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# Quantitative Flow-microfluorometric Analysis of the DNA in Cells from Neoplasms of the Urinary Bladder: Correlation of Aneuploidy with Histological Grading and the Cytological Findings

B. Tribukait[1] and P. L. Esposti[2]

[1] Institute of medical radiobiology, Karolinska institute, Stockholm,
[2] Radiumhemmet, Karolinska sjukhuset, Stockholm, Sweden

Summary. The total DNA content in single cells from bladder washings of patients with tumours in the bladder has been analysed by means of a rapid-flow cytofluorometer. The frequency of occurrence of aneuploid cell populations has been related to the cytological findings and the histological grading according to Bergkvist et al. The frequency of occurrence of aneuploid cell populations in 228 patients increased from 7% for tumours of grades 0 and 1 to 79% for grade III tumours. In cell material cytologically classed as benign the frequency of occurrence was 8% increasing to 96% for poorly differentiated carcinomas. In patients with tumours of histological grade II, subdivided into one group with minor atypical features with no invasion and one with major deviation from the normal cell picture and suspected or manifest invasion, the frequencies of occurrence of aneuploidy were 9% and 68% respectively. The degree of aneuploidy has been determined by comparison of the position of the DNA values in relation to the normal DNA values. In 54 irradiated patients with persistent or recurrent tumours the position of the aneuploid cell lines changed in the diploid direction in comparison with that for 65 non-irradiated patients for the majority of which the aneuploid cell lines were in the tri-tetraploid region. The biological significance of the different degrees of aneuploidy is as yet unknown.

Key words: DNA-analysis - Bladder neoplasms - Malignancy grading - Aneuploidy.

Studies of human malignant tumours originating in solid tissues have shown a clear tendency for a certain degree of chromosome abnormality. In well-differentiated carcinomas of the bladder a diploid mode of chromosomes has been found; moderately well-differentiated or poorly differentiated tumours on the other hand mainly showed modes in the tetraploid range (5, 7). Since quantitative DNA measurements in single cells indicate any changes in the numbers of chromosomes, aneuploidy and its degree can be analysed in this way.

In the current studies the amount of DNA in a large number of single cells from bladder washings has been analysed by means of a rapid-flow cytofluorometric method. In this way the frequency of occurence of aneuploid cell populations in a large number of patients with different types of bladder tumours has been assessed. There are two questions of particular interest: 1) Is there any relation between the frequency of occurrence of aneuploidy and the histological grading of the tumours or the cytological findings in the bladder washings? 2) Are there indications that the degree of aneuploidy found is of significance for the biological behaviour of the tumour?

## MATERIAL AND METHODS

The material consisted of cells from bladder washings of patients examined at the Karolinska Sjukhuset with tumours in the bladder. The method used for cell preparation, fixation and staining for DNA-analysis using a rapid-flow

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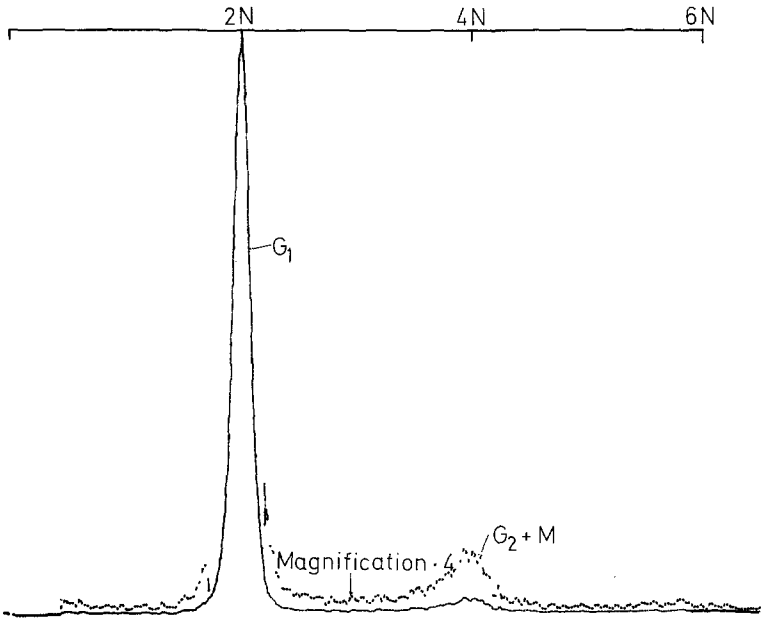


Fig. 1. DNA-histogram from a patient with normal bladder mucosa The largest peak represent the diploid $G_1$-cells, the smaller peak the tetraploid $G_2$+M cells. In between are the cells in S-phase. 35 000 cells were measured

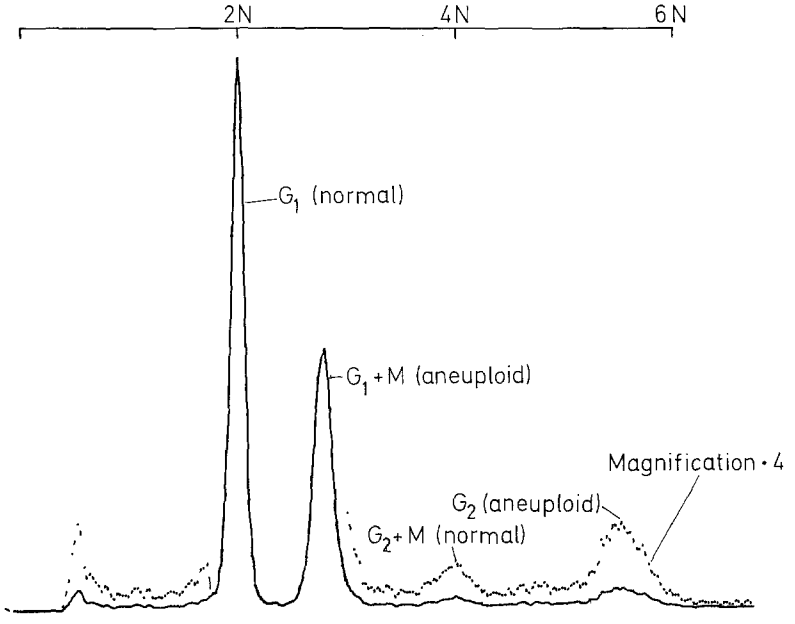


Fig. 2. DNA-histogram from a patient with a bladder tumour $T_2$ grade III. In between the normal $G_1$ and $G_2$+M peaks there is the $G_1$ peak of an aneuploid cell line with a $G_2$+M peak at a point representing almost hexaploid DNA-content

cytofluorometer has been described earlier (8). Briefly, fresh cell material in 0.9% NaCl solution was centrifuged, the erythrocytes were removed by haemolysis with water and the cells fixed in 96% ethanol. The fixed cells were washed in a Tris-EDTA-buffer together with RNase. Suspensions of single cell nuclei were obtained by pepsin treatment. After washing in the buffer the nuclei were stained using ethidium bromide in Tris-EDTA-buffer with high molarity. The DNA contents of the cell nuclei were then analysed using a rapid-flow cytofluorometer ICP 11 (Phywe, W. Germany) with a flow rate of up to 1,000 cells/sec. The cells in the same material had been classified cytologically without knowledge of the clinical findings or previous cytological results. The cells were grouped into the categories "benign", "suspected malignant" and "malignant" (2). The malignant cells were subdivided into the classes "well differentiated", "moderately differentiated" and "poorly differentiated" according to their degree of deviation from the features of normal urothelium. This subdivision was not

Table 1. Frequency of occurrence of aneuploid cell populations related to histological grading and cytological findings in 228 patients with bladder tumours

| Cytological findings | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Carcinoma | | | | | |
| Histological grading | Benign cells | Suspected malignancy | Not graduated | Well diff. | Moderately diff. | Poorly diff. | Total | |
| 0 | 0/2 | - | - | - | - | - | 0/2 | ( 0%) |
| I | 2/41 | 0/8 | 2/2 | 0/2 | - | - | 4/53 | ( 7%) |
| II | 5/49 | 3/19 | 0/2 | 5/7 | 9/14 | 16/16 | 38/107 | (35%) |
| III | 1/9 | 0/4 | 3/3 | - | 12/12 | 36/38 | 52/66 | (79%) |
| Total | 8/101 | 3/31 | 5/7 | 5/9 | 21/26 | 52/54 | 94/228 | |
| | (8%) | (10%) | (71%) | (56%) | (81%) | (96%) | | |

Table 2. Frequency of occurrence of aneuploid cell populations in non-invasive neoplasms grade II with slight atypia (II a) and in possibly invasive or invasive neoplasms grade II with more severe nuclear atypia (II b) in 85 patients with bladder tumours

| Histological grading | Frequency of aneuploidy |
|---|---|
| II a | 5/54 ( 9%) |
| II b | 21/31 (68%) |

possible in all cases, especially in the case of squamous cell carcinomas.

Since there were only a few cases in which the histological assessments were made at the same time as the cytological investigations and the cytofluorometric measurements, the histopathological result closest in time to the above mentioned investigations was accepted as representative for the type of tumour. The histological grading was based on the classification according to Bergkvist et al. (1). The grade II groups have in a number of cases been subdivided into one group with minor atypical features with no invasion (II a) and one with major deviation from the normal cellular picture, possibly invasive or invasive (II b).

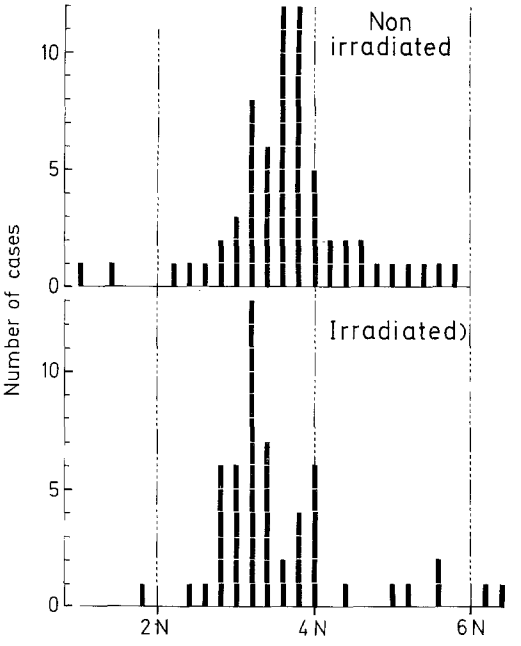


Fig. 3. Position of the aneuploid $G_1$-peaks for 65 non-irradiated patients (upper part) and for 54 irradiated patients with persistent or recurrent tumours (lower part)

## RESULTS

The majority of cells in normal somatic cell populations are in a state of rest, even in proliferating cell populations such as the bone marrow. All these resting somatic cells have 46 chromosomes and consequently a constant

amount of DNA of about 7 pg. In proliferating cells the amount of DNA doubles. The number of cells in the population engaged in proliferation, and the time spent in $G_1/G_0$, S, $G_2$ and M will influence the frequency of cells with a particular nuclear DNA content.

Figure 1 shows a typical example of a DNA histogram from a patient with urothelial cells cytologically assessed as benign. A total of 35,000 cells has been measured. The large peak to the left containing 95% of all the cells represents $G_1/G_0$ cells. At twice the distance, at 4 N, there is a smaller peak representing $G_2$+M cells; this peak contains about 4% of all the cells, a typical value for normal cases. Between these two peak we find the few S-phase cells.

Figure 2 shows a typical example of an aneuploid cell line from a patient with a bladder tumour histologically classified as grade III. In all cases with aneuploidy there were always normal cells in varying amounts from 5 to 97%. Cells with normal diploid quantities of DNA consitute here the largest maximum at 2N, whereas the $G_2$+M cell population is at 4N. Of the total of 46,000 cells measured, 57% were cells with normal di-tetraploid DNA values. Half-way between these two maxima is the $G_1$ maximum formed by an aneuploid stem cell line with an almost triploid DNA content the $G_2$+M maximum of which stands out with an almost hexaploid DNA content. In about 10% of all cases with aneuploidy two or more unambiguous aneuploid cell stem cell lines can be distinguished.

Table 1 presents an analysis of the occurrence of aneuploidy for 228 patients with bladder tumours in relation to the histological grading and to the cytological classification. The few cases of histological grading grade IV have been combined with the grade III cases. The frequency of occurrence of aneuploidy in relationship to the histological grading increases from 7% for grade 0+I to 79% for grade III. Grade II cases have an intermediate position with 35%. When grade II is subdivided into grade II a for less and II b for more pronounced atypia, it is found that the dividing line for the presence of aneuploidy coincides with this subdivision; thus the frequency of occurrence of aneuploidy is 9% in grade II a and 68% in grade II b (Table 2). The occurrence of aneuploidy in relationship to the cytological classification increases from 8% in the "benign" group to 96% for the group with "poorly differentiated" malignant cells. In the limited number of cases reported in Table 1 there is an apparent increase in occurrence of aneuploidy when the classification changes from "well differentiated" to "poorly differentiated" carcinomas; this increase has been confirmed in a larger study (9). The frequency of occurrence of aneuploidy is greatest when the cytological picture indicates "poorly differentiated" cells and when at the same time the histological grading is grade II or III. Only two such cases show no aneuploidy and for these the morphological description was almost anaplastic.

So far there are only indications that the degree of aneuploidy may be associated with the biological behaviour of the tumour. The position of the $G_1$ peak in relation to that of the normal diploid peak may be used as an expression for the degree of aneuploidy. A change in the position of the $G_1$ peak for the aneuploid cells for irradiated patients with persistent or recurrent tumours may be an expression of the appearance of cell stem lines with new properties such as altered radiation sensitivity. Such a change is shown in Figure 3. In the upper part of this figure, the position of the aneuploid $G_1$ peak for 65 non-irradiated patients has been shown; for the majority of them it is within the tri-tetraploid region. For 54 irradiated cases with persistent tumours or recurrent tumours the position has moved significantly in the direction of the diploid cells.

## CONCLUSIONS

The development of automated procedures for the determination of the DNA contents in individual cells and of the development of cell preparation procedures makes it possible to analyse large numbers of cells and thus to obtain the characteristics of aneuploid cell populations far better than has hitherto been the case.

Comparison of the frequency of occurrence of aneuploidy in the urothelium of patients with bladder tumours with the histological grade of malignancy and the frequency of cytological diagnoses of cancer make it highly suggestive that aneuploidy is an indication of malignancy. A contrary indication, namely that non-invasive tumours are generally near-diploid, is given by chromosome analyses (4, 5, 6, 7). The number of pseudo-diploid malignant tumours in the material presented here cannot be more than a few per cent since the frequency of occurrence of aneuploid cell lines from histologically poorly differentiated cases which were also cytologically classed as malignant is almost 100%. The borderline of aneuploidy passes through the histological grade II. A similar borderline has been observed in exfoliative studies of bladder tumours of grade II. The

frequency of occurrence of malignant cells in smears from bladder washings with neoplasms of grades 0, I and II a is less than 10% but is 70% in cases with tumours of grade II b (3).

While quantitative DNA analyses for the detection of aneuploidy appear to offer information of practical value as a compliment to the histological diagnosis and the cytological findings in cases of tumour disease, the significance of the degree of aneuploidy and its changes, for example subsequent to irradiation, remains uncertain. Clinical observation and other observations on the behaviour of the tumour must be the guiding factor.

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B. Tribukait
Institute of Medical Radiobiology
Karolinska Institute
S-104 01 Stockholm
Sweden