Current Practice of Urinary Bladder Cytology

William J. Frable, Lois Paxson, Jo A. Barksdale, and Warren W. Koontz, Jr.

Division of Surgical and Cytopathology [W. J. F., L. P., J. A. B.] and Department of Urology [W. W. K.], Virginia Commonwealth University, Health Sciences Division, Medical College of Virginia, Richmond, Virginia 23298

Summary

Six years of experience with the cytological diagnosis of carcinoma of the urinary tract are reviewed. This includes 2 years of participation in the National Bladder Cancer Project. With increasing experience of the cytopathologist and cytotechnologist, attention to detail of specimen collection and preparation, the increasing use of bladder washing and cystoscopic urine specimen, and the interest and cooperation of the urologist, the false-positive rate of diagnosis of transitional cell carcinoma of the urinary tract has decreased.

Papillomas and low-grade papillary, noninvasive transitional cell carcinomas have proven impossible to diagnose by cytology alone but may be diagnosed rarely from cell block material prepared from the bladder washings. A residual of cases of chronic cystitis, benign prostatic hyperplasia, renal calculi, chemotherapy effects, and cancer-associated atypias provide the cytopathologist with diagnostic problems, since they remain nearly inseparable from transitional cell carcinoma on the basis of cytology alone.

Cytology has proven sensitive to the detection of recurrent and persistent transitional cell carcinoma following treatment with chemotherapy, radiation, or surgery. In such cases the abnormal area of epithelium in the bladder may be extremely difficult for the urologist to find by biopsy. He should not be lulled by a normal-appearing bladder and negative biopsies in these cases into believing that the cytology report is incorrect.

There are many factors that affect the efficiency, accuracy, and specificity of the cytological diagnosis of cancer of the entire urinary tract and urinary bladder: collection and preparation of the cytological specimen, expertise and interest of the cytopathologist and cytotechnologist, clinician interest in obtaining good specimens and providing accurate clinical data, and even participation in a special study of the National Bladder Cancer Project. This report documents the interaction of these factors on the performance of 1 participating cytology laboratory in Clinical Collaborative Group A of the National Bladder Cancer Project.

Materials and Methods

All of the urinary tract cytology specimens submitted to our laboratory at the Medical College of Virginia between January 1, 1970, and December 31, 1975, were reviewed and correlated with clinical and histological data as completely as possible. This period includes 2 years of participation in Clinical Collaborative Group A, 1974 and 1975. During these 2 years specific protocols for the collection of urine specimens for cytological study were in force. The actual degree of compliance with these protocols cannot be determined. Voided urine samples were to be collected as fresh morning specimens and placed directly in an equal volume of 50% ethyl alcohol before being transported to the cytology laboratory. Over weekends and holidays these prefixed specimens were to be stored in the cytology laboratory refrigerator, but in some cases they were undoubtedly left at room temperature in various areas of the hospital.

Urine was collected at the beginning of cystoscopy, and bladder washings were then obtained. Both specimens were collected without fixation and transported immediately to the cytology laboratory for preparation of membrane filters. Collection of the bladder washing specimen was performed according to the protocol of Clinical Collaborative Group A, using 50.0 ml of 0.9% NaCl solution and irrigating the bladder 3 to 5 times with this same fluid. In the laboratory 4.0 ml of well-mixed cystoscopic urine specimen were used to make 1 filter preparation, and 1 filter preparation was made for each 25.0 ml of bladder washing. Unclear specimens were diluted with 0.9% NaCl solution until nearly clear before filtering an aliquot. If the specimen was to be diluted, it was necessary for the cytotechnologist to exercise judgment concerning the volume of specimen that could be filtered to make a good cytological preparation without overloading the filter and/or distorting the cells. Two filter preparations were made of each voided or cystoscopic urine submitted to the laboratory. The Nucleopore membrane filter (General Electric Co., Pleasanton, Calif.) was used (7). The remaining sample of bladder washings not used to make filter preparations was centrifuged and prepared as a cell block by the method of Harris et al. (7).

Also included in this study are a small number of ureteral and renal pelvis washings prepared in the same manner as bladder washings and ureteral and renal pelvis brushings prepared as direct smears from the brush and as filter preparations by agitating the brush in 4.0 ml of 0.9% NaCl solution.

Results

The results of this review are summarized in Tables 1 to 4. The cytological diagnosis of suspected or definite carcinoma refers to individual specimens. The number of histological diagnoses also refers to individual specimens and...
The primary type of specimen. The false-positive rate is similar for both cystoscopic and voided urine samples in comparison with the diagnosis of bladder cancer. The correlation of type of cytological specimen with positive versus false-positive diagnoses is shown in Table 2. The number of false-positive diagnoses related to different benign urinary tract processes is listed in Table 3.

In summary, the cytological study of patients with cancer of the urinary tract, primary or metastatic, has shown that the false-positive rate is similar for both types of material in 68% of the cases. This is documented in Table 3. Inflammatory atypias were equally difficult to interpret in bladder washings and voided urine. Certain urinary tract lesions are responsible for the majority of false-positive cytology findings from urine specimens. This is a curious phenomenon of malignant-appearing cells in patients with known cancer that does not involve the epithelial surface from which the cancer could have microscopic metastases in the urinary tract to account for the presence of tumor cells in the urine. In 1 case in this series, the positive urine cytology occurred in a patient with primary lung cancer without evidence of distant metastases.

Examples of false-positive cytology are illustrated in Figs. 1 through 4, and comparison may be made between them and a typical cytological membrane preparation with the diagnosis of transitional cell carcinoma (Figs. 5 and 6). In all of the false-positive cases, there is a similarity in nuclear structure and cell arrangement. The nuclear detail

Table 1

<table>
<thead>
<tr>
<th>Yr</th>
<th>Patients with known cancer</th>
<th>Cytology positive or suspected cancer, individual submitted specimens</th>
<th>Cytology diagnosis confirmed histologically</th>
<th>False positive/suspicious, individual submitted specimens</th>
<th>False negative</th>
<th>Total specimens submitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970</td>
<td>12</td>
<td>19</td>
<td>10</td>
<td>7 (36%)</td>
<td>0</td>
<td>255</td>
</tr>
<tr>
<td>1971</td>
<td>31</td>
<td>42 (3)*</td>
<td>28</td>
<td>12 (28%)</td>
<td>0</td>
<td>548</td>
</tr>
<tr>
<td>1972</td>
<td>22</td>
<td>39 (6)</td>
<td>34</td>
<td>4 (10%)</td>
<td>3</td>
<td>576</td>
</tr>
<tr>
<td>1973</td>
<td>24</td>
<td>37 (15)</td>
<td>30</td>
<td>7 (18%)</td>
<td>1</td>
<td>711</td>
</tr>
<tr>
<td>1974</td>
<td>48</td>
<td>59 (28)</td>
<td>45</td>
<td>10 (16%)</td>
<td>1</td>
<td>739</td>
</tr>
<tr>
<td>1975</td>
<td>51</td>
<td>101 (57)</td>
<td>89</td>
<td>12 (11%)</td>
<td>3</td>
<td>699</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>3488</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* Number in parentheses, number of bladder, ureteral, or renal pelvic washing specimens of the total positive or suspected cytological diagnoses of cancer.

Table 2

<table>
<thead>
<tr>
<th>Type of specimen</th>
<th>Positive cytology for carcinoma</th>
<th>Confirmed False positive</th>
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<tbody>
<tr>
<td>Cystoscopic or voided urine</td>
<td>191</td>
<td>153</td>
</tr>
<tr>
<td>Bladder washings</td>
<td>93</td>
<td>79</td>
</tr>
<tr>
<td>Ureter or pelvic washings</td>
<td>16</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 3

<table>
<thead>
<tr>
<th>Benign urinary tract processes</th>
<th>Cystoscopic or voided urine</th>
<th>Bladder washings</th>
<th>Ureter or pelvic washings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>12</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>10</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Calculus</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cancer-associated changes</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal bladder</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
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Table 4
Correlation of positive cytological diagnosis and tissue diagnosis

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Histological diagnosis</td>
<td>73</td>
<td>4</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Transitional cell carcinoma (bladder)</td>
<td>4</td>
<td>13</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Squamous cell carcinoma (bladder)</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma (bladder)</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Mixed carcinoma (bladder)</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prostatic carcinoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Metastatic carcinoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

is not usually as well preserved in the false-positive cases. The differences between cystoscopic urines and bladder washings are slight. Quantitatively, there are usually fewer abnormal cells in the false-positive cases, but if membrane filters are used these quantitative differences disappear or depend upon the amount of specimen used in preparing the specimen for study.

This laboratory has reported its abnormal cytology in as specific a diagnostic terminology as possible, using both ancillary clinical information when available and the cell block material from bladder washing specimens. Even when cytology allowed only a diagnosis of suspected carcinoma, an effort was made to determine the histological type. Comparing only the positive diagnoses of carcinoma, primary or metastatic to the urinary tract, with the corresponding histology, Table 4 reveals the degree of specificity of cytology in this laboratory. Thus, 112 of 150 (74%) of the positive samples were cytologically and histologically identical. This is a slightly better result than the specificity of cervical vaginal cytology in the same laboratory. The degree of specificity in this particular investigation is undoubtedly influenced by the care taken in procuring the specimen and the simultaneous comparison of cystoscopic urine, bladder washing, and cell block material when making a cytological diagnosis. In essence, cancer can be diagnosed from urine specimens, but the type of tumor can usually not be established by cytology alone.

Discussion

Obviously, the combination of factors previously outlined results in improved diagnostic accuracy of urinary cytology. Probably most important is improved preservation of cellular detail through collection of both cystoscopic urine and bladder washings and their prompt preparation for cytological examination. Urine is a very inhospitable fluid for cells, but the basis for the deleterious effect is not known (10). Kern and Bales (8) attempted unsuccessfully to correlate cell size with urine tonicity, urine pH, and the number of inflammatory cells. Quick removal of the fluid portion of urine and prompt fixation of the freshly desquamated cells are the main reasons for improved preservation. This must be the reason that bladder washings provide the most satisfactory specimen for cytological study.

Even when excellent specimens are available, the number of false-positive diagnoses reported varies from less than 1% to more than 20% (2, 5, 14, 15, 17, 19). Most series show a range of 5 to 15% false-positive cases, although the authors seldom indicate how the rate is calculated (i.e., as a percentage of all cancer diagnoses or a percentage of all specimens examined). The latter method gives very impressive but unrealistically low rates. The causes of false-positive diagnoses in the reported series are the same as those that plagued the present study, namely, benign prostatic hyperplasia, infections of the urinary tract, and calculi (2, 5, 17, 18).

False-negative diagnoses are also a problem, and useful information in this regard is difficult to evaluate from the literature. Long-term follow-up of a large series of negative cases is required. Series with low false-positive rates (high specificity of diagnosis) have high false-negative rates (poor sensitivity to the detection of cancer) (17). If papillomas and Grade I noninvasive transitional cell carcinomas comprise a significant part of the series, the rate of false-negative diagnoses will be high, up to 66% of papillomas and 30% of Grade I carcinomas in 1 series (2). Esposti et al. (3, 4) reported in 2 separate series false-negative cytology with Grade I tumors of 45 and 25%. Bladder washings in the present series allowed recognition of 2 of 3 well-differentiated noninvasive transitional cell carcinomas, but 2 cases of papilloma were not recognized by us. Harris et al. (6) did not recognize 1 case of papilloma in a large series of bladder washings from symptomatic patients. It is of interest from Kern’s cell measurements that there is only a 5.0-μm average difference in cell and nuclear diameter between Grades I and II versus Grades III and IV transitional cell carcinoma (9). False-negative cytology in low-grade lesions is therefore not unexpected.

Umbrella cells are seen in papillomas, low-grade carcinomas, and fragments of bladder epithelium dislodged from the normal bladder during washing. The loss of that cell arrangement in some cases of cystitis and calculi probably accounts for false-positive diagnoses (Figs. 3 and 4).
False-positive diagnoses, although higher here than in other areas of cytology, are probably not important. A positive cytological diagnosis of bladder cancer does not lead directly to cystectomy or other radical therapy. Patients in this series were symptomatic, most of them with at least hematuria. Some type of urinary tract disease was expected. False-negative cytology in such a situation can be more damaging. As Levy and Jerusalem (11) have determined, false-negative cytology should be low even in the screening situation in the totally asymptomatic, non-high-risk population. In fact, it should be so low (2/1000 screened in his series) that routine screening from such a population is not indicated (11). It may not be indicated because the false-positive rate in such a population could be high.

Although not part of the bladder cancer project, ureteral and pelvic tumors were included in the analysis of cytology because they make up part of the differential diagnosis. These cases proved difficult, with a false-positive error rate of 50%. Pelvic or ureteral calculi produced clusters of cells that the authors could not distinguish from different-differentiated transitional cell carcinoma. In a preliminary report, Bibbo et al. (1) did not have any false-positive diagnoses of cancer from 20 ureteral brushing specimens. There were 8 cancers in that series, all diagnosed from the cytology or, in 1 case, from the histology of the cell block material (1). Five cases had infections of the kidney and renal pelvis, and 4 had calculi, so that there was ample opportunity for error (1).

A final and important part of the cytology studies within the Bladder Cancer Project is the follow-up of patients treated for bladder cancer. The literature indicates some success with the prediction of persistent and/or recurrent tumor following radiation, chemotherapy, or surgery. Recurrence may be detected well in advance of clinical or histological evidence of tumor (3, 15, 16). Diagnosis depends on finding anaplastic tumor cells without the appearance of radiation or chemotherapeutic effects on even normal transitional epithelial cells (12). Esposti et al. (3) followed 182 bladder cancer patients treated by X-ray with detection of 27 out of 29 nonresponders, 52 of 57 patients with response followed by recurrence, and a 2.5% false-positive error. There was an indeterminate group of 24 cases in this series, 13 with cytology positive for recurrence and 3 other patients suspected of having recurrence. Persistent tumor (or recurrence?) was detected from cytological studies between 3 and 24 months prior to clinical or histological proof (3). From a smaller series reported by Orell (14), the data are similar.

In the authors' series there are 17 cases for analysis following therapy by radiation, surgery (partial cystectomy, fulguration), chemotherapy, or a combination of these. There are 2 negative reports, both from recurrent papillary well-differentiated transitional cell carcinoma. Two other cases of this tumor were detected, along with 6 cases of invasive transitional cell carcinoma, 1 invasive squamous cell carcinoma, and 2 invasive mixed carcinomas of transitional and squamous type. These recurrences have been identified cytologically on an average of 4 months prior to clinical or histological detection. One case had a positive cytology 16 months before the recurrence was found. Cystoscopy was frequently normal, or the changes in the bladder mucosa were minimal and nonspecific.

Particularly deceptive in follow-up cytology is chemotherapy with triethylenethiophosphoramide, which may destroy low-grade papillary tumors, leaving a normal-appearing urinary bladder. Tumor cells may still exfoliate from such a bladder along with bizarre-appearing cells that represent benign but polyploid transitional cells. Recent experimental work by Murphy et al. (3) shows that the effects of this drug on normal urothelium are very transient. Histologically, the bladder returns to normal rapidly, after an initial short period of cell swelling. Malignant-appearing cells are not exfoliated from the normal bladder following instillation of triethylenethiophosphoramide (thio-tepa). In a patient with bladder cancer treated with this drug, there is a tendency to regard abnormal cells as indicating a drug effect, probably because this relationship has been established for other chemotherapeutic agents instilled into the bladder (10). That is probably not true with triethylenethiophosphoramide. When abnormal cells are seen following treatment with this drug, they correlate with recurrent or persistent tumor, even if the bladder looks essentially normal.

Acknowledgments

This study and report could not have been realized without the cooperation of the entire faculty and housestaff of the Department of Urology.

References


Fig. 1. Bladder washing with papillary clusters of atypical transitional cells reported as indicating low-grade papillary transitional cell carcinoma. Patient has only benign prostatic hyperplasia. Papanicolaou, × 600.

Fig. 2. Bladder washing with severely atypical papillary clusters of transitional cells reported as carcinoma. Patient with metastatic lymphoepithelioma being treated by chemotherapy. Cells could represent cancer-associated atypia or chemotherapeutic effect on transitional epithelium. Papanicolaou, × 600.

Fig. 3. Cystoscopy urine from a patient with hematuria. Papillary clusters of atypical transitional cells suggesting a low-grade papillary transitional cell carcinoma. Multiple biopsies indicate only chronic follicular cystitis. Papanicolaou, × 600.

Fig. 4. Bladder washing. Patient with hematuria. Papillary clusters of malignant-appearing transitional cells indicating papillary transitional cell carcinoma. Bladder mucosa appears red and inflamed. Multiple biopsies reveal only severe chronic cystitis. Papanicolaou, × 600.

Fig. 5. Bladder washing. Malignant-appearing cells from papillary transitional cell carcinoma, moderately well differentiated, noninvasive; for comparison with Figs. 1 through 4. Papanicolaou, × 600.

Fig. 6. Same case as Fig. 5. Papanicolaou, × 600.
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