal pelvis and ureter.

	No. Pts. (%)
Renal pelvis	52 (72)
Ureter	20 (28)
Multiplicity:	
Singel	54 (75)
Multiple	18 (25)
Grade:	
1	17 (24)
2	36 (50)
3	19 (26)
Stage:	
1	32 (45)
2	19 (26)
3	21 (29)
DNA ploidy:	
Diploid	23 (32)
Tetraploid	23 (32)
Aneuploid	26 (36)
Analgesics abuse (phenacetin)	10 (15)
Status:	
Alive	41 (57)
Dead	31 (43)
Cause of death:	
Transitional cell Ca	26 (36)
Other	5 (7)

measured were printed together with the mean value, standard deviation, variance and coefficient of variation.

The results are printed out in the form of a histogram in which the number of tumor cell nuclei measured is marked as 'n' on the ordinate, and the relative DNA content of the individual cell populations, given in relative c and expressed in arbitrary units, is plotted against the abscissa. Figures 1a-d show typical DNA histograms. The tumors exhibit either a diploid or several aneuploid or tetraploid DNA cell lines.

RESULTS

Tumor stage - histological and cytomorphological classification

The dissemination of the tumor was determined according to the tumor, nodes and metastasis classification recommended by the International Union Against Cancer (14). Of the patients 32 (45%) had stage pT1 pNO, 19 (26% stage pT2 pNO, 19% stage pT3 pNO and 7 (10% stage pT3 pN+ disease (table 1). The tumors were classified histologically

according to the guidelines issued by the World Health Organization (15), whereas the evaluation of cellular anaplasia in the cytological preparations was assessed according to the classification suggested by Mostofi et al and Bennington and Beckwith (16). The histological malignancy grade 2 was found to prevail at 50%, in the entire case material, followed by malignancy grades 3 (26%) and 2 (24%, table 1).

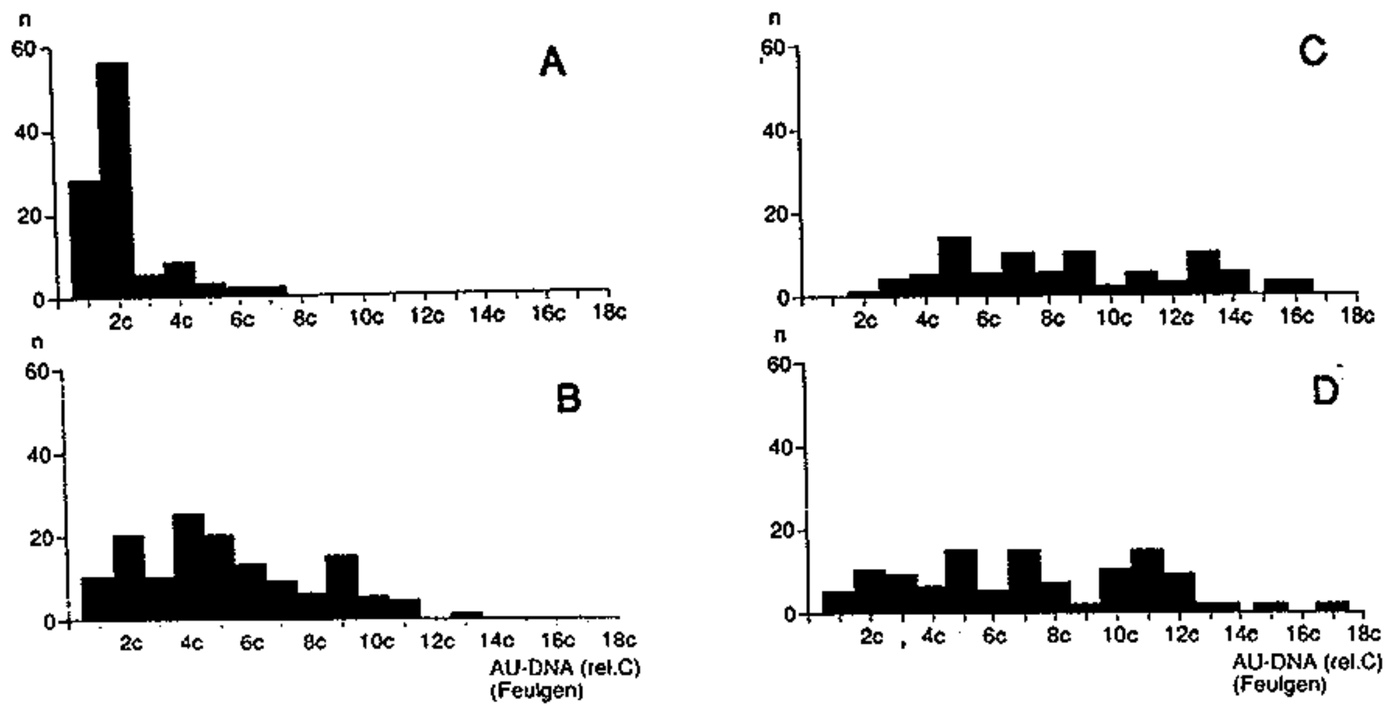

Fig. 1. *A*, malignancy grade 1. DNA histogram with frequency peak in diploid range and scattered values up to 7c. *B*, malignancy grade 2. DNA histogram with broad heterogeneous distribution ranging from 2c to 13c. *C*, malignancy grade 3. DNA histogram with heterogeneous distribution shows frequency peaks in 5c, 7c, 9c, 13c, and widely scattered values up to 16c. *D*, DNA histogram of lymph node metastasis shows aneuploid DNA distribution. *AU-DNA* (*rel. C*), arbitrary units.

DNA Ploidy and Grade of Malignancy

A positive correlation was found between the cellular anaplasia of the tumor cells and their DNA content. A total of 117 DNA histograms, 11 of which were histograms of lymph node metastases, was analyzed. In the 17 patients (24%) with grade 1 tumors, 88% of the tumor cell nuclei measured exhibited diploid (2c), 5% tetraploid (4c) and 7% aneuploid DNA distribution (Fig. 1a and 2).

The 36 patients with grade 2 tumors showed heterogeneous DNA distribution patterns, with DNA frequency peaks in the diploid, tetraploid and aneuploid ranges, although the carcinomas exhibited the same morphological degree of differentiation. The DNA content measured in the tumor cell nuclei was in the diploid range in 23%, in the tetraploid range in 35%, in the aneuploid range in 42%. (Figs 1b and 2)

The DNA content measured in the tumor cell nuclei of the 19 patients with grade 3 tumors was tetraploid in 29% and aneuploid in 71% as demonstrated by the ploidy distribution (Figs. 1 and 2).

DNA Ploidy and Pathological Tumor Stage

There was also a positive correlation between DNA ploidy and tumor stage. The DNA cell lines were aneuploid in 38% of the patients with stage T1 tumors, 56% of the patients with stage T2 tumors and almost 85% of the patients with stage T3 tumors, with or without lymph node infiltration (Fig. 3).

DNA Ploidy and Abuse of Analgesics

It is noteworthy that the nuclear DNA distribution measured in the 11 patients with carcinoma of the renal pelvis or ureter who had reported phenacetin abuse was aneuploid. In

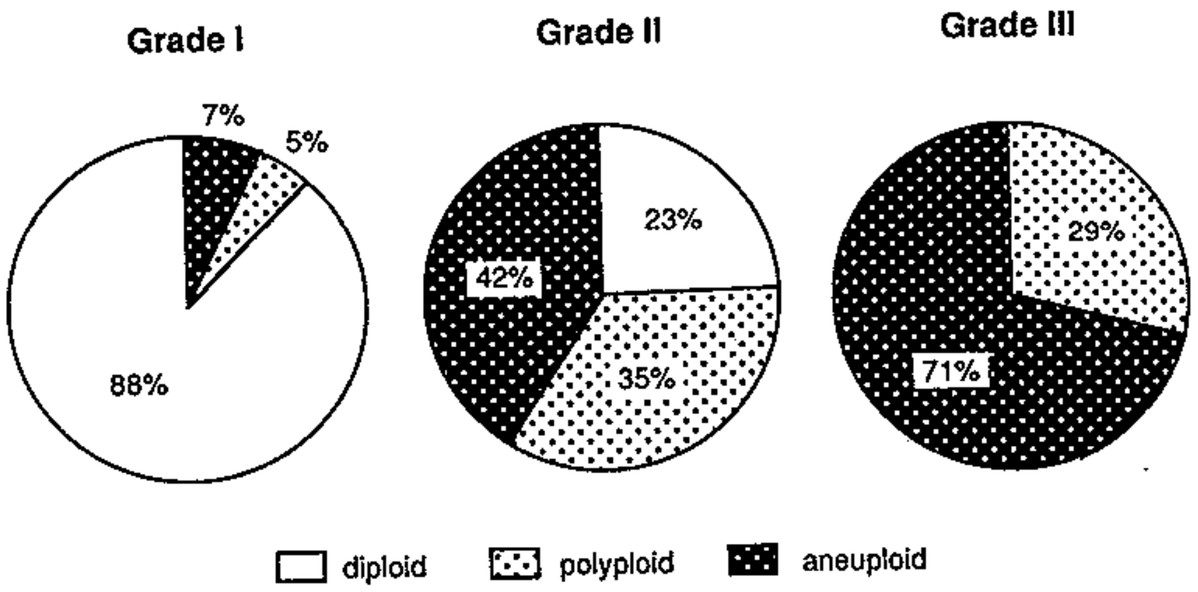

Fig. 2. Correlation of DNA ploidy and grades of malignancy in 72 patients with cacinoma of renal pelvis and ureter.

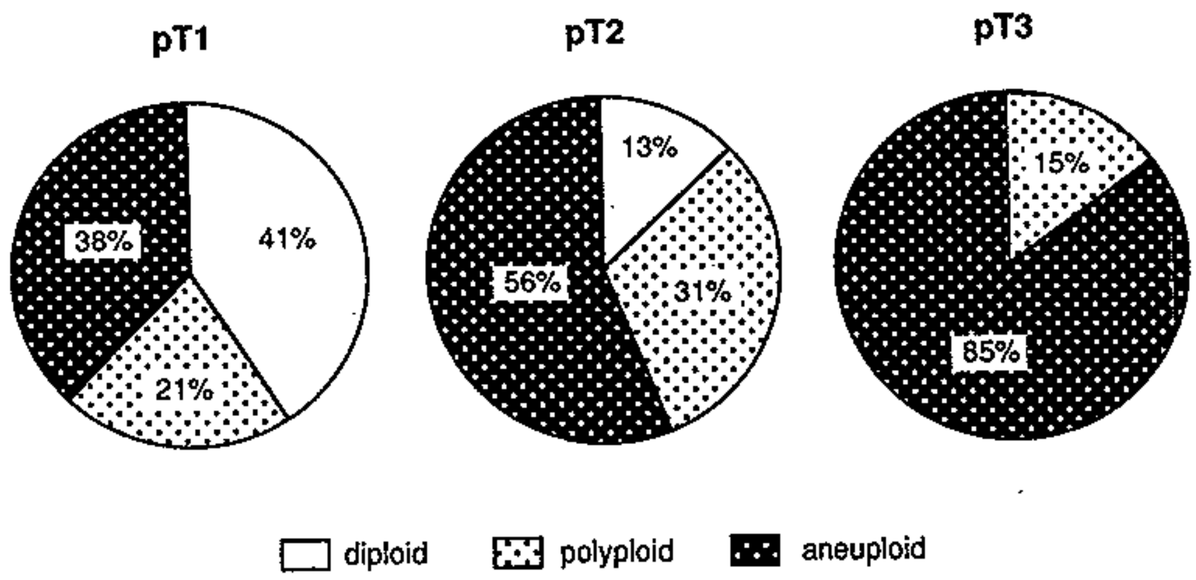

Fig. 3. Correlation of DNA ploidy and histological staging of urothelialcarcinoma of renal pelvis and ureter in 72 patients.

addition to multifocal tumor dissemination, these patients exhibited severe cellular anaplasie even at 6 cm from the tumor. These findings were established by scrape cytology and confirmed by the broad DNA distributions demonstrated by DNA cytophotometry (Fig. 1c and 2). 8 of the 11 patients had DNA distribution patterns with several cell lines.

DNA Ploidy and Multiplicity of the Tumor

Of the 26 patients whose tumors showed DNA aneuploidy 18 (69%) exhibited multifocal spreading (table 2). In these patients, cytology even showed microscopic changes indicative of carcinoma in situ in the urothelium in the immediate vicinity of the tumor.

Table 2. Multiplicity distribution of transitional cell carcinoma of the renal pelvis and ureter in 18 patients

Multiplicity	No. Patients	(%)
Renal pelvis	5	(28)
Ureter	3	(17)
Renal pelvis + bladder	4	(22)
Bilateral renal pelvis	4	(22)
Bilateral renal pelvis + ureter + bladder	2	(11)

DNA Ploidy and Metastatic Dissemination

A total of 23 patients with diploid DNA distribution patterns (DNA frequency peak in the 2c range) had no metastases during the 10-year observation period and none died of carcinoma. Of the patients with aneuploid tumors, however, and especially patients with reported phenacetin abuse, 84% suffered tumor progression with metastases to other organs (that is retroperitoneal lymph nodes in 43%, lungs in 32% and bones in 26%) within 28 to 36 months and they died of the tumors.

Table 3. Correlation of nuclear DNA patterns clinical course in 72 patients with transitional cell carcinoma of the renal pelvis and ureter.

DNA Histogram Pattern	No. Pts.	Died of Metastatic Transitional Cell Ca No. (%)
Diploid	23	–
Tetraploid	23	4 (17)
Aneuploid	26	22 (84)

Prognostic Significance of DNA Ploidy

The DNA ploidy and cell line heterogeneity were significant for the prognosis. Patients with aneuploid DNA distribution patterns died sooner than patients with diploid cell lines. Of the patients with diploid or tetraploid DNA distribution patterns 5 (7%) died of cardioplumonary disease: 3 had diploid and 2 had tetraploid DNA distribution patterns. Of the 26 patients with aneuploid DNA distribution patterns 22 (84%) died of metastasis of the primary disease. Two patients had metastases in the lymph nodes, bones and lungs within 16 and 21 months after tumor nephrectomy but they have been clinically stable with methotrexate, vinblastine, doxorubicin and cisplatin chemotherapy (17). Two patients were clinically stable while exhibiting neither metastases nor tumor progression at 11 and 17 months postoperatively. The remaining 41 patients (57%) have shown no signs of progression of the primary disease (table 3).

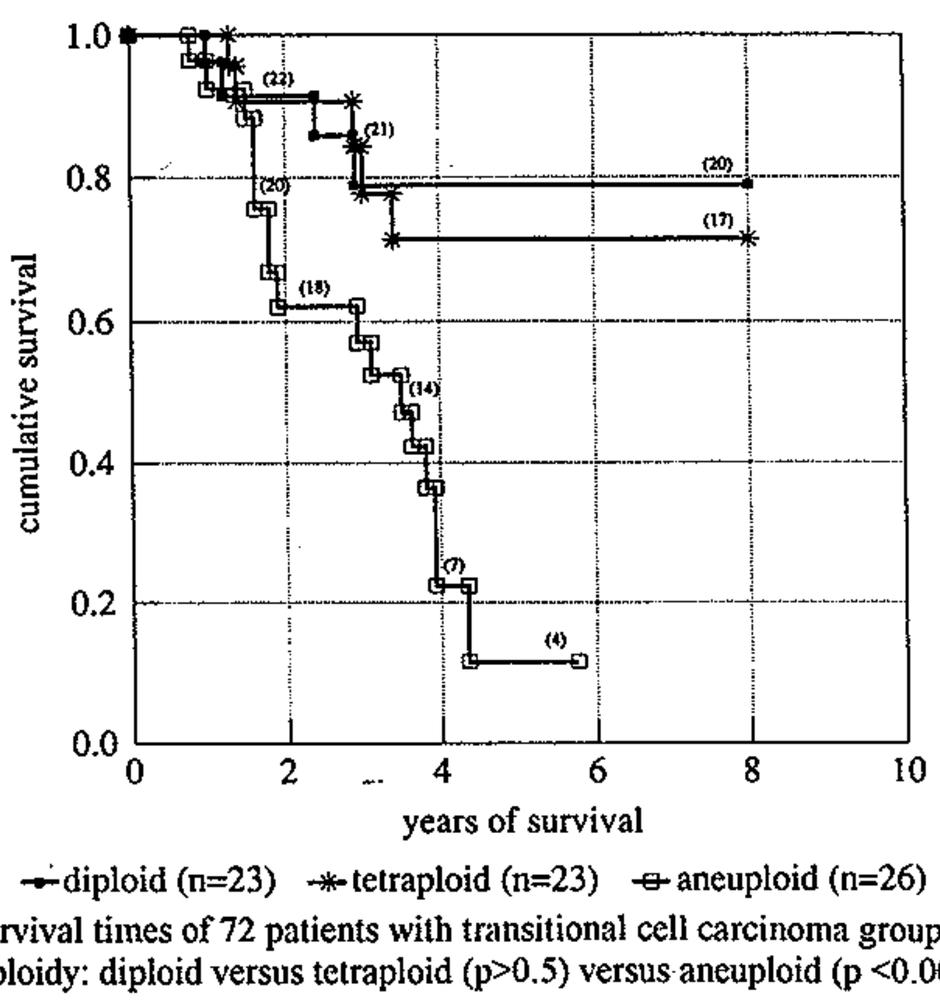

Fig 4. Survival times of 72 patients with transitional cell carcinoma grouped according to DNA ploidy: diploid versus tetraploid ($p>0.5$) versus aneuploid ($p<0.001$).

DISCUSSION

According to Bergkvist et al, (1) the stage and pathohistological grading of a tumor do not suffice to judge its biological aggressiveness in respect of recidivation, invasion and metastatic growth. Since histological grading is subjective and not uniform (2, 3), it is necessary to assess the grade of malignancy and tumor stage objectively by means of prognostic methods of investigation (4, 5, 6, 10, 12).

Presently there are 2 different methods of measuring the nuclear DNA content of malignant and benign tumors: flow-through cytophotometry, which is the most common method and single cell cytometry.

Researchers often employ auto-radiographic examinations to assess the proliferate activity of a tumor. This method is not suitable for clinical application, though, on account of the procedure it involves.

This is the first prospective study on urothelial carcinomas of the renal pelvis and ureter in which DNA analyses have been conducted by single-cell cytophotometry over a follow-up period of 10 years.

Our results show that the DNA ploidy, tumor heterogeneity and the distribution of the individual cell cycle phases of the tumors are independent factors which are of considerable value for predicting patient's survival times.

Detailed investigations have shown that nuclear DNA analysis can contribute to the existing clinical and morphological parameters by the prognostically supplementary information it affords. In this context we would like to mention our previous studies on 329 patients with prostatic carcinoma, 112 patients with renal cell carcinoma and on 127 patients with urothelial carcinoma of the urinary bladder conducted over a follow-up period of 9 years (6, 18, 19).

The results of the study on 409 patients with mammary carcinoma conducted by "Fallenius (1987)" (20) underline the prognostic significance of DNA ploidy.

Other investigations based on flow-through cytophotometry point to a correlation between the histological grade of malignancy of a tumor and the DNA content of the tumor cell nuclei (4, 7, 8).

In the past few years various working groups have reported that it is possible to measure DNA by flow-through cytophotometry in cell material embedded in paraffin. In 1988, a research team at the Mayo Clinic employed this method in 119 cases of urothelial carcinoma of the renal pelvis and confirmed the prognostic relevance of DNA ploidy that we had shown using single-cell cytophotometry (13).

In our study we found an aneuploidy rate of 7% in highly differentiated urothelial tumors and one of 71% in poorly differentiated tumors. Other authors who employed flow-through cytophotometry to investigate tumors of the lungs found aneuploidy rates of up to 85% (21).

A negative turn of prognosis in the individual case can be deduced from a broad scattering of DNA values as manifest in the histogram. A similar conclusion can be drawn from the ratio of diploid cells to aneuploid cells. This ratio is particularly important for carcinomas with grade 2 malignancy which are uniform with regard to morphology but heterogeneous with respect to malignancy potentials.

DNA histograms show also the distribution of the cell cycle phases in a cell population measured. The individual tumor cell nuclei are assigned to the different phases of the cell cycle (G0/G1 phase, S phase and G2/M phase) according to their DNA content. Changes in cellular genetics result in corresponding changes in the DNA histograms (12, 13). The prognosis for patients with more than 88% diploid cells (G0/G1 phase) is better than for those with a lower percentage of diploid cells. As a rule, a high percentage of G0/G1 cells is a sign of a slow growth rate of the tumor. The differences in survival times associated with the rates of G0/G1 and proliferating cells (S and G2/M) are not only evident in the group of patients with malignancy grade 1 but also in the ones with malignancy grades 2 and 3.

The patients with nephropathy caused by analgesics abuse who also showed multifocal tumor dissemination exhibited high DNA values with predominantly aneuploid DNA distribution and several DNA cell lines in DNA cytophotometry. Prolonged phenacetin abuse presumably provokes aggressive cytochemical and morphological changes in the epithelial cells which lead to the development of a biologically highly aggressive tumor.

CONCLUSIONS

DNA histograms define the biological characteristics of a tumor more precisely than the criteria by which the clinical status of the disease is judged. Only in the case of grade 1 and grade 3 tumors, the histological grading correlates with the nuclear DNA content. The prognosis for grade 1 tumors is good whereas it is very unfavorable for grade 3 tumors. For both groups (patients with grade 1 and grade 3 tumors) DNA ploidy affords no additional prognostic information. Grade II tumors, on the other hand, are very heterogeneous in respect of DNA ploidy although they exhibit the same histomorphological degree of differentiation. These tumors can be sub classified in aneuploid (biologically aggressive) and diploid or tetraploid (biologically less aggressive) tumors with the aid of DNA cytometry.

Analyses of DNA ploidy, tumor cell heterogeneity and cell cycle phases by means of DNA cytophotometry afford additional valuable information as to prognosis and treatment.

Acknowledgements

We thank Mrs. Ikonija Rotter for her excellent technical assistance and Prof. Küster for helping with the statistics.

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