Morphometrical analysis of urothelial cells in voided urine of patients with low grade and high grade bladder tumours

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SUMMARY The morphometric differences between the urothelial cells (wet-fixed Papanicolaou-stained) in the voided urine of 20 patients with low grade and high grade bladder tumours were measured. The morphometrical data of this learning set resulted in a cytomorphometrical classification rule, which was applied to a test set of 21 cases with low grade and high grade bladder tumours.

The results of the cytomorphometrical classification rule correspond very well with the histomorphometrical classification and the histological grade of the parent tumours.

The results indicate that it is feasible to classify bladder tumours using the cytomorphometrical data of the exfoliated urothelial cells alone.

In a previous study it was shown that cellular and nuclear dimensions of 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. However, it was not possible to separate all grade I tumours from all grade II tumours. For therapeutic reasons it is important to discriminate between these low grade bladder tumours (grade I and II) and high grade (grade III) tumours; it is clinically less important to separate grade I and II tumours. An objective method of distinguishing low grade from high grade tumours is desirable.

For the classification rule (learning set) were used: mean nuclear area, mean nuclear diameter, standard deviation nuclear area, mean nuclear/cytoplasm (N/C) area ratio, mean N/C diameter ratio, standard deviation N/C area ratio and mean cytoplasmic area.

Material and methods

LEARNING SET
Twenty cases of bladder tumour were selected for the learning set: 10 patients with a low grade tumour (grade I and II) and 10 cases with a high grade bladder tumour (grade III).

All these patients belong to a reference set of bladder tumours, in which there is no doubt about the histological grade.

TEST SET
The test set consisted of 21 cases, of which voided urine and the transurethrally resected bladder tumour were available for this study.

HISTOMORPHOMETRY
The parent tumours of the test set were histomorphometrically classified using a classification rule. For this classification the nuclear areas of the superficial cells are measured.

CYTOLOGICAL MATERIAL
Voided urine was used for this study. Two consecutive samples were taken from each patient and the smears with the highest cytological grading were used. Wet-fixed Papanicolaou-stained smears were used for this study. The wet-fixation was achieved by spray-fixing (coating fixative 80 ml polyethylene glycol (MW 300), 690 ml isopropanol, 170 ml acetone, 60 ml distilled water).

CYTOMORPHOMETRY
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 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 diameter (2√(area/π)), cell diameter, N/C area ratio, N/C diameter ratio. From each slide the mean and the standard deviation of the assessed features were calculated. This gave 16 sets of values per case.

**Statistical analysis**

Statistical analysis was carried out on a PDP 11 computer DEC with part of the program STP (statistical package) developed by one of us (PHJK).

The descriptive statistics of the measurements in the wet-fixed Papanicolaou-stained smears were computed for the two groups (low grade v high grade).

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**

The morphometric parameters in the learning set show many differences between the exfoliated cells from the low grade and the high grade tumors respectively (low grade and high grade). (Table 1).

The results of histomorphometrical classification, histological grade and cytomorphometrical classification of the test set are shown in Table 2. There is a good correlation between cytomorphometrical classification, histomorphometrical classification and established histological grade in each patient.

The histomorphometrical classification results in only one doubtful case (9); histological re-examination of the slides gave no explanation for this. More interesting was case 3. The histomorphometry resulted in a definite low grade classification whereas the cytomorphometry was in accordance with the established histological grade. This case was the only carcinoma-in-situ case in this series. In the cases 6, 8, 16, and 19, there was good correlation between histomorphometry and established histological grade whereas the cytomorphometrical classification was doubtful. In the remaining cases there was no discrepancy between cytomorphometry, histomorphometry, and established histological grade.

**Discussion**

One of the main problems in quantitative studies is to obtain a large reference set of tumors of which the grade is known. This is best ensured if grading is done by several authorities in the field followed by selection of only those cases about which there is a general agreement. Therefore, we organised several sessions where a large number of tumors was graded by several pathologists with special experience in bladder cancer. Such a procedure is essen-

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**Table 1** Descriptive statistics and significant differences between wet-fixed Papanicolaou-stained cells in low grade bladder and high grade tumors in the learning set

<table>
<thead>
<tr>
<th>Parameter (mean)</th>
<th>Low grade</th>
<th>High grade</th>
<th>Probability of difference (two sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean nuclear perimeter (μm)</td>
<td>37.5</td>
<td>43.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Standard deviation mean nuclear perimeter (μm)</td>
<td>6.2</td>
<td>8.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean nuclear area (μm²)</td>
<td>76.6</td>
<td>107.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Standard deviation mean nuclear area (μm²)</td>
<td>25.3</td>
<td>44.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean nuclear diameter (μm)</td>
<td>9.6</td>
<td>11.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Standard deviation mean nuclear diameter (μm)</td>
<td>1.5</td>
<td>2.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Mean N/C area ratio</td>
<td>0.51</td>
<td>0.58</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Mean N/C diameter ratio</td>
<td>0.71</td>
<td>0.76</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Table 2** Cytomorphometrical and histomorphometrical classification

<table>
<thead>
<tr>
<th>Case</th>
<th>Morphometry-histology probability to be low grade</th>
<th>Histological grade (WHO)</th>
<th>Morphometry-cytomology probability to be low grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>3</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>1</td>
<td>0.99</td>
</tr>
<tr>
<td>3</td>
<td>0.99</td>
<td>3</td>
<td>0.12</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>2</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>2</td>
<td>0.95</td>
</tr>
<tr>
<td>6</td>
<td>0.99</td>
<td>2</td>
<td>0.71</td>
</tr>
<tr>
<td>7</td>
<td>0.06</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>8</td>
<td>0.01</td>
<td>3</td>
<td>0.38</td>
</tr>
<tr>
<td>9</td>
<td>0.23</td>
<td>3</td>
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</tr>
<tr>
<td>10</td>
<td>0.98</td>
<td>2</td>
<td>0.98</td>
</tr>
<tr>
<td>11</td>
<td>1.00</td>
<td>2</td>
<td>0.99</td>
</tr>
<tr>
<td>12</td>
<td>0.98</td>
<td>2</td>
<td>0.96</td>
</tr>
<tr>
<td>13</td>
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<td>3</td>
<td>0.14</td>
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<tr>
<td>14</td>
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<td>2</td>
<td>0.93</td>
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<tr>
<td>15</td>
<td>1.00</td>
<td>2</td>
<td>1.00</td>
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<tr>
<td>16</td>
<td>0.04</td>
<td>3</td>
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<tr>
<td>17</td>
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<td>3</td>
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</tr>
<tr>
<td>18</td>
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<td>3</td>
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<tr>
<td>19</td>
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<td>2</td>
<td>0.47</td>
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<tr>
<td>20</td>
<td>0.00</td>
<td>3</td>
<td>0.02</td>
</tr>
<tr>
<td>21</td>
<td>0.99</td>
<td>2</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Morphometrical analysis of urothelial cells in voided urine

1065

tial in validating quantitative studies. The cases in the
learning set all belong to this reference material, so
that there is minimal doubt about the histological
grade of each case. The only case in which there was
no agreement between the histomorphometry and
the established histological grade was a carcinoma-
in-situ. This is in accordance with our previous study\(^8\)
in which all carcinoma-in-situ cases resulted in a
lower grade by histomorphometry. It is not surprising
that this carcinoma-in-situ was incorrectly classified
since it is known that the superficial cells in
carcinoma-in-situ exfoliate easily, whereas the
histomorphometrical classification rule used by us\(^8\)
is based on the superficial cell layer. In contrast the
cytomorphometrical classification of this carcinoma-
in-situ case resulted in a correct grade.

The fact that the mean nuclear area in the exfoli-
cated cell was largest in the high grade tumours is in
agreement with our findings in histological
specimen\(^8\) in which the mean nuclear area of the
superficial cell layer was also significantly larger in
these tumours. It must be kept in mind that the exact
values of histomorphometry and cytomorphometry
differ from each other due to difference in processing
methods. The results of this study indicate that mor-
phometry of wet-fixed Papanicolaou-stained cells in
voided urine of patients with bladder tumours can be
of practical value to discriminate patients with low
grade and high grade bladder tumours, and that the
results of the cytomorphometry correspond very well
with the results of histomorphometry and the estab-
lished histological grade, which is in accordance with
the findings of Tribukait et al, who investigated biop-
sies from bladder tumours by flow cytomorphometric
DNA analysis and compared the results with measure-
ments of exfoliated cells.\(^6\,\,7\)

Especially in the follow-up of patients with trans-
urethrally resected bladder tumours this objective
cytological method can be of great value, in the
detection of the development of areas of high grade
flat carcinomas, from which high grade invasive car-
cinoma might develop.\(^9\)

If the cytomorphometrical grade is lower than the
histomorphometrical grade, a repeat urine specimen
should be investigated: in the measured urine of the
first sample the diagnostic cells were absent, but
might appear in the repeat sample.\(^8\) Thus cyto diag-
nosis and histodiagnosis can be used as complement-
techniques, as well in objective analyses.

References
1 Boon ME, Kurver PHJ, Baak JPA, Ooms ECM. Morphometric
differences between urothelial cells in voided urine of patients
with grade I and grade II bladder tumours. J Clin Pathol
1981;34:612-5.
2 Soloway MS, Ikard M, Ford K. Cis-diaminedichloroplatinum (II)
in locally advanced and metastatic urothelial cancer. Cancer
3 Matsumoto K, Kakizoe T, Mikuriya S, Tanaka T, Kondo I,
Umegaki Y. Clinical evaluation of intraoperative radiotherapy
4 Cooley WW, Lohnes PR. Multivariate data analysis. New York:
5 de Voogt HJ, Rothert P, Beyer-Boon ME. Urinary cytology.
6 Tribukait B, Esposti PL. Quantitative flow-microfluorometric
analysis of the DNA in cells from neoplasms of the grading and
7 Tribukait B, Gustafson F, Esposti P. Ploidy and proliferation in
human bladder tumours as measured by flow-cytofluorometric
DNA analysis and its relations to histopathology and cytology.
Cancer 1979;43:1742-51.
8 Koss LG. Mapping of the urinary bladder: its impact on the con-
9 Beyer-Boon ME, de Voogt HJ, van der Velde EA, Brussee JAM,
Schaberg A. The efficacy of urinary cytology in the detection of
10 Ooms ECM. Bladder carcinoma: a morphometrical study. Thesis,
University of Amsterdam. 1981.

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