Morphometric differences between urothelial cells in voided urine of patients with grade I and grade II bladder tumours

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SUMMARY The morphometric differences between the urothelial cells in the voided urine of 24 patients with grade I and grade II bladder tumours were measured. In both wet-fixed Papanicolaou-stained smears as in air-dried Giemsa-stained preparations, the cells in the grade I tumours are larger and have more pronounced anisocytosis and a smaller nucleus to cytoplasm area (N/C) ratio. Although absolute dimensions of cells and nuclei in the two preparatory techniques differ significantly, the average N/C ratios are similar. In both methods almost all grade I tumours have average N/C cell size ratios of less than 0.6 and grade II tumours more than 0.6. Morphometrically urothelial cells could not be distinguished from cells exfoliated from grade I bladder tumours. The results indicate that it is feasible to classify bladder tumours using the morphometric values of the exfoliated urothelial cells alone.

Grade I bladder tumours do not shed identifiable tumour cells and so cannot usually be diagnosed cytologically. On the other hand, a significant proportion of the grade II tumour can be detected cytologically¹ because the exfoliated cells can be recognised as malignant. The neoplastic cells from grade II tumours can be identified as cancer cells because of certain quantitative features. For example, recent studies have shown that a large proportion of cells in grade II tumours have a raised DNA content.² It is difficult, however, to translate the findings from DNA studies into the microscopical images with which the cytopathologist is confronted in his diagnostic work. On the other hand, the results from morphometry can be easily assimilated by the histopathologist.^{3,4} Therefore we carried out morphometry measurements on urothelial cells in voided urine of cases with grade I and grade II bladder tumours to discover the most distinctive and objective diagnostic criteria, and to compare the cell features in the wet-fix Papanicolaou method with those in the air-dried Giemsa method.

Material and methods

CYTOLOGY AND HISTOLOGY

Voided urine was used for this study. Two con-

secutive samples were taken from each patient and the smears with the highest cytological grading were used. In eight patients with grade I tumours and 17 with grade II tumours, both the air-dried Giemsa-stained smear and the wet-fixed Papanicolaou-stained smear were used; in another case of grade II tumour, only the air-dried Giemsa-stained preparation was available for study. Samples made from bladder scraping of 25 patients without urological complaints were used as control smears.

The wet-fixation was achieved by spray-fixing (coating fixative 80 ml polyethyleneglycol (MW 300), 690 ml isopropanol, 170 ml acetone, 60 ml distilled water). The smears for the Giemsa method were air-dried before staining. The histological sections of the bladder tumours were classified according to the WHO classification.⁵ There were six borderline cases, which were classified by six pathologists independently: when the mean score was 1.0-1.5, the case was classified as a grade I tumour, and when the mean score was 1.6-2.0, as a grade II tumour.

MORPHOMETRY

In each smear, 50 urothelial cells were measured. Except for the exclusion of cells with degenerative features, cells were selected at random. The measurements were performed with a graphic tablet (ASM, Leitz, West Germany) equipped with a camera lucida system. The cursor could be seen through the

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camera lucida drawing tube, and the contour of the nuclei and cells outlined. The computer calculated the following features from the two delineated areas: nuclear perimeter, cell perimeter, nuclear area, cell

area, nuclear size $\left(2\sqrt{\frac{\text{area}}{\pi}}\right)$, cell size, nucleus to cytoplasm area (N/C) ratio, N/C size ratio. From each slide the mean and the standard deviation of the assessed features was calculated. This gave 16 sets of values per case.

STATISTICAL ANALYSIS

Statistical analysis was carried out on a PDP11 computer DEC with a part of the program STP (statistical package) developed by one of us (PHJK). The descriptive statistics of the measurements in the air-dried Giemsa-stained and the wet-fixed Papanicolaou-stained smears were computed for the two groups. Wilcoxon's test was used to establish the significance of differences between the two groups. A level of significance of $p < 0.05$ was adopted.

Results

WET-FIXED PAPANICOLAOU-STAINED SMEARS
The morphometric parameters show many differences between the two tumour groups (Table 1). Significant differences were established for the nine sets of values: mean cell perimeter, standard deviation cell perimeter, mean cell area, standard deviation cell area, mean cell size, standard deviation cell size, mean N/C area ratio, mean N/C size ratio and standard deviation N/C size ratio. All the cases were plotted in a scattergram with the mean N/C size ratio on the x-coordinate and the mean nuclear area on the y-coordinate (Fig. 1).

Fourteen of the 16 grade II cases, and one of the 8 grade I cases had N/C size ratios of over 0.60. There

Table 1 Descriptive statistics: wet-fixed Papanicolaou-stained cells in cases of grade I and grade II tumours

Parameters (mean)	Mean (μm) $\pm$ SD		*Probability of difference (two-sided)
	Grade I	Grade II	
Nuclear perimeter	31.8 $\pm$ 6.46	31.1 $\pm$ 5.85	0.69 (0.10)
Cell perimeter	63.2 $\pm$ 19.7	52.0 $\pm$ 12.1	*0.0001 *(0.01)
Nuclear area	56.8 $\pm$ 23.0	53.3 $\pm$ 21.2	0.60 (0.15)
Cell area	223 $\pm$ 154	138 $\pm$ 62	*0.0003 *(0.01)
†Nuclear size	8.3 $\pm$ 1.65	8.01 $\pm$ 1.46	0.56 (0.08)
Cell size	15.8 $\pm$ 4.80	12.8 $\pm$ 2.67	*0.0002 *(0.02)
N/C area ratio	0.32 $\pm$ 0.12	0.40 $\pm$ 0.10	*0.001 (0.50)
N/C size ratio	0.55 $\pm$ 0.11	0.63 $\pm$ 0.08	*0.0003 *(0.02)

*Wilcoxon's test. The asterisks indicate probabilities < 0.05 in differing from grade I tumours.

†Nuclear size = $2\sqrt{\frac{\text{area}}{\pi}}$.

Fig. 1 Scattergram of the mean N/C size ratios and mean nuclear areas of the wet-fixed Papanicolaou-stained urothelial cells. Each symbol represents one case.

was considerable overlap between the grade I tumours and normal urothelium (Table 2). Significant differences were established only for the standard deviations of nuclear size and perimeter.

Table 2 Descriptive statistics of normal urothelial cells prepared with the Papanicolaou and with the Giemsa method

Parameters (mean)	Mean (μm) $\pm$ SD	
	Papanicolaou	Giemsa
Nuclear perimeter	36.2 $\pm$ 5.9	41.9 $\pm$ 5.8*
Cell perimeter	80.1 $\pm$ 19.0	88.9 $\pm$ 19.2
Nuclear area	71.5 $\pm$ 25.1	100.0 $\pm$ 27.5
Cell area	337.7 $\pm$ 177.0	415.3 $\pm$ 153.7
†Nuclear size	9.2 $\pm$ 1.5	10.8 $\pm$ 1.4*
†Cell size	18.8 $\pm$ 4.5	21.6 $\pm$ 3.9
N/C area ratio	0.29 $\pm$ 0.07*	0.28 $\pm$ 0.08
N/C size ratio	0.53 $\pm$ 0.07*	0.53 $\pm$ 0.08

*Wilcoxon's test. The asterisks indicate probabilities < 0.05 in differing from grade I tumours.

†Size was computed as follows: Nuclear size = $2\sqrt{\frac{\text{area}}{\pi}}$.

AIR-DRIED GIEMSA-STAINED SMEARS

Table 3 shows that in the Giemsa-stained cells, six sets of values proved to be significantly different: mean cell perimeter, mean cell area, standard deviation cell area, mean cell size, mean N/C area ratio and mean N/C size ratio. The cases plotted in the scattergram (Fig. 2) display that all cases of grade

II tumours have mean N/C size ratios of over 0.59, and, in addition, two of the grade I cases. Also in this method there was considerable overlap between normal urothelium and grade I tumours (Table 2). Significant differences were established for only the standard deviations of the N/C ratios.

Table 3 Descriptive statistics: air-dried Giemsa-stained cells in cases of grade I and grade II tumours

Parameters (mean)	Mean (μm) $\pm$ SD		*Probability of difference (two-sided)
	Grade I	Grade II	
Nuclear perimeter	40.3 $\pm$ 7.69	39.2 $\pm$ 7.56	0.67 (0.77)
Cell perimeter	81.2 $\pm$ 21.3	64.1 $\pm$ 16.9	*0.02 (0.16)
Nuclear area	88.2 $\pm$ 32.9	89.2 $\pm$ 36.0	0.92 (0.72)
Cell area	363 $\pm$ 208	229 $\pm$ 122	*0.03 *(0.04)
†Nuclear size	10.3 $\pm$ 1.94	10.3 $\pm$ 1.95	0.97 (0.72)
†Cell size	20.3 $\pm$ 5.65	16.1 $\pm$ 3.96	*0.03 (0.05)
N/C area ratio	0.31 $\pm$ 0.12	0.34 $\pm$ 0.12	*0.00 (0.62)
N/C size ratio	0.54 $\pm$ 0.11	0.66 $\pm$ 0.09	*0.00 (0.41)

*Wilcoxon's test. The asterisks indicate probabilities < 0.05 in differing from grade I tumours.

†Nuclear size = $2 \sqrt{\frac{\text{area}}{\pi}}$.

Fig. 2 Scattergram of the mean N/C size ratios and mean nuclear areas of the air-dried Giemsa-stained urothelial cells. Each symbol represents one case.

Discussion

The results of this investigation show that the cellular and nuclear dimensions of the urothelial cells in the voided urine of patients with grade I and

grade II tumours differ, and that normal urothelial cells cannot be distinguished from cells exfoliated from grade I tumours. In routine diagnosis the nuclear and cellular dimensions are used as diagnostic criteria, and therefore the results of this study can be of value in the investigation of future cases.

In both the air-dried Giemsa and the wet-fixed Papanicolaou methods the best discriminative factor was the N/C ratio. The N/C ratio has proved to be of major importance in distinguishing other benign and malignant lesions^{3,6} and as a major indicator in the survival of T-I bladder tumours.⁷ In other quantitative studies of cells from bladder tumours the N/C ratio as such was not calculated but only the nuclear size^{8,9} which in our study proved to have no value as an indicator (Table 1 and Table 3). A higher N/C ratio was also found in Kern's study¹⁰ in the grade II tumours. In his study the mean nuclear area was larger in the grade II tumours, although only grade II tumour cases were measured. Moreover, "characteristic" cells were selected, in contrast to our study in which urothelial cells were measured at random. The outcome of the two studies are thus not fully comparable. Flow cytometry also allows assessment of the relative nuclear size,¹¹ and therefore can be applied in the non-selective measurements of N/C ratios in grade I and II tumours. The results of such studies are highly suitable for comparison with our results.

To summarise, therefore, we can say that when compared with grade II, the N/C ratio of grade I tumour cells is smaller and while the nuclear dimensions are not important, the cell dimensions are. In grade I tumours, cells are invariably larger and associated with a pronounced anisocytosis.

Interesting comparisons can be made between the wet-fixed Papanicolaou-stained smears and the air-dried Giemsa-stained smears. As expected, both the cell and the nuclear areas are larger in the Giemsa method, in which the cells flatten out in the drying process. This is thought to be due to the extreme pressures exerted on cellular structures by the surface tension of evaporating water, thereby causing collapse of the cells.¹² This phenomenon was seen in the normal urothelial cells, as well as in the neoplastic ones.

The application of morphometry also allows the grading of the parent tumour in individual cases. Using mean N/C size ratio as a single discriminating factor, three of the 24 cases were misclassified using the Papanicolaou-stained smear, and four other cases using the Giemsa smear.

These results are highly encouraging, considering we are dealing with a morphological continuum of bladder tumours which have been subjectively classified. We hope to develop a classification rule for the

(computer-aided) grading of individual bladder tumours in our laboratory, similar to that which we already use for the distinction between endometrial hyperplasia and carcinoma in histological sections⁴ and between follicular adenoma and carcinoma of the thyroid⁶ on cytological material.

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References

- ¹ Esposti PL, Zajicek J. Grading of transitional cell neoplasms of the urinary bladder from smears of bladder washings. A critical review of 326 tumors. *Acta Cytol* 1972;16:529-36.
- ² Boon ME. DNA content of normal and neoplastic urothelial cells. *Tumori* 1980;66:37-49.
- ³ Boon ME, Baak JPA, Kurver PH, Overdiep SH, Verdonk GW. Adenocarcinoma in situ of the cervix: an underdiagnosed lesion. *Cancer* 1980; (in press).
- ⁴ Baak JPA, Kurver PHJ, Overdiep SH, *et al*. Quantitative microscopical (computer-aided) diagnosis of endometrial hyperplasia or carcinoma in individual patients. *Histopathology* 1980; (in press).
- ⁵ World Health Organization. Histological typing of urinary bladder tumours. International histological classification of tumours. Geneva, 1973.
- ⁶ Boon ME, Löwhagen T, Lopes Cardozo P, Blonk DI, Kurver PJH, Baak JPA. Computation of pre-operative diagnosis probability for follicular adenoma and carcinoma of the thyroid on aspiration smears. *Analytical and Quantitative Cytology* 1980; (in press).
- ⁷ Ooms ECM, Essed E, Veldhuizen RW, Alons CL, Kurver P, Boon ME. The prognostic significance of morphometry in T-I bladder tumors. *Histopathology* 1980;480-5.
- ⁸ Fossa SD, Kaalhus O. Nuclear size and chromatin in transitional cell carcinoma of the urinary bladder. *Beitraege zur Pathologie* 1976;157:109-25.
- ⁹ Levi PE, Cooper EH, Phil D, Anderson CK, Path MC, Williams RE. Analysis of DNA content, nuclear size and cell proliferation of transitional cell carcinoma in man. *Cancer* 1969;23:1074-85.
- ¹⁰ Kern WH. The cytology of transitional cell carcinoma of the urinary bladder. *Acta Cytol* 1975;19:420-8.
- ¹¹ Collste LG, Devonec M, Darzynkiewicz Z, *et al*. Bladder cancer by flow cytometry. *Cancer* 1980;45:2389-94.
- ¹² Beyer-Boon ME, Voorn-den Hollander MJA, Arentz PW, Cornelisse CJ, Schaberg A, Fox CH. Effect of various cytopreparatory techniques on normal urothelial cells and their nuclei. *Acta Pathol Microbiol Scand [A]* 1979; 87:63-9.

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