Urinary Sediment

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- Columnar Cells
- Mucus-Containing Epithelial Cells
- Squamous Cells
- Renal Epithelial Cells
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The Problem of Hematuria
- Routine Laboratory Investigation of Hematuria
- Dysmorphic Red Blood Cells Indicate Glomerular Disease
- Comprehensive Analysis of Urine for Evaluation of Hematuria

Introduction
Examination of urine is one of the oldest medical tests, used by Samarians, Babylonians, Egyptians, Indians, and Greeks in their traditional medicine. It was not until after Papanicolaou and Marshall published the first article in 1945 that urine cytology was used to detect urothelial carcinoma. Subsequently, Koss, Melamed, and colleagues characterized urine cytology and histology in 1960. Numerous classification systems have been introduced, and those before 2013 are nicely reviewed by Owens et al. The greatest contemporary issues with urine cytology are low sensitivity detection of low-grade cancer, poor interobserver agreement (especially with atypia), and lack of standardized diagnostic criteria. Recent efforts described in this chapter offer great promise in resolving these concerns.

This chapter discusses the spectrum of cytologic abnormalities in voided urine samples and washings to allow comparison with biopsy findings described in Chapters 5 and 6, and presents classifications published after the last edition of this text. The clinically significant and common problem of hematuria is also addressed from the perspective of the cytopathologist.

Utility of Urine Cytology
Indications
Cytologic examination of the urine sediment is of value in the diagnosis of a wide variety of benign and malignant diseases of the bladder, urethra, ureter, and kidney. The chief indications for the use of cytology in disorders of the urinary tract include:
1. Screening and diagnosis of carcinoma in situ and high-grade carcinoma
2. Follow-up of patients with atypical cytology evaluation or urothelial tumor, regardless of grade
3. Monitoring of patients with urothelial tumor undergoing or after treatment, including active surveillance
4. Evaluation of hematuria, including separation of kidney (upper tract disease) and nonkidney (lower tract) causes

Sources
The sources of urologic cytology specimens include voided urine, catheterized urine, bladder washing (barbotage), brushing, ureteral and renal pelvic brushing and washing, and neobladder urine from
an ileal conduit or colonic pouch. Initial morning first-void urine includes exfoliated cells, debris, and impurities that have collected in the urinary tract and urethral opening during the night, and may optimize yield of potential pathogens such as human papillomavirus. Ureteral washings and other instrumented specimens require caution because they may produce artifactually clustered urothelium.

The specimen source is critically important for diagnosis. For example, upper urinary tract washings were superior to voided samples in detection of upper tract high-grade carcinoma (90% versus 50% yield, respectively). Similarly, urinary diversion cytology specimens from patients who undergo radical cystectomy are often submitted for screening for recurrent urothelial carcinoma, and those with carcinoma (2% to 6% incidence rate) revealed scant, well-preserved urothelial cells either alone or in clusters with high-grade features, including eccentrically located, enlarged, hyperchromatic nuclei; irregular nuclear borders; and high nuclear-to-cytoplasmic (N/C) ratios, often with an inflammatory background. The sensitivity, specificity, positive predictive value, and negative predictive value of cytology for high-grade carcinoma in diversion remnants were 82%, 97%, 75%, and 98%, respectively; 21% of patients with atypia were eventually diagnosed with carcinoma. In another study, bladder washing specimens were more predictive of high-grade cancer than voided urine specimens.

**Specimen Adequacy**

Adequacy is a reflection of how representative the specimen is based chiefly on cellularity, although the presence of obscuring elements is also important. Regardless of the specimen type (voided urine or instrumented), an unsatisfactory or inadequate specimen is one that is poorly cellular, completely obscured, or predominately degenerate. Obscuring elements include neutrophils, lubricants, other foreign debris, crystals, bacteria, squames, and spermatozoa. Conversely, according to one group, if there are any atypical cells, regardless of the overall cellularity, this represents a satisfactory specimen. Brief exposure to contrast agents does not influence adequacy; thus, contrast washings of the urinary tract and may optimize yield of potential pathogens such as human papillomavirus. Contemporary processing methods include conventional cytospin, Meiers improved filter method, ThinPrep, and SurePath methods (Table 7.1); direct smear has been largely abandoned.

The patient’s underlying condition and his or her indication for cytologic evaluation influences specimen adequacy. Increased cellularity is observed in specimens from patients with cancer, calculi, or infection compared with those with only hematuria or irritative voiding symptoms. Other adequacy factors include level of hydration, micturition before specimen acquisition, the use of diuretics or other medications, the presence of obstructive conditions such as benign prostatic hyperplasia, with consequent reduction of bladder capacity, and medical problems resulting in oliguria.

Operator-dependent factors refer to expertise of the examiner and the potential for human bias and error. Logistic factors that influence adequacy include length of time from collection to processing, container leakage with potential drying artifacts, and many others.

**Reporting and Classification**

Several reporting and classification systems for urine cytology have been published, each of which has relative strengths and weaknesses. Unlike cervical cytology, there has not been widespread acceptance and use of any single reporting system for urine cytology studies. Thus, terminology and criteria for urine cytology reporting are not uniform among pathologists. The major diagnostic categories that we use at our laboratory are presented in Table 7.2.

Recently two international consensus conferences published their classifications: the Paris System 2013 and the International Consultation on Urologic Disease–European Association of Urology 2015 (Table 7.2). Both were based on expert consensus by small, self-selected academic groups with minimal input from other cytopathologists, urologists, oncologists, or others.
It should be noted that evidence-based guidelines have supplanted such expert panels and simple consensus conference-based conclusions, and are now considered to be the contemporary standard for defining the practice of medicine; thus it is surprising that these recent efforts failed to abide by even the most basic tenets of evidence-based medicine, instead resorting to “biology by democracy.” All proper methods of systematic review and guideline generation share certain core concepts, including careful selection of the guideline topic, thorough structured review of the evidence with grading and synthesis, creation of recommendations, consultation and peer review, dissemination and implementation, revision, and updating. According to the U.S. Agency for Healthcare Research and Quality, three key principles are required for successful conduct of systematic reviews: (1) the review must be relevant and timely, focusing on the most important issues and the optimal time to initiate a review; (2) the review must be objective and scientifically rigorous, free from conflicts of interest; and (3) the review must include public participation and transparency to ensure confidence and credibility, and provide for accountability. Thus the recent cytopathology consensus statements should be considered below the standards of current practice of evidence-based medicine. Nonetheless, any efforts to create standardized terminology are laudable and generate renewed interest in refinement of diagnostic criteria, continuing the work of the Papanicolaou Society at creation of uniformity in cytopathology practice.

In Paris System 2013, the recommended diagnostic words are also problematic. The words “negative for high-grade urothelial carcinoma” on a report could easily be mistyped or misinterpreted by the transcriptionist, cytopathologist, or urologist if the word negative is overlooked while the word carcinoma registers, potentially resulting in serious consequences for the patient. Reasonable alternatives include “negative for high-grade malignancy,” “negative for high-grade neoplasia,” and “no definite evidence of malignancy.”

### The Paris System 2013

The Paris System 2013 focused chiefly on accuracy of identification of high-grade carcinoma, requiring five criteria for a definitive diagnosis: at least 5 malignant cells (10 cells for upper tract cancer), elevated N/C ratio (≥0.7), markedly atypical nuclear borders, moderate to severe hyperchromasia, and coarse chromatin (Tables 7.2 and 7.3). However, malignant specimens often contain degenerative changes, and this may limit the number of diagnostic cells; consequently, about half of positive cases failed to fulfill this criterion in a report from Johns Hopkins University. Furthermore, less than 20% of cells present in positive specimens fulfilled all five of the criteria. The second most restrictive criterion, N/C ratio ≥0.7, was present in only 78% of positive specimens. Nonetheless, the Paris System 2013 upgraded about 40% of indeterminate specimens and did not change the frequency of diagnosis of high-grade carcinoma.

N/C ratio is a critical component of the Paris System, but just how reliable is it? Hang et al. confirmed the importance of the ≥0.5 cut point for the diagnosis of atypical urothelial cells using digital image analysis; receiver operating characteristic analysis demonstrated that the maximum N/C ratio alone was highly predictive of high-grade carcinoma on follow-up (area under the curve [AUC], 79%), with a sensitivity of 73% and a specificity of 85%. However, visual quantitation of N/C ratio showed only a fair

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
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<tbody>
<tr>
<td>Cytospin smear (two-step</td>
<td>Urine samples are sedimented at 700 g for 10 minutes. The supernatant is removed to within approximately 1-2 mL of the cell pellet, and the pellet is then resuspended and rinsed with 10 mL of hypotonic solution (0.075 M potassium chloride) for 10 minutes. The cells are resedimented at 600 g for 10 minutes, and the supernatant is removed to within 0.5 mL of the pellet. The pellet is then gently vortexed and resuspended in 10 mL 3:1 methanol/glacial acetic acid fixative. Fixed specimens are left at room temperature for 30 minutes. The urinary cells are then sedimented at 600 g for 5 minutes, aspirated, and transferred to a 2-mL microfuge tube. The final cell pellet is left in approximately 100-500 μL of residual methanol/glacial acetic acid fixative, depending on the size of the cell pellet. A total of 10 μL of cell sediment is placed on the slide, and the specimen is allowed to dry.</td>
</tr>
<tr>
<td>centrifugation/fixation method</td>
<td></td>
</tr>
<tr>
<td>Meiers improved filter method</td>
<td>Urine samples are fixed in ethanol and drawn up into a 60-mL syringe threaded with a Luer lock tip. An 8.0-μm filter mounted in a filter holder is subsequently attached to the syringe tip. The urine sample is then pushed gently through the filter until complete. The membrane filter is placed on a positively charged glass slide. Gauze is placed over the membrane and slight pressure applied with the palm of the hand to transfer the cell filtrate. The cell filtrate is placed on a slide in a manner similar to Cytospin. The membrane is discarded and the filter holder was deposited in a 10% bleach solution overnight until next use.</td>
</tr>
<tr>
<td>ThinPrep (Hologic, Bedford, MA)</td>
<td>ThinPrep test is performed with a proprietary automated liquid-based monolayer cell preparation system. Urine samples are immersed in a buffered preservative solution, transferred to a bowl, and a cylinder with a filtration membrane is then placed in the bowl to ensure that the cells are homogeneously distributed. Using negative pressure, the erythrocytes and mucus penetrate the filtration membrane, leaving only the filtration membranes for the diagnostic procedure. This maneuver is repeated until an appropriate number of cells (2000-50,000) is collected. Thereafter the cylinder is removed from the bowl; cells left on the filtration membrane are attached to the slide and then fixed in 95% alcohol.</td>
</tr>
<tr>
<td>SurePath (BD Diagnostics,</td>
<td>The SurePath test is performed with a proprietary liquid-based monolayer cell preparation system density gradient-based cell enrichment. Urine samples are immersed in ethanolic preservative solution and a device is placed into the vial to ensure that cells are homogeneously distributed. A polysaccharide-based density gradient reagent is used to filter debris, centrifuged, resuspended, and centrifuged again. The PrepStain processor creates and stains the slides.</td>
</tr>
<tr>
<td>Burlington, NC)</td>
<td></td>
</tr>
</tbody>
</table>
correlation with actual N/C ratio, with correlation decreasing with increasing N/C ratio. In the critical range, N/C ratio of 0.5 to 0.7, interobserver correlation (75%), and correlation with true N/C ratio (53%) may be insufficiently accurate for precise category assignment in the Paris System.

Compared with previous classification systems, the Paris System 2013 resulted in a great increase in the rate of “atypical” cases while improving sensitivity but lowering specificity. Granados et al. found that the incidence of “atypical” increased from 3% to 24% in benign cases, from 2.5% to 25% in low-grade carcinoma, and from 6.6% to 16% in high-grade carcinoma. The false-positive rate (abnormal cytology in negative or low-grade carcinoma cases) increased from 11% to 34%. Sensitivity was higher (63% versus 49%) at the expense of lower specificity (73% versus 91%). The agreement between prior classification and Paris System 2013 was moderate for negative and high-grade carcinoma.

**TABLE 7.2** Comparison of Cytologic Diagnostic Categories in Urine Sediment

<table>
<thead>
<tr>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic Negative</td>
<td>Nondiagnostic Negative for high-grade urothelial carcinoma</td>
<td>Nondiagnostic Negative for epithelial cell abnormality</td>
<td>Repeat within 3 months</td>
</tr>
<tr>
<td>Atypical</td>
<td>Atypical urothelial cells</td>
<td>Atypical urothelial cells of undetermined significance</td>
<td>Clinical follow-up as needed</td>
</tr>
<tr>
<td>Suspicious</td>
<td>Low-grade urothelial neoplasma</td>
<td>Low-grade urothelial carcinoma</td>
<td>Clinical follow-up; consider ancillary tests</td>
</tr>
<tr>
<td>Malignant cells present</td>
<td>High-grade urothelial carcinoma</td>
<td>High-grade urothelial carcinoma</td>
<td>Cystoscopy and biopsy</td>
</tr>
<tr>
<td>Other (specify)</td>
<td>Others: primary and secondary malignancies and miscellaneous lesions</td>
<td>Other (specify)</td>
<td>Cystoscopy and biopsy, depending on specificity of findings</td>
</tr>
</tbody>
</table>

4Expanded terminology: These are the templated words that appear on the Bostwick Laboratories’ reports.

Nontumor-associated cytology:
- Normal cells/negative for malignant cells
- Inflammatory changes: specific type or nonspecific

Tumor-associated cytology:
- Rare single cells and clusters of mildly to moderately atypical urothelial cells; this may represent a reactive process, but neoplasm should be considered; clinical correlation is indicated
- Rare highly atypical urothelial cells suspicious for neoplasm; reactive process cannot be excluded; repeat study and/or further investigation may be of value
- Severely atypical urothelial cells highly suspicious for neoplasm; clinical correlation is recommended
- Malignant cells present most suggestive of urothelial carcinoma
- Malignant cells present (specify squamous cell carcinoma, adenocarcinoma, prostatic adenocarcinoma, renal cell carcinoma, other)
- Malignant cells present, not otherwise specified

4Modified from Amin et al.262
4Modified from Barkan et al.24
4In the Paris System, the presence of fibrovascular cores is rare and is the only instance in which the diagnosis of low-grade urothelial neoplasm in instrumented urine can be made. Low-grade urothelial neoplasm should be used sparingly and in conjunction with the negative category to clarify the absence of high-grade carcinoma in the Paris System. In the Bostwick Laboratories classification, the presence of fibrovascular cores is considered suspicious.

**TABLE 7.3** Comparison of Morphologic Criteria of Abnormal Cells in the Paris System 2013 for Reporting Urinary Cytology Category

<table>
<thead>
<tr>
<th>Category</th>
<th>Nuclear-to-Cytoplasmic Ratio (Feature 1)</th>
<th>Nuclear Chromasia (Feature 2)</th>
<th>Chromatinic Rim/Nuclear Membrane (Feature 3)</th>
<th>Chromatin Quality (Feature 4)</th>
<th>Mandatory (Major) Features</th>
<th>Minor Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical urothelial cells</td>
<td>&gt;0.5</td>
<td>Similar to umbrella cells or dark/very darka</td>
<td>Fine and even or uneven shape and thicknessb</td>
<td>Finely granular or coarsely clumpedc</td>
<td>1</td>
<td>2-4 (one of the features)</td>
</tr>
<tr>
<td>Suspicious and high-grade urothelial carcinoma</td>
<td>&gt;0.7</td>
<td>Very dark</td>
<td>Uneven shape and thickness</td>
<td>Coarsely clumped</td>
<td>1, 2</td>
<td>3, 4 (at least one of the above must be a third feature identified)</td>
</tr>
</tbody>
</table>

4Only one minor feature required.
4Only difference is the cellular quantity: suspicious, very few cells; high-grade carcinoma, 5-10 cells or more.

cases (κ = 0.42 and 0.56, respectively) and weak for low-grade tumors (κ = 0.35). Conversely, Hassan et al. found fewer cases were diagnosed as “atypical” with the Paris System compared with their original diagnoses (26% versus 39%), whereas the correlation of “atypical” with subsequent high-grade cancer increased from 33% to 53%. The new system also resulted in a higher number of low-grade carcinomas diagnosed as “negative” (40%) rather than “atypical” (22%). In another study, 70% of cases of “atypical” cases were reclassified by Paris System 2013 as “negative”; however, 18% of these were found to have high-grade cancer. The sensitivity and specificity of fluorescence in situ hybridization (FISH) with Paris System 2013 were 86% and 33%, respectively, in the “atypical” group and 63% and 100%, respectively, in the “negative” group.

The category of “atypical urothelial cells” no longer includes cellular changes attributed to the BK polyomavirus cytopathic effect, according to the Paris System 2013. Reclassification of such cases as “negative” decreased the rate of “atypical” from 25% to 21%, although the high rate of subsequent “high-grade cancer” among nonsurveillance patients suggested that the reclassification may be “inappropriate.”

In Paris System 2013, nonatypical urothelial cell groups are classified as “negative for high-grade carcinoma” except in cases that display fibrovascular cores that are now diagnosed as “low-grade urothelial neoplasm.” However, because of the correlation of nonatypical urothelial cells with high-grade carcinoma (high specificity and negative predictive value [87.1% and 94%, respectively]) despite low sensitivity (30.4%), Granados et al. concluded that the presence of nonatypical urothelial cell clusters in voided urine (even without fibrovascular cores) should not be diagnosed as “negative.”

The predictive values of “suspicious” and “high-grade carcinoma” diagnoses were unchanged (94% each) after reclassification with Paris System 2013 despite the new exclusion criterion of cellular degeneration for “suspicious.” Joudi et al. found that “high-grade carcinoma” with the Paris System 2013 yielded a higher predictive value for carcinoma than the cytoplogic diagnosis of “suspicious” (79% versus 55%, respectively), similar to results with the Bostwick Laboratories Classification (74% versus 54%, respectively).

Addition of anisokaryosis and India ink nuclei (but not tumor diathesis, ragged edge of urothelial cells, apoptotic bodies, or pleomorphism) significantly improved the predictive accuracy of the Paris System 2013 according to Suh et al. With their modification the reporting rate of “atypical” decreased from 25% in their original system to 15% in Paris System 2013 and 11% in Suh’s proposed modification; likewise, sensitivity increased from 59% to 71% and 90.0%, respectively.

Interobserver agreement with the Paris System 2013 was adequate for the category of “negative for high-grade carcinoma,” but not for the other categories, with mean absolute agreement of 65% and a mean expected agreement of 44%; the mean chance-corrected agreement (κ) was only 0.32. Approximately 15% of disagreements were classified as high clinical impact. The authors concluded that this low level of diagnostic precision may negatively impact the applicability of Paris System 2013 for widespread clinical application.

Normal Components of the Urinary Sediment

The most common cellular elements are benign superficial urothelial cells, followed by intermediate and basal urothelial cells that are more commonly observed in instrumented specimens. Superficial squamous cells from the female genital tract often outnumber urothelial cells. Benign glandular cells (from cystitis glandularis), squamous cells originating in squamous metaplasia of urothelium or external genital tract skin, and, rarely, benign seminal vesical cells also fall into this category. Clusters or fragments of urothelial cells that may be seen in both instrumented and noninstrumented urine specimens should be classified as “negative” unless the cytomorphology of the cells forming the group fulfills the criteria for “atypical.” Similarly, changes associated with urolithiasis, treatment-related changes, and polyomavirus cytopathic changes should all be classified as “negative,” according to Paris System 2013.

Urothelial cells are the most variably sized cells in the urinary sediment, ranging from 20 μm in diameter for intermediate and basal cells up to 100 μm for typical “umbrella” or superficial cells. Urothelial cells typically have single round to oval nuclei with abundant, homogenous, predominately basophilic cytoplasm. Cells from the basal urothelium are smaller, round, and display well-defined thickened cytoplasmic membranes. Chromocenters and multiple eosinophilic micronucleoli may be prominent, especially in cases with accompanying inflammation.

Fragments of urothelial cells are commonly found in catheterized specimens, as well as bladder washes; however, it is abnormal to see urothelial fragments in spontaneously voided urine, and their presence may be associated with papilloma or low-grade urothelial cancer. Occasionally large urothelial fragments may display cytoplasmic vacuoles containing mertrophils. Multinucleation, nuclear enlargement, and hyperchromasia can be found in inflammatory processes within the lower urinary tract.

Superficial (Umbrella) Cells

Regardless of the type of sample and collection technique used, superficial urothelial cells are a common component of the urine sediment. These cells have one or more nuclei that are large, measuring up to 3 μm in diameter, comparable with superficial squamous cells (Fig. 7.1A). Binucleate and multinucleate cells are common. Such cells are often larger than the mononucleate superficial cells, and their nuclei are somewhat smaller. Large multinucleate superficial cells are by far the most striking component of the urinary sediment, particularly in washings or brushings of the bladder or ureter. Multinucleate superficial cells are particularly large and may be mistaken for giant cells. A potential error in diagnosis is misinterpretation of large superficial cells as macrophages or tumor cells. The DNA content of superficial cells may be polyploid.

The chromatric rim of the nucleus is thick and sharply demarcated. The chromatin is finely granular, with a "salt and pepper" appearance, and may contain one or more prominent chromocenters. The structure of the nucleus is better preserved in bladder washings than in voided urine. In women there may be a sex chromatin body attached to the nuclear membrane. The cytoplasm of these cells is usually basophilic, often finely granular, and sometimes vacuolated. The cell border is convex (luminal) and concave (deep).

Cells Originating From the Deeper Layers of the Urothelium

All other urothelial cells are smaller than the superficial cells, and often exfoliate in clusters, particularly in instrumented specimens. Small single urothelial cells are observed in voided urine. Clusters
of urothelial cells may be tightly packed and assume spherical “pseudopapillary” configurations with sharp borders. Such clusters are often misinterpreted as low-grade papillary carcinoma.\textsuperscript{52,53} When deep (basal) cells are removed by instrument, they often appear in loose clusters. These cells are polygonal or elongate, sometimes columnar, and almost always display cytoplasmic extensions in contact with other cells. The amount of basophilic cytoplasm in such cells depends on the layer of origin and is more abundant in cells derived from upper layers. Single cells resemble parabasal squamous cells in size and configuration. These cells are often spherical or round, particularly in voided urine, but may also show cytoplasmic extensions.\textsuperscript{8} The nuclei of the smaller urothelial cells are approximately the same size, measuring about 2 to 5 μm in diameter (Fig. 7.1B). They are usually finely granular and benign appearing, containing one or rarely two small chromocenters. In voided urine the nuclei may be pale or opaque and occasionally somewhat darker.

### Columnar Cells

Columnar urothelial cells are common, particularly in specimens obtained by instrumentation.\textsuperscript{54} Columnar cells often derive from cystitis cystica or the urethra. They can be single or in small groups, often with a tail by which they are attached to the basement membrane (Fig. 7.2).

### Mucus-Containing Epithelial Cells

Occasionally urine specimens contain mucus-secreting columnar epithelial cells with peripheral nuclei and distended clear cytoplasm. These cells may be ciliated. Such cells often derive from cystitis cystica or cystitis glandularis but may represent cells from urachal remnant, nephrogenic metaplasia, or Müllerian rest (endometriosis or endocervicosis).

### Squamous Cells

Squamous cells of varying size and degrees of maturation are common in urine sediment, particularly in voided specimens (Fig. 7.3). Such cells are more abundant in female than male patients.\textsuperscript{8} In
women these cells originate in the urethral squamous epithelium and in the trigone of the urinary bladder, and are often glycogenated. Voided urine sediment may also contain squamous cells derived from the vulva, vagina, or uterine cervix. In men the origin of the squamous cells is the terminal portion of the urethra or, in rare cases, vaginal type of squamous metaplasia with bladder origin. Among the benign squamous cells, there may be superficial cells, intermediate cells, and small parabasal cells. Navicular cells are intermediate squamous cells with abundant cytoplasmic glycogen content and peripheral nuclei; these cells stain yellow with Papanicolaou stain. Such cells may be observed during pregnancy, early menopause, and sometimes in women or men receiving hormonal therapy (androgen deprivation therapy for prostate cancer). Squamous cells may also be anucleate and fully keratinized. In such cases these should be reported, because the presence of such “ghost” cells may be of considerable significance, representing leukoplakia or squamous cell carcinoma of the bladder.

Renal Epithelial Cells

Cells derived from renal tubules sometimes appear in the urine sediment. These cells are small and usually poorly preserved, with pyknotic, hyperchromatic, condensed, spherical nuclei, and granular eosinophilic cytoplasm. Occasionally the tubular cells form small clusters or casts. The significance of tubular cells in urine sediment remains uncertain. In patients after kidney transplant the presence of renal tubular cells may indicate rejection of the allograft.

Convoluted Tubular Cells

Cells from the convoluted tubular epithelium are the largest cells in the nephron, present at the entrance to the Bowman capsule and extending to the beginning of the loop of Henle. These cells are rarely seen in healthy individuals but are shed in large numbers in cases of renal toxicity and renal ischemia caused by a wide variety of drugs, heavy metals, immunosuppressant, and other toxins.

Proximal tubular cells in urine are easily identified by their large size (20 to 60 µm in diameter); irregular, elongate, or cigar-like appearance; and coarsely granular basophilic cytoplasm (Fig. 7.4A). Cytoplasmic borders are indistinct and may be ragged or torn. The granular cytoplasm contains large numbers of mitochondria by ultrastructure. Nuclei are slightly larger than erythrocytes and may occasionally be multinucleate. Interestingly, proximal and distal tubular cells appear singly, never in fragments or clusters. These cells are often mistaken for granular casts in unstained bright-field microscopy. Proximal and distal renal tubular cells slough from their basement membranes and can be found in urine as intact preserved cells or as “ghost” or necrotic forms that retain their size and cytoplasmic characteristics (Fig. 7.4B).

Collecting Duct Cells

Renal tubular cells lining the proximal and distal collecting ducts are small (12 to 18 µm in diameter), and each contains a single slightly eccentric nucleus with coarse and evenly distributed chromatin. There may be an occasional prominent nucleolus, because these cells may be reactive, but they are never multinucleate. The cytoplasm is polygonal to columnar, finely granular, and uniform.
basophilic, with distinct borders (Fig. 7.4C). Vacuolization may occasionally be seen, especially in reactive states. The cells may phagocytize castlike material, crystals, and pigments.

Collecting duct cells in urine may be present in very low numbers in normal individuals, but are significant when found with renal casts or as fragments. An abnormal number (greater than one per hpf) may be found in a wide variety of clinical conditions, including shock, trauma, burn, and exposure to toxins; also, an increased number of cells in renal transplant patients heralds clinical rejection up to 48 hours early.56

Renal epithelial cell fragments in urine indicate a severe form of renal tubular injury (“ischemic necrosis”) and are exclusively from the collecting duct. This reflects loss of blood flow (ischemic injury) to the renal tubules and subsequent sloughing of entire segments or portions of the renal tubules with regeneration of lost epithelium, a process similar to repair in cervical smears. There are five types of fragments, and these are classified according to morphology: (1) spindle fragments; (2) fragments attached to or surrounding cast material; (3) pavement or “en face” fragments; (4) fragments with reactive cellular or noncellular inclusions (castlike, crystal, or pigmented [ bile] inclusions); and (5) cylindrical, tubelike fragments.

Other Benign Cells
Occasionally cells of prostatic and seminal vesicle (Fig. 7.5) origin may be present in the urinary sediment. Such cells accompany spermatozoa and are common after prostatic massage.57,58 Erythrocytes are a frequent component of the urinary sediment, particularly in patients with clinical evidence of hematuria (see later).7

Inflammatory Cells
Macrophages are often observed in inflammatory reactions of the urinary tract. The cells may be mononucleate or multinucleate and contain fine cytoplasmic vacuoles, sometimes with phagocytic debris. Normal urine sediment contains very few lymphocytes or neutrophils. The presence of large numbers of such cells may precede clinical evidence of inflammation. For example, when there were more than 12.5 white blood cells/μL by image analysis, sensitivity and specificity for predicting Chlamydia infection were 87% and 89%, respectively, in first voided urines in men at high risk.59

Noncellular Components of the Urinary Sediment
In addition to viral inclusions, a variety of intracellular and extracellular findings may be diagnostically valuable in the urine sediment.

Pigment and Pigmented Cells
Numerous normal and pathologic processes result in extracellular pigmented material in the urine and pigmented cells (Table 7.4).60

Cytoplasmic Eosinophilic Inclusions (Melamed-Wolinska Bodies)
Non specific cytoplasmic inclusions may appear as products of degenerating cells in multiple body fluids and can be seen with careful examination in 43% of urine samples.61 There is no relationship with any disease. The round, opaque bodies are 12 to 15 μm in diameter, and may be single or multiple, with eosinophilia standing in contrast with the pale-staining urothelial cytoplasm. Nuclei are usually degenerate, with hyperchromasia, karyorrhexis, or pyknosis, but may also be intact.

Non specific cytoplasmic eosinophilic inclusions should be distinguished from acid-fast–positive nuclear inclusions in renal tubular cells associated with lead poisoning, as well as nonspecific acid-fast–negative red nuclear inclusions of uncertain significance in older women.62,63

Crystals
Polygonal transparent crystalline precipitates of urates are common in voided urine. Their presence results from changes in the acidity of urine after collection but has no diagnostic significance. Crystals derived from true uric acid are rare, and other crystals are rarely of diagnostic value.64 Voided urine and occasional specimens obtained by instrumentation may contain contaminants and renal casts. For a complete review, refer to other texts.

Casts and Other Findings Attributable to Renal Diseases in Urine
Renal casts are observed in urine sediment in patients with glomerular and renal parenchymal diseases. Casts are composed of Tamm-Horsfall protein and originate in the distal tubules and collecting ducts. In healthy individuals hyaline and rare granular casts may

<table>
<thead>
<tr>
<th>Table 7.4 Pigmented Cells in Urine: Differential Diagnosis</th>
</tr>
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<tbody>
<tr>
<td>Finding</td>
</tr>
<tr>
<td>Lipofuscin pigment</td>
</tr>
<tr>
<td>Hemosiderin</td>
</tr>
<tr>
<td>Melanin</td>
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</tbody>
</table>

Fig. 7.5 Seminal vesicle cells and sperm in voided urine.
occasionally appear because of dehydration, fever, exercise, and other factors; these casts are considered physiologic. Conversely, nonphysiologic casts made of abnormal urine protein and those that contain cells of various types are easily identified. The type of cells contained within the cast matrix, the width of the casts, and the number of casts is indicative of the severity of the underlying disease. The presence of abnormal amounts of protein, blood, leukocytes, nitrites, and bilirubin all correlate with the type of cast.

“Round cells” are recently described cells in patients with end-stage renal failure that appear to be predictive of early hemodialysis.65 They are distinct from known cells in sediment and are similar to proximal convoluted tubule-derived cells based on morphology and molecular marker expression (GGT1, but not podocalyxin). These cells also express PAX2, Wilms tumor 1 (WT1), OSR1, and SIX2. The number of round cells correlates with the severity of chronic kidney disease.

The severity of lupus nephritis correlates strongly with voided urine cytology findings, including erythrocytes (isomorphic and dysmorphic), acanthocytes, and leukocytes (0.65 for each); classification tree has an accuracy rate of 84.3%.66

Dysmorphic red blood cells may be indicative of urologic or glomerular diseases (see later).

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Diagnostic Criteria

Infections

Bacteria

A wide variety of bacteria may affect the epithelium of the urinary tract. Most are coliforms and other gram-negative rods. Cystitis may be acute or chronic. Acute cystitis is usually associated with symptoms that rarely require confirmatory tissue biopsy or cytologic examination. The sediment may contain numerous exfoliated urothelial cells, necrotic material, and inflammatory cells, with a predominance of neutrophils (Fig. 7.6A to C). Marked necrosis and inflammation may also occur in the presence of necrotic tumors, particularly high-grade urothelial carcinoma and squamous cell carcinoma.

The sediment in chronic cystitis usually contains a background of chronic inflammation with macrophages and erythrocytes.7 Urothelial cells may be abundant and poorly preserved, occasionally forming small clusters. The cytoplasm in these cells tends to be granular and vacuolated; when the cells are degenerate, the cytoplasm contains spherical eosinophilic inclusions (Melamed-Wolinska bodies) (Fig. 7.7).67 There may be slight nuclear...
enlargement and hyperchromasia, but the contours of the nuclei are usually regular and the chromatin texture is finely granular without the coarse granularity of cancer cells. There may be necrosis of urothelial cells, with nuclear pyknosis and marked cytoplasmic vacuolization. In ulcerative cystitis, large sheets of urothelial cells may exfoliate.

Interstitial cystitis, a form of chronic cystitis associated with chronic inflammation, displays nonspecific cytologic changes.8 Eosinophilic cystitis has a predominance of eosinophils, a pattern that may be seen in patients with allergic disorders, previous biopsies, or after mitomycin C treatment.68

Tuberculous cystitis may be observed in patients with AIDS and those receiving treatment for urothelial carcinoma with bacillus Calmette–Guérin (BCG). In such patients the urine has inflammatory cells, necrosis (Fig. 7.6B), and rarely contains fragments of tubercles consisting of clusters of elongate carrot-shaped epithelioid cells, sometimes accompanied by multinucleated Langhans-type giant cells, and reactive atypia of urothelial cells.69-71 Ziehl-Neelsen staining may reveal acid-fast bacilli (Fig. 7.6C). The sediment occasionally contains “decoy” cells with glassy hyperchromatic nuclei.70 Similar findings may occur in patients with tuberculosis of the bladder.

Fungi

Fungi occasionally affect the lower urinary tract, particularly the urinary bladder, and Candida albicans is the most common, usually seen in pregnant women, diabetics, and those with impaired immunity such as patients with AIDS, those undergoing chemotherapy for cancer, and bone marrow transplant recipients. In the sediment the fungi may appear as yeast forms, with small oval bodies, or pseudohyphae, with oblong branching nonencapsulated filaments (Fig. 7.8A and B). Other fungi are uncommon, including Blastomyces dermatitidis, Aspergillus, and mucormycosis. A fungus of the species Alternaria is a common laboratory contaminant.8

Viruses

Several important viruses cause significant morphologic changes in the urothelial cells, many of which may be confused with malignancy. The dominant feature of viral infection is the formation of nuclear and cytoplasmic inclusions (Table 7.5).

Herpes simplex is an obligate intracellular virus, and florid infection with permissive replication of the virus causes abnormalities in urothelial cells that are readily recognized. In the early stages of viral replication the nuclei of infected cells appear hazy with a ground-glass appearance. Multinucleation is commonly observed in such cells. Multiple nuclei are often densely packed, with nuclear molding and tightly fitting contoured nuclei (Fig. 7.9). In later stages of infection the viral particles concentrate in the center of the nuclei, forming bright eosinophilic inclusions with a narrow clear zone or halo at the periphery. Infected cells may contain single or multiple nuclei.8,64

Cytomegalovirus is usually seen in newborn infants with impaired immunity. The infection is common in adults with AIDS. The characteristic changes are readily recognized in the sediment, including large cells with prominent basophilic nuclear inclusions surrounded by an abundant peripheral clear zone (Fig. 7.10). There is a distinct outer band of condensed nuclear chromatin.

Polyomavirus infection is widespread, according to serologic studies of adults. The BK polyomavirus may cause hemorrhagic cystitis in patients with allogeneic hematopoietic stem cell
transplant and virus-associated nephritis in patients with renal transplant. The occult virus can become activated and recognized in voided urine sediment. Polyomavirus plays a major role in urine cytology because it produces cell abnormalities that may be readily confused with cancer; these cells are also known as “decoy” cells (Fig. 7.11A).72 In permissive infections, the virus produces large homogeneous basophilic nuclear inclusions that occupy almost the entire volume of the nuclei with only a thin chromatinic rim.73,74 The background usually contains abundant inflammatory and cellular debris. Infected cells are often enlarged and usually contain a single nucleus, but binucleation and occasional large multinucleated cells may be seen.75 Elongate cells are referred to as “comet” cells. Nonspecific eosinophilic inclusions (Melamed-Wolinska bodies) may be present in the cytoplasm.66 Late infections may contain pale-staining degenerated inclusion-bearing cells in cases in which the virus may be detected in voided urine.66 When the inclusions regress, the chromatin acquires a distinctive, coarsely clumped appearance. Clearance of decoy cells from urine is closely related to histologic remission of polyomavirus nephropathy.77 The cytologic picture in some cases may be quite dramatic and has led to misdiagnosis of carcinoma.78 Decoy cells do not exhibit aneuploidy by FISH, and acid hematoxylin stain appeared to be superior to Papanicolaou stain in identifying and confirming the presence of infection (Fig. 7.11B).79 Decoy cells occurred in 14% of samples from patients with histologically proven viral nephropathy, with a sensitivity of 67%, specificity of 89%, positive predictive value of 12%, and negative predictive value of 99%.80 Quantification of decoy cells improved the positive predictive value to 32% (threshold ≥10 cells). Immunohistochemical staining of urinary exfoliated cells for SV40T improved sensitivity to 86% for detecting atypical or degenerate infected cells (specificity of 93% and positive predictive value of 33%).

More than 70 types of human papillomavirus have been identified, and types 6 and 11 are associated with condyloma acuminatum. Condyloma may also appear in the urethra and invariably induces koilocytosis. Urothelial carcinoma exhibits a low incidence of human papillomavirus types 16 and 18 infection (Fig. 7.12).81

Trematodes and Other Parasites

The most common urine parasite is Schistosoma haematobia (Bilharzia). There are two important cytologic manifestations of infection with S. haematobia: recognition of the ova and the malignant tumors that may be associated with it.68 The ova are elongate structures with a thick transparent capsule and a sword-shaped protrusion known as the terminal spine located at the narrow end of the ovum. Fresh or calcified ova may be readily recognized in the sediment. The embryonal form of the parasite, known as miracidium, is released in human stool and urine, retaining the shape of the ovum with its terminal spine. Other common intestinal parasites that affect the bladder include Ascaris lumbricoides, Enterobius vermicularis, and agents of filariasis. Trichomonas vaginalis is a sexually transmitted parasite that is rarely found in urine (0.1% incidence), appearing as round to oval organisms with eccentric nuclei and cytoplasmic granules; acute inflammation is usually present.82 Hassan et al. described microfilariae of Wuchereria bancrofti in an 18-year-old boy in India who presented with chylous hematuria.273
Reactive Cytologic Changes

Numerous reactive changes involving the urothelium may be misinterpreted as “atypical” or “suspicious/malignant” (Table 7.6).

Lithiasis

About 40% of patients with calculi have abnormal cytologic findings in voided urine. These patients have numerous large, smooth-bordered clusters of benign urothelial cells with an abundance of superficial cells (Fig. 7.13A). These changes may overlap with the spectrum of findings with low-grade urothelial carcinoma, but the cells tend to cluster, with fewer single cells. Calculi are abrasive to the mucosa when present in the renal pelvis, ureter, or urinary bladder, and the resultant cytologic specimens closely resemble the effects of instrumentation. Significant atypia of urothelial cells due to lithiasis is uncommon, and the clusters have smooth borders (Fig. 7.13B and C). Nonetheless, lithiasis remains a major diagnostic pitfall in urine cytology interpretation.

Drug Effects

Intravesically administered agents and drugs, including BCG (see earlier Bacteria section), mitomycin C, and thiopeta, are commonly used for treatment of primary and recurrent bladder tumors (Figs. 7.14A and B and 7.15). They may induce cell enlargement, cytoplasmic vacuolization, and other reactive changes, including nuclear enlargement of cells, wrinkled nuclear membranes, mild hyperchromasia, pleomorphism, abnormal nuclear morphology, disordered orientation of the urothelium, and eosinophilic inflammation. Intravesical chemotherapy can contribute to false-positive results in urine cytology.

Systemically administered drugs, such as the alkylating agents cyclophosphamide and busulfan, have a marked effect on the urothelium, inducing significant cytologic abnormalities (Fig. 7.16A to C). These drugs may cause changes that include bizarre urothelial cells with marked nuclear and nucleolar enlargement, mimicking poorly differentiated carcinoma. Large doses of cyclophosphamide have been shown to induce urothelial carcinoma, leiomyosarcoma, and carcinosarcoma.
Effects of Radiation Therapy

Radiation therapy typically induces marked cell enlargement, with bizarre cell shapes and vacuolated nuclei, polychromatic cytoplasm, and sometimes multiple nucleoli (Fig. 7.17A and B). These findings may persist for years after treatment. Clinical history is essential for diagnosis.

Degenerative Changes

Degenerating cells with pyknotic, crenated nuclei are often a source of concern in urine cytology caused by inflammation, stone, and trauma, among others. Although these changes mimic malignancy, the chromatin is usually smudged and degenerated (Fig. 7.18), in contrast with the cancerous cells in which the chromatin is crisp and distinct. Such changes are occasionally observed in polyomavirus infection.

Instrumentation Atypia

Large numbers of superficial cells and intermediate cells can be seen in catheterized urine, bladder washings, and brushings (Fig. 7.19A). Small pseudopapillae, cellular enlargement, and
pleomorphism with large nucleoli can be intimidating features (Fig. 7.19B), but careful examination of the entire sample may be helpful for distinguishing reactive changes from malignancy (Tables 7.6 and 7.7).

**Laser-Induced Changes and Other Ablation Changes**

Marked cellular spindling is common in post-laser coagulation of the bladder. The spindled cells occur singly, in loose clusters, and in lamellar stacks, and have elongate nuclei with dense chromatin and bipolar cytoplasm (Fig. 7.20). Cytologic interpretation should not be undertaken during the immediate posttreatment period.89

Irreversible electroporation, an apoptosis-inducing ablation method used for small renal masses, preserves the urinary collecting system with unaltered normal morphology, temporarily inducing degeneration with vacuolization of detached urothelial cells.90 Electromotive drug administration and chemohyperthermia represent minimally invasive methods of intravesical instillation of therapeutic agents such as mitomycin C. In “negative” or “atypical” voided urines, these treatments induce a unique characteristic pattern of increased cellularity with enlarged nuclear size, irregular nuclear membranes, and altered N/C; hyperchromasia and irregular nuclear chromatin are rarely observed.91

Electromagnetic and electrohydraulic extracorporeal shockwave lithotripsy for treatment of calculi causes a transient (4 to 10 days) increase (≥10-fold) in red blood cells and epithelial cells that is not observed in basal cells or myocytes.

**Neobladder and Ileal Conduit Urine**

The urine is dominated by degenerated glandular cells. Nuclei are usually dense and hyperchromatic due to degeneration. Urothelial cells are usually sparse or absent. Eosinophilic cytoplasmic inclusions (Melamed-Wolinska bodies) are common (Fig. 7.21A and B). Debris, cytoplasmic fragments, granular deposits, bacteria, occasional inflammatory cells, red blood cells, and small intestinal cells are seen in the background.92,93 Vegetable cells in urines from Bricker ileal conduit originate from the ostomy adhesive.96 Fresh specimens should be examined; urine from the collection bag is unsatisfactory for cytologic examination.

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**Fig. 7.15** Thiotepa-induced changes, including urothelial detachment with nuclear atypia and cytoplasmic vacuolization.

**Fig. 7.16** Cyclophosphamide changes. Atypical urothelial cells with large hyperchromatic nuclei that may be mistaken for malignant cells (A to C).

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CHAPTER 7  Urine Cytology
Urine Cytology in Renal Transplant Recipients

Urine cytology is an effective screening method for monitoring patients with renal transplant, with high sensitivity and high negative predictive value, and can be routinely used in follow-up. The epithelial cells of collecting tubules are well preserved. The cells that appear in urine specimens have scant vacuolated cytoplasm with spherical and somewhat opaque nuclei. A feature of impending rejection is the presence of numerous T lymphocytes and erythrocytes in the urine. The erythrocytes have a thick outer border and clear center suggestive of renal origin. In rejection, tissue fragments may be present, including necrotic renal tubules and hyaline casts.

Other Benign Conditions

A wide variety of benign conditions induce unique findings in the urine. Partial or complete keratinization of the squamous epithelium, referred to clinically as leukoplakia, often replaces the urothelium, resulting in a cystoscopic gray-white appearance of the mucosa. In the urinary sediment, anucleated keratinized cells,
so-called ghost cells, may be present. When these cells are present, one should exclude the possibility of squamous cell carcinoma.7,8 Cystitis glandularis may shed ciliated mucus-containing epithelial cells that contain peripheral nuclei and clear cytoplasm. Such cells may be mistaken for adenocarcinoma. Endometriosis of the bladder may result in urine shedding of diagnostic glandular and spindle cells.9 8 Large numbers of macrophages may be present in urine samples in patients with malakoplakia, but the release of such inflammatory cells usually occurs after biopsy and is detected in the urine stream (Fig. 7.22A). The spherical, intracytoplasmic, eosinophilic, or calcified Michaelis-Guttmann bodies associated with malakoplakia in the cytoplasm of the macrophages are usually readily identified (Fig. 7.22B). Urinary mulberry cells indicate Fabry disease, a lysosomal storage disorder caused by a deficiency of α-galactosidase A.99

### Benign Tumors and Tumor-like Processes

There are no cell changes that are characteristic of inverted papilloma (Fig. 7.23A and B) or nephrogenic adenoma, and cytologic

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**TABLE 7.7** Features of Reactive Changes and Urothelial Carcinoma (World Health Organization 1973 Classification)

<table>
<thead>
<tr>
<th>Feature</th>
<th>Reactive Changes</th>
<th>Grade 1 Carcinoma</th>
<th>Grades 2 and 3 Carcinoma and Carcinoma In Situ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell arrangements</td>
<td>Pseudopapillae</td>
<td>Papillae and tight clusters</td>
<td>Single cells and loose clusters</td>
</tr>
<tr>
<td>Cell configuration</td>
<td>Flat groups</td>
<td>Papillary</td>
<td>Variable</td>
</tr>
<tr>
<td>Size</td>
<td>Enlarged, pleomorphic</td>
<td>Slightly enlarged, uniform</td>
<td>Markedly enlarged, pleomorphic</td>
</tr>
<tr>
<td>Numbers</td>
<td>Few cells</td>
<td>Few groups</td>
<td>Variable</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>Vacuolated</td>
<td>Central, uniform</td>
<td>Variable, vacuolated</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Normal to slightly increased</td>
<td>Eccentric, enlarged</td>
<td>Eccentric, pleomorphic</td>
</tr>
<tr>
<td>Nuclear-to-cytoplasmic</td>
<td>Slightly enlarged</td>
<td>Increased</td>
<td>Moderate to markedly increased</td>
</tr>
<tr>
<td>ratio</td>
<td>Smooth, thick</td>
<td></td>
<td>Enlarged</td>
</tr>
<tr>
<td>Size</td>
<td>Fine, evenly distributed</td>
<td>Granular, evenly distributed</td>
<td>Moderate to markedly irregular, thin</td>
</tr>
<tr>
<td>Border</td>
<td>Often large</td>
<td>Small, absent</td>
<td>Coarse, unevenly distributed</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Diploid</td>
<td>Usually diploid</td>
<td>Large, variable</td>
</tr>
<tr>
<td>Nucleoli</td>
<td></td>
<td></td>
<td>Aneuploid</td>
</tr>
<tr>
<td>DNA content</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 7.20** Laser-induced changes.

**Fig. 7.21** Ileal conduit urine. (A) Cellular sample with many degenerating cells (low magnification). (B) Columnar cells, degenerating cells, red blood cells, and melamed-wolinska bodies.
findings from these processes and other benign processes may be difficult to differentiate. Paraganglioma presents with tumor nests composed of epithelioid cells with fine chromatin and moderate cytoplasm admixed with occasional spindle sustentacular cells. Single cells are discohesive and large with moderate cytoplasm and inconspicuous nucleoli.

Condyloma acuminatum of the urinary bladder is uncommon and may be associated with condyloma of the urethra or external genitalia. Koilocytosis is characterized by squamous cells with large hyperchromatic nuclei and perinuclear clear zones or halos. These changes result from infection by human papillomavirus types 6 and 11. The presence of koilocytes in voided urine sediment in males often indicates a lesion in the bladder or urethra (Fig. 7.12). In women, such cells may also indicate contamination from the lower genital tract. Occasionally koilocytes may mimic squamous cell carcinoma.

Endometrial-type glandular cells in urine sediment have been reported in women with endometriosis.

Fragments of benign urachal remnant were found in an unusual voided urine. The specimen was moderately cellular, consisting of sheets, small strips, and clusters of benign-appearing glandular cells with a moderate amount of cytoplasm and smooth round to oval eccentric polarized nuclei, lightly stippled fine chromatin, and inconspicuous nucleoli. There was an absence of mitotic figures, apoptotic bodies, blood, or background inflammation.

**Atypical Urothelial Cells**

One of the greatest challenges in urine cytology interpretation concerns the category of “atypical.” There is a lack of consensus on diagnostic criteria, terminology, clinical significance, and benchmark incidence rate, although all authors agree that the rate should be as low as possible. The incidence of “atypical” is widely variable, with a range of 11% to 33% in large published series with more than 100 patients (Table 7.8). The diagnostic incidence differs according to multiple variables, including patient age, gender, type of cytology specimen (instrumented versus void versus washing), patient selection (hematuria versus urinary tract symptoms versus cancer follow-up), sample processing (routine centrifugation versus liquid-based preparation), and number of cytology
Further, it is compounded by variance in subsequent predictive accuracy of “atypical” for carcinoma. The “atypical” category encompasses findings that may include from low- to intermediate-grade dysplasia (an uncommon histologic finding) (Figs. 7.24 through 7.28) at the low end of the spectrum, although it is difficult, if not impossible, to recognize specific cyto-
logic changes corresponding to histologic low- to intermediate-
grade dysplasia.8,106 High-grade (severe) dysplasia and carcinoma
in situ are considered to be equivalent, and the findings in urine
may be underinterpreted as “atypical” when they are best classified

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**TABLE 7.8** Incidence and Cancer Yield of “Atypical Urine Cytology” and Other Categories

<table>
<thead>
<tr>
<th>Authors (Date)</th>
<th>Total Cases of Atypical (n)</th>
<th>Atypical Rate (%)</th>
<th>Cases of Atypical with Follow-up Biopsy (n)</th>
<th>Time Interval to Biopsy (months)</th>
<th>All Atypical, Excluding “Favor Neoplasm” and “Favor High-Grade Carcinoma”</th>
<th>“Suspicious”</th>
<th>“Positive”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deshpande and McKee (2005)104</td>
<td>238</td>
<td>N/A</td>
<td>102</td>
<td>≤12</td>
<td>N/A</td>
<td>23.4</td>
<td>N/A</td>
</tr>
<tr>
<td>Raab et al. (2007)109</td>
<td>710</td>
<td>11.1</td>
<td>133</td>
<td>≤14</td>
<td>42</td>
<td>65.4</td>
<td>81.8</td>
</tr>
<tr>
<td>Voss et al. (2008)103</td>
<td>b</td>
<td>b</td>
<td>128</td>
<td>≤12</td>
<td>56.9</td>
<td>60.9</td>
<td>73.8</td>
</tr>
<tr>
<td>Brimo et al. (2009)167</td>
<td>691</td>
<td>23.2</td>
<td>110</td>
<td>≤12</td>
<td>68.9</td>
<td>78</td>
<td>87</td>
</tr>
<tr>
<td>Siddappa et al. (2012)160</td>
<td>464</td>
<td>32.5</td>
<td>464</td>
<td>N/A</td>
<td>N/A</td>
<td>11.5</td>
<td>N/A</td>
</tr>
<tr>
<td>Dimashkieh et al. (2013)264</td>
<td>296</td>
<td>16.1</td>
<td>296</td>
<td>≤36</td>
<td>8.3</td>
<td>32.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Bostwick and Hossain (2014)17</td>
<td>1074</td>
<td>10.2</td>
<td>1074</td>
<td>≤12</td>
<td>13.5</td>
<td>31.1</td>
<td>54.3</td>
</tr>
<tr>
<td>Musu Ubago et al. (2013)265</td>
<td>1320</td>
<td>8.1</td>
<td>N/A</td>
<td>≤138</td>
<td>N/A</td>
<td>21.0</td>
<td>N/A</td>
</tr>
<tr>
<td>Chau et al. (2015)110</td>
<td>159</td>
<td>22.8</td>
<td>159</td>
<td>≤24</td>
<td>41.9</td>
<td>61.3</td>
<td>78.3</td>
</tr>
<tr>
<td>Virk et al. (2017)262</td>
<td>377</td>
<td>N/A</td>
<td>377</td>
<td>≤12</td>
<td>N/A</td>
<td>16.5</td>
<td>N/A</td>
</tr>
</tbody>
</table>

The atypical rate may be misleading because many articles reported only cases with matched cytology–histology pairs, and most negative biopsies would be excluded because they do not trigger subsequent biopsy. N/A, Data not available.

*Excludes studies with less than 100 “atypical” cytologies with follow-up biopsy.45,265-269 Excludes upper tract cases and renal cell carcinoma.52 Excludes Piaton et al. (2011)271 and (2014)270 with an “atypical” rate of 0.8% to 2%.

This study126 included an additional category of cellular “clusters” that accounted for 579 cases, so the results cannot be directly compared with other studies.

Positive includes 44 cases diagnosed by subsequent positive cytology only (no biopsy) and 17 secondary (nonurothelial) carcinomas.

Cancer yield on biopsy includes suspicious for malignancy and diagnostic of malignancy because both categories require immediate clinical intervention.

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specimens obtained.105 Further, it is compounded by variance in subsequent predictive accuracy of “atypical” for carcinoma. The “atypical” category encompasses findings that may include from low- to intermediate-grade dysplasia (an uncommon histologic finding) (Figs. 7.24 through 7.28) at the low end of the spectrum,
Fig. 7.25 Atypical, uncertain. (A) Groups of atypical cells with mildly hyperchromatic nuclei and mild nuclear membrane irregularity (Papanicolaou stain). (B) Same case as in (A) depicting nuclear details (acid hematoxylin stain).

Fig. 7.26 Atypical cells in bladder wash. (A) Papanicolaou stain. (B) Acid hematoxylin stain.

Fig. 7.27 Atypical cells in voided urine. (A) Papanicolaou stain. (B) Acid hematoxylin stain.
as at least “suspicious” and, preferably, “high-grade carcinoma” (see later).

Reported diagnostic criteria for “atypical” usually include specimens in which the N/C ratio is greater than 50%, a criterion adopted by the Paris System 2013 (see earlier). Cell clusters are usually classified as “atypical, favor reactive” in combination with smooth uniform nuclear membranes; nucleoli may or may not be enlarged. Deshpande and McKee recognized three groups of urothelial cell clusters: group 1 consisted of flat clusters, group 2 had overlapping clusters with two or more cell layers that may be three-dimensional, and group 3 had overlapping clusters with smooth borders. “Atypical, unclear if reactive or neoplastic” described urothelial cells without degenerate features with irregular intact nuclear membranes, chromatic clumping, or the presence of black structureless nuclei referred to as “India ink–type nuclei.” Degenerate cells (poorly preserved cells) were excluded from classification by some observers, but not others, as were decoy cells of polyomavirus infection. Increased cellularity, nuclear membrane irregularities, number of “India ink–type nuclei,” and number of cell clusters correlated with adverse outcome.

We found that the “atypical” category had significant predictive value for urothelial carcinoma (about 36% overall), especially in patients under active surveillance for recurrent malignancy. Accordingly we believe that it is critical for clinicians to undertake follow-up of these patients. Unfortunately, “atypical” has become a wastebasket category because it is often overused by pathologists, contributing to complacency and lack of response on the part of many urologists when confronted with this diagnosis. Further, others have suggested that “atypical” is equivalent to “negative,” although such studies were based on an insufficient number of cases to reach such a conclusion. Brimo et al. undertook logistic regression and found that “atypical” was not significantly predictive of urothelial carcinoma on follow-up biopsy, but they failed to provide the variables weighed in their multivariate model, so their conclusion cannot be independently confirmed.

Comparison of the two “atypical” categories (“atypical, favor reactive” [see Fig. 7.24A and B] and “atypical, uncertain” [see Fig. 7.25A and B]) revealed similar predictive values for urothelial cancer on subsequent biopsy in our study of more than 9000 consecutive urine cytologies within 12 months (31.1% versus 37.7%), suggesting that this stratification is not very useful clinically, similar to the recommendation of other authors. However, some have reported significant stratification of “atypical”; Rosenthal et al. reported predictive values for cancer of 10% and 38% for “atypical, favor reactive” and “atypical, favor high-grade carcinoma,” respectively, although they apparently did not recognize the “suspicious” category, lumping those patients into “atypical, favor high-grade carcinoma.”

Our rate of atypia (10.2%) was significantly lower than others’ (up to 32.5%) (Table 7.8). This difference cannot be attributed to the use of liquid-based cellular enhancement methods, because we used the Meiers improved filter method. Other reports come

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**Fig. 7.28** Atypical urothelial cells consistent with dysplasia (A to D). Multiple biopsies of the bladder revealed dysplasia, but no evidence of carcinoma in situ.
from academic medical centers with a high level of experience in cytopathology, especially urine cytology, so this is an unlikely source of significant variation. Further, our comparison group did not rely on expert re-review of cases, but rather used the real-life method of reviewing existing diagnoses from reports. Rodgers et al. found that urine cytology was unable to rule out malignancy or exclude patients from further investigation despite ability to confirm the presence of cancer. Interobserver disagreement was “moderate to good” using Kappa statistics, but there were considerable differences in accuracy according to the level of expertise and reporting bias.

Kappa statistics, but there were considerable differences in accuracy the Papanicolaou stain. Nuclear details are critical for diagnosis, and the use of two stains: the acid hematoxylin stain in combination with an

World Health Organization (WHO) 1973 grade 1 and 2 carcinoma, the urothelial cell clusters are often arranged in a papillary configuration and are difficult to distinguish from those shed from normal benign urothelium after palpation, instrumentation, or irritation by calculi or inflammation (Figs. 7.29 and 7.30). In voided urine, spontaneously shed complex clusters of morphologically benign urothelial cells may be suggestive of a papillary tumor, provided that trauma is excluded clinically. Diagnostic features of WHO grade 1 carcinoma (papillary neoplasm of low malignant potential) include the presence of tumor fragments with connective tissue stalks or central capillary vessels (Tables 7.7 and 7.10). Numerous attempts to define the precise microscopic features of tumor fragments that separate benign urothelial cell clusters from WHO grade 1 carcinoma have met with limited success. Some authors claim that low- to intermediate-grade papillary urothelial tumors shed recognizable cells in the urinary sediment; they note that the characteristic features include increased N/C ratio, enlarged and eccentric nuclei, and inconspicuous nucleoli, features present in 70% of such tumors. Mai et al. found that low-grade urothelial carcinoma in urine frequently contained three-dimensional cell clusters with disordered nuclei and cellular dyscohesion, findings that collectively had sensitivity of 70% and specificity of 94%. Scant cellularity was observed in 20% of cases. Others reported correct cytologic diagnosis in 33% of such cases.

Differentiation of WHO grade 1 carcinoma (papillary urothelial neoplasm of low malignant potential) from instrumentation artifact is based on the presence of cell clusters with ragged borders, unlike the smooth borders lined by densely stained cytoplasm at the edge of benign cell clusters. Grade 1 carcinoma can be identified with 45% sensitivity and 98% specificity based on cytologic criteria of increased N/C ratio, irregular nuclear borders, and cytoplasmic homogeneity. In one study, overall observer accuracy was 76%, with a sensitivity of 82% for a definitive negative diagnosis and specificity for a definitive positive diagnosis of 96%. In another study, sensitivity of 90% and specificity of 65% for grade 1 carcinoma was based on the absence of inflammation, the presence of single and

**TABLE 7.9 Incidence and Cancer Yield of “Suspicious” in Recent Reports**

<table>
<thead>
<tr>
<th>Authors (Date)</th>
<th>Cases of Suspicious With Follow-up Biopsy (n)</th>
<th>Incidence of Suspicious (%)</th>
<th>Time Interval to Biopsy (months)</th>
<th>Cancer Yield on Biopsy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voss et al. (2008)</td>
<td>96</td>
<td>4.1</td>
<td>≤12</td>
<td>56.9</td>
</tr>
<tr>
<td>Siddappa et al. (2012)</td>
<td>28</td>
<td>2.0</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>Vandenbussche et al. (2013)</td>
<td>62</td>
<td>6.5</td>
<td>36-60</td>
<td>11</td>
</tr>
<tr>
<td>Bostwick and Hossain (2014)</td>
<td>593</td>
<td>4.1</td>
<td>≤12</td>
<td>16.6</td>
</tr>
<tr>
<td>Platon et al. (2014)</td>
<td>185</td>
<td>2.0</td>
<td>4-56</td>
<td>N/A</td>
</tr>
<tr>
<td>Ton Nu et al. (2014)</td>
<td>191</td>
<td>2.5</td>
<td>&gt;6</td>
<td>6.3</td>
</tr>
<tr>
<td>Joudi et al. (2016)</td>
<td>150</td>
<td>N/A</td>
<td>≤6</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A, Data not available.

- Suspicious is reported by some as “atypical urothelial cells, cannot exclude high-grade urothelial carcinoma”; the similarity of incidence rates and described criteria suggest that these groups are comparable with “suspicious.” The suspicious rate may be misleading because many articles only reported cases with matched cytology-histology pairs, and most negative biopsies would be excluded because they do not trigger subsequent biopsy.
- This series included only cases of hematuria.
- High-grade carcinoma only (excluded low-grade carcinoma).
overlapping groups of cells with high N/C ratio, hyperchromasia, nuclear grooves and notches, and small nucleoli. A third study showed 26% sensitivity for grade I urothelial carcinoma, and a fourth showed 37% sensitivity (suspicious or malignant diagnoses) and 94% specificity; 48% of these cytology specimens were classified as atypical. WHO grade 1 carcinoma is a major source of false-negative results in urine cytology. Chung et al. reported that five distinctive cytologic criteria were helpful in cases in which other conventional criteria for low-grade carcinoma were insufficient for diagnosis (loss of polarity of papillaroid clusters, irregular contours, absence of columnar cells, hobnail features, and hyperchromasia). Stepwise logistic regression analysis revealed that four features distinguished low-grade carcinoma from reactive urothelial cells: increased numbers of monotonous single (nonumbrella) cells, increased N/C ratio, hyperchromasia, and presence of small and large urothelial cells.

The Paris System 2013 recognizes the diagnosis of low-grade urothelial neoplasm (not low-grade carcinoma) based on the presence of fibrovascular cores in urothelial cell groups and an absence of cytologic atypia. However, in a series of select histologically proven low-grade carcinomas and negative control urines, McCroskey et al. found low sensitivity (21% to 53%) but relatively high specificity (81% to 95%), resulting in poor-to-fair accuracy for the diagnosis of low-grade urothelial carcinoma; overall agreement was fair ($\kappa = 0.30$). Ancillary techniques (see later) that may be valuable for separating benign and neoplastic urothelial cells include FISH, immunocytochemical tests, and DNA ploidy analysis. Digital image analysis of voided urine was superior to bladder wash cytology for prediction of tumor recurrence.

**TABLE 7.10** Criteria for Cytologic Grading of Urothelial Cancer

<table>
<thead>
<tr>
<th>Morphologic Features</th>
<th>Carcinoma In Situ</th>
<th>Grade 1 Carcinoma (Papillary Neoplasm of Low Malignant Potential)</th>
<th>Grade 2 Carcinoma (Low-Grade Urothelial Carcinoma)</th>
<th>Grade 3 Carcinoma (High-Grade Urothelial Carcinoma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>Clean</td>
<td>Clean</td>
<td>Clean</td>
<td>Dirty, tumor diathesis</td>
</tr>
<tr>
<td>Cellular arrangement</td>
<td>Numerous single cells, rare fragments</td>
<td>Large fragments of urothelium</td>
<td>Large fragments of urothelium and single cells</td>
<td>Large fragments and numerous single cells</td>
</tr>
<tr>
<td>Nuclear features</td>
<td>Syncytia, cannibalism</td>
<td>Slightly enlarged</td>
<td>Nuclear crowding and overlap</td>
<td>Syncytia, cannibalism</td>
</tr>
<tr>
<td>Nuclear membrane</td>
<td>Marked membrane irregularity</td>
<td>Regular, round or oval</td>
<td>Minimal membrane irregularity</td>
<td>Marked membrane irregularity</td>
</tr>
<tr>
<td>Chromatin</td>
<td>Increased chromatin, coarsely granular, evenly distributed</td>
<td>Finely granular, vesicular</td>
<td>Finely granular, evenly distributed</td>
<td>Increased chromatin, coarsely granular, unevenly distributed</td>
</tr>
<tr>
<td>Nucleolus</td>
<td>Rare nucleoli</td>
<td>Occasional micronucleoli</td>
<td>Variable micronucleoli</td>
<td>Macronucleoli</td>
</tr>
<tr>
<td>Cytoplasmic features</td>
<td>Variable maturation</td>
<td>Cell maturation present</td>
<td>Moderate degree of maturation</td>
<td>Maturation absent, squamoid and/or glandular features</td>
</tr>
</tbody>
</table>

*Loss of cell borders.


**Fig. 7.29** Examples of grade 1 (of 3) papillary urothelial carcinoma (A and B). In each case the diagnosis was confirmed by biopsy.

**High-Grade Carcinoma**

“High-grade carcinoma” includes the findings from urothelial carcinoma in situ, characterized by the presence of malignant cells that...
Fig. 7.30 Examples of grade 2 (of 3) papillary urothelial carcinoma (A to H). In each case the diagnosis was confirmed by biopsy.
that look like high-grade urothelial carcinoma and are often uniform in size and may be small or large (Fig. 7.31A to E; Table 7.10). The cells show markedly enlarged nuclei with high N/C ratio, coarse and dark chromatin, and irregular nuclear membranes. Nucleoli may or may not be present. The background is often clean, free of necrotic debris, and lacks inflammation. Occasionally the cells may be heterogeneous and large, particularly after biopsies. When there is prominent inflammation present, it is often prudent not to attempt to separate carcinoma in situ from invasive carcinoma. Microinvasive carcinoma may not be recognizable in cytologic samples, particularly when carcinoma in situ is present. Carcinoma in situ may persist after intravesical therapy such as BCG (Fig. 7.32A and B).

This category also accounts for the urine findings from WHO 1973 grade 2 (intermediate- to high-grade) carcinoma, WHO 1973 grade 3 (high-grade) carcinoma, and carcinoma in situ. It may be difficult to separate grades 2 (Fig. 7.30A to H) and 3 (Fig. 7.33A to F) carcinoma from carcinoma in situ in urine samples (Table 7.10). Unlike benign urothelial cells, these cells have substantial nuclear and cytoplasmic abnormalities. The principal value of urine cytology is the diagnosis and monitoring of high-grade tumors that may not be evident cystoscopically, including carcinoma in situ and occult invasive carcinoma.142,143

In voided urine, low-grade and high-grade urothelial carcinoma cells vary in size and shape, and may be small or large. The nuclei are enlarged, with coarsely granular chromatin, hyperchromasia,
abnormal nuclear contours, and prominent nucleoli. Multinucleate cancer cells and mitotic figures are often readily identified.144

In washings, urothelial carcinoma may demonstrate a lower degree of nuclear hyperchromasia, perhaps resulting in more prominent large nucleoli. The cells may be poorly preserved, particularly when there is inflammation or necrosis, and a variety of changes may be present, including frayed or vacuolated cytoplasm, nonspecific eosinophilic cytoplasmic inclusions, and pyknotic nuclei. In some high-grade papillary tumors the dominant cytologic finding may be the presence of isolated cancer cells, either singly or in groups of two or three.

Grade 2 carcinoma may present a diagnostic challenge because it is often similar cytologically to grade 1 carcinoma.81,128,143,145 Fortunately, in most cases, atypical urothelial cells are observed, alerting the clinician of the need for cystoscopic examination. For high-grade urothelial carcinoma, digital image analysis and bladder wash cytology are equally predictive.140

Correlation of Urine Cytology and Biopsy Findings (Diagnostic Accuracy)
The diagnostic accuracy of urine cytology is generally high in patients who are symptomatic or being managed after treatment for bladder cancer. However, reported results vary considerably, especially in different patient cohorts. For example, most series of bladder tumors indicate that papilloma and papillary urothelial neoplasm of low malignant potential cannot be reliably diagnosed by urine cytology despite inclusion of several key cytologic findings. Reported sensitivity of urine cytology for grade 1 urothelial carcinoma varied from 26% to 90%.26,84 The sensitivity increased to 80% for grade 2 and 95% for grade 3.

Carcinoma in situ is usually diagnosed as “suspicious” or “positive” in almost all instances. The overall sensitivity of urine cytology for primary carcinoma of the bladder ranges from 45% to 97%. In a recent report, urine cytology predicted 82% of all recurrent tumors in the bladder.146 Two major drawbacks of urinary cytology are the high rate of false-positive results in patients receiving intravesical chemotherapy and the high rate of false-negative results in those with grade 1 carcinoma. Scarcity of diagnostic or malignant cells is arguably the greatest single limitation of urine cytology, according to Frost and colleagues.47 Urine cytology findings, cystoscopy, and possible diagnostic outcomes are summarized in Table 7.11.

False-negative diagnosis of high-grade carcinoma can be attributed to underdiagnosis as “negative,” “atypical,” or “suspicious”; interestingly, in about 20% of cases, false-negative result was attributed to overdiagnosis on biopsy of high-grade carcinoma.148

If poor preservation and obscured samples were considered non-diagnostic, the sensitivity and specificity of cytology for high-grade cancer would be as high as 94% and 71%, respectively.

“Positive” voided urine predicted progression and cancer-specific mortality for non–muscle-invasive carcinoma, independent of and outperforming histologic grade on biopsy.149 The 5-year cumulative progression and cancer-specific mortality rates for patients with “positive” cytology were 20% and 15%, respectively, compared with 2% and 2%, respectively, for those with “negative” results.

Urine cytology after BCG therapy had sensitivity and specificity of 56% and 56% for cancer recurrence; when combined with cystoscopy, results were 88% and 82%, respectively, obviating the need in many patients for routine biopsy.150 Urine cytology after radical cystectomy is an early indicator of cancer recurrence, preceding radiographic evidence by a mean of 2.1 years.151

Urinary Cells Originating From Other Sites

Prostate
Prostatic adenocarcinoma (Fig. 7.34) may yield cells in voided urine spontaneously or after prostatic massage, particularly when the carcinoma is high grade. Cancer cells in the urine sediment are usually small, often spherical, and columnar, sometimes in small clusters. The cytoplasm is usually basophilic with open vesicular nuclei and prominent nucleoli.

Urethra
Primary cancer of the urethra is rare, and may be urothelial, squamous cell, or adenocarcinoma. Other rare cancers include malignant melanoma and clear cell adenocarcinoma.

Cytologic examination of the urethra after cystectomy for bladder cancer sometimes reveals carcinoma in situ or early invasive carcinoma.142

Upper Tract
Urine cytology is usually diagnostic when there is urothelial carcinoma of the renal pelvis and ureter, particularly when the cancers are high grade. With low-grade urothelial malignancies, the same diagnostic problems are encountered as in the bladder. Urine cytology rarely identifies renal cell carcinoma. When malignant cells are present, they are large, with clear or vacuolated cytoplasm and distinct nucleoli.

Among patients with clinical suspicion of upper tract malignancy with positive urine cytology, 42% experienced upper
tract cancer and an additional 33% had cancer limited to the bladder. Voided urine, selective urine cytology, ureteral washings, and computed tomography (CT) scan predicted upper tract cancer with a sensitivity and specificity of 63% and 67%, 76% and 73%, 50% and 95%, and 95% and 26%, respectively. Patients who had combined abnormal CT and “positive” voided urine had cancer in 83% of cases, whereas 100% of those with combined normal CT and “negative” voided urine (investigated for ongoing symptoms) were cancer free. FISH hypertetrasomy showed sensitivity and specificity for diagnosis of upper tract carcinoma of 50% and 89%, respectively. Urine cytology alone cannot differentiate upper tract cancer from bladder involvement.

Preoperative “positive” voided urine cytology was predictive of intravesical recurrence after radical nephroureterectomy for upper tract carcinoma. Recurrence-free survival at 1 and 3 years after surgery was 61% and 46% in patients with “positive” urine and 71% and 52% in those with “negative” urine, respectively. Multivariate

Fig. 7.33 Grade 3 (of 3) papillary urothelial carcinoma with marked cytologic abnormalities (A to F). In (D), note cell cannibalism.
analysis showed that gender, tumor multifocality in surgical specimens, and “positive” urine cytology were independent risk factors. Ureteral cytology had no additional efficacy beyond voided urine.

**Kidney Medulla and Cortex**

The strong correlation of chronic kidney disease (58% to 59%) with upper tract urothelial carcinoma, especially among patients receiving dialysis, may result from old age, aristolochic acid nephropathy, and increased risk status after nephroureterectomy, standard treatment for such tumors. Urine cytology is associated with a low detection rate (0.0% to 33.3%) in patients receiving dialysis for all urothelial cancers, especially those with upper tract involvement, because most have anuria.

Renal carcinoma associated with Xp11.2 translocation/TFE3 gene fusions in catheterized urine from the renal pelvis appeared as clusters of cells with abundant clear or eosinophilic granular cytoplasm, large, round nuclei, and prominent nucleoli. Papillary clusters containing thin fibrous stroma were occasionally seen. Voided urine showed similar cell clusters but was obscured by degenerative findings. Immunohistochemistry and FISH were useful diagnostic adjuncts.

**Secondary Tumors**

Numerous secondary malignancies may be observed in the urinary sediment, the most common arising from adjacent or contiguous organs, including the kidney, uterine cervix, endometrium, (Fig. 7.35), ovary, prostate (see earlier), and colon. Clinicopathologic correlation is usually required for diagnosis. Rare cases of carcinoid (low-grade neuroendocrine carcinoma) have been diagnosed by urine cytology. Other rare cancers may be diagnosed by cytology, including bladder and prostatic leiomyosarcoma, primary and secondary lymphoma, mantle cell lymphoma, ALK-negative anaplastic large cell lymphoma, posttransplant lymphoproliferative disorder in patients with renal transplant, melanoma, endometrioid adenocarcinoma with squamous differentiation, and choriocarcinoma. In many cases urine cytology may not be diagnostic. A recent case of pediatric adrenal neuroblastoma was diagnosed by the presence of highly cellular clusters composed of small, round, atypical cells with scant cytoplasm and high N/C ratio; nuclear molding was also noted. Immunostains were positive for synaptophysin and chromogranin A.

**Anticipatory Positive Cytology**

Urine-based tests for bladder cancer are frequently apparently falsely positive (“positive” cytology but no clinical or cystoscopic evidence of cancer). However, with further follow-up time, some of these false-positive tests are vindicated as true (anticipatory) positive tests. Among patients with “positive” cytology and initially negative cystoscopy, the hazard ratio of development of a bladder tumor at 1 year was 1.8; 76% of these patients had a tumor within 1 year. Similarly, among patients with a positive FISH and initially negative cystoscopy, the hazard ratio of development of a bladder tumor at 1 year was 1.6; 40% of these patients had a tumor within 1 year. Yafi et al. reported an anticipatory positive rate of 44% after a median time of 15 months.

**Other Types of Carcinoma**

**Squamous Cell Carcinoma**

Abnormal squamous cells in the urine may result from squamous metaplasia of the urethelium, cervicovaginal squamous intraepithelial lesion, condyloma acuminatum of the bladder, urothelial carcinoma with squamous differentiation, endometrial adenocarcinoma with squamous differentiation, and squamous cell carcinoma of the urinary tract. Squamous cell carcinoma is common in Africa and the Middle East, particularly in patients infected with...
Observations in voided urine are distinctive, often consisting of keratin, and may be squamous cell carcinoma, including sharply demarcated cells with eosinophilic cytoplasm and large nuclei. Most cases are aneuploid.

**Adenocarcinoma**

In colonic-type adenocarcinoma of the bladder, the sediment contains colloid cancer cells with large hyperchromatic nuclei and large nucleoli, sometimes in clusters. In poorly differentiated mucus-producing carcinoma, the cancer cells are small, spherical or cuboidal in shape, and contain large hyperchromatic nuclei, often with prominent nucleoli. The cytoplasm is usually basophilic, often scant, and sometimes poorly preserved. When there are large cytospin vacuoles containing mucus, the nuclei may be pushed to the periphery of the cell, features suggestive of or diagnostic of signet ring cell carcinoma.

In clear cell adenocarcinoma the cancer cells are large, with abundant finely vacuolated or granular cytoplasm, open vesicular nuclei, and prominent nucleoli. Such cells usually form round papillary clusters. Most cases are aneuploid. When numerous mucin-producing goblet cells are present, another consideration is villous adenoma.

**Small Cell Undifferentiated Carcinoma (Oat Cell Carcinoma)**

In small cell carcinoma, the cancer cells are small and round to oval, about four times the size of lymphocytes, contain compact and finely granular chromatin, often with pyknotic nuclei, high N/C ratio, and scant basophilic cytoplasm, and are set in a background of inflammatory and necrotic material. Nuclear molding may be prominent; nucleoli are not visible. The presence of small clusters of tightly packed tumor cells with nuclear molding is diagnostically helpful. The presence of cell clusters without prominent nucleoli is useful in differentiating these cells from malignant lymphoma; in the latter, cells do not cluster and usually contain small nucleoli. The demonstration of neuroendocrine differentiation in small cell carcinoma may require immunocytologic or ultrastructural studies.

**Mixed Carcinoma**

Urothelial cancer may contain foci with more than one histologic type, including squamous cell carcinoma, adenocarcinoma, and small cell carcinoma. The cytologic findings in such tumors rarely allow the diagnosis of mixed carcinoma. Usually one pattern is dominant, although a mixed population of cancer cells may be observed, including some of the rare variants.

**Rare Variants of Urothelial Carcinoma**

Signet ring cell, micropapillary, plasmacytoid, urothelial carcinoma with oncocytic features, and sarcomatoid variants have been reported in the cytology literature, mostly as case reports. Recognition of variants is critical because many are associated with different clinical outcomes or therapeutic approaches.

Signet ring cell carcinoma consists of scattered malignant epithelial cells displaying distinct cell borders, abundant cytoplasm with single large, discrete mucin vacuoles, and eccentric irregular nuclei with prominent nucleoli. In contrast, metastatic colonic signet ring cell carcinoma displayed predominantly single dispersed malignant cells containing eccentrically placed, oval nuclei with occasional small nucleoli and a moderate amount of vacuolated cytoplasm.

Micropapillary urothelial carcinoma is rarely identified, appearing as numerous small, cohesive groups and single neoplastic cells. Pseudopapillae were present in 17 of 20 cases, and in 9 they were a relevant finding; morules were present in 15 cases; isolated microacini were seen in 14 cases; cellular atypia was prominent in 17 cases. In 15 cases a cytologic diagnosis of urothelial carcinoma was made, 1 case was diagnosed as adenocarcinoma, and the remaining 4 cases were considered suspicious of malignancy.

Plasmacytoid carcinoma contains single cells with eosinophilic cytoplasm and characteristic eccentric hyperchromatic nuclei. Immunoreactivity for CD138 is helpful but represents a pitfall because it is also positive in plasma cell dyscrasias.

Urothelial carcinoma with oncocytic features appeared as delicate papillae with cells displaying oncocytic cytoplasm and relatively low N/C ratio; immunostains showed strong p53 immunoreactivity and low Ki-67 labeling.

**Major Diagnostic Pitfalls**

Most errors in urine cytology are overdiagnosis of benign cellular changes as malignant (Table 7.12). Knowledge of these changes is fundamental to the practice of cytology. The College of American Pathologists Interlaboratory Comparison project of more than 46,000 pathologists found that participants performed well in accurately classifying cases as benign or malignant (overall 92.4% concordance) (Table 7.13). However, the greatest difficulties were with correct identification of adenocarcinoma and squamous cell carcinoma cases, and with overinterpretation of ileal loop and polyomavirus as high-grade carcinoma.
which are also described and illustrated elsewhere in this chapter.

Table 7.12: Major Diagnostic Pitfalls in Lower Urinary Tract Cytology

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Responses</th>
<th>Concordance Rate (%)</th>
<th>False Responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overdiagnosis of normal and degenerated urothelium as malignant</td>
<td>71,581</td>
<td>83.8</td>
<td>Atypical urothelial cells (4.1), reactive (3.2), adenocarcinoma (3.1), negative for malignancy (1.3), squamous cell carcinoma (1.1), polyomavirus (0.9), inflammation (0.7), and other (1.8)</td>
</tr>
<tr>
<td>Overdiagnosis of human polyomavirus infection as malignant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overdiagnosis of effects of cyclophosphamide as malignant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Underdiagnosis of grade 1 or 2 urothelial carcinoma (papillary urothelial neoplasia of low malignant potential or low-grade urothelial carcinoma) as benign</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.13: Concordance with the Urine Cytology Reference Standard in the College of American Pathologists Interlaboratory Comparison Program 2000 to 2010

<table>
<thead>
<tr>
<th>Reference Diagnosis</th>
<th>No. of Responses</th>
<th>Concordance Rate (%)</th>
<th>False Responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-grade urothelial carcinoma</td>
<td>71,581</td>
<td>83.8</td>
<td>Atypical urothelial cells (4.1), reactive (3.2), adenocarcinoma (3.1), negative for malignancy (1.3), squamous cell carcinoma (1.1), polyomavirus (0.9), inflammation (0.7), and other (1.8)</td>
</tr>
<tr>
<td>Negative for malignancy</td>
<td>2852</td>
<td>73.9</td>
<td></td>
</tr>
<tr>
<td>Polyomavirus</td>
<td>3535</td>
<td>71.7</td>
<td>Reactive (6.4), high-grade carcinoma (6.2), and cytomegalovirus/ herpes (3.1)</td>
</tr>
<tr>
<td>Ileal loop urine</td>
<td>5291</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>756</td>
<td>49.1</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1667</td>
<td>42.9</td>
<td></td>
</tr>
<tr>
<td>Treatment-related changes</td>
<td>1293</td>
<td>36.3</td>
<td></td>
</tr>
</tbody>
</table>

Following is a summary of some of the most vexing problems, which are also described and illustrated elsewhere in this chapter.

**Trauma or Instrumentation**

The normal urothelium tends to exfoliate in the form of tissue fragments that are round or oval, commonly designated as papillary clusters. Vigorous palpation, catheterization, or any form of instrumentation may result in the formation of such epithelial clusters. When present in large numbers, these clusters may be misinterpreted as carcinoma. Another source of error is the presence of numerous superficial urothelial cells that may be mistaken for cancer because of their variable nuclear features. Careful consideration of chromatin pattern, N/C ratio, and nuclear membrane irregularity should enable differentiation of benign and malignant processes in the vast majority of cases.

**Cell Preservation**

Cells in voided urine sediment, particularly in the first morning void, are often poorly preserved, compounding the diagnostic difficulty. The diagnosis of cancer in voided urine should be avoided unless the findings are unequivocal.

**Human Polyomavirus**

Polyomavirus (BK virus) infection creates large intranuclear inclusions that may mimic cancer nuclei. However, the inclusions are homogeneous and lack the coarse chromatin granularity of malignancy. This is an important source of diagnostic errors that can contribute to costly and lengthy patient investigations. Polyomavirus-infected cells and malignant cells may coexist in urine cytology.

**Lithiasis**

Calculi in the lower urinary tract are abrasive, dislodging epithelial fragments that may be quite large and display papillary appearance mimicking low-grade carcinoma. The presence of numerous superficial cells may also create diagnostic difficulty because of nuclear abnormalities.

**Drugs and Other Therapeutic Procedures**

Urothelial cell changes may result from a wide variety of inciting agents, including chemotherapy, radiotherapy, and other interventions. Intravesical chemotherapy is responsible for a high rate of false-positive results. A further source of diagnostic difficulty may be synchronous infection with polyomavirus in patients who are immunocompromised. It should be remembered that urothelial carcinoma or sarcoma may develop in patients who are receiving cyclophosphamide for treatment of lymphoma.

**Ancillary Studies and Immunocytology**

This section is limited to discussion of cell-based assays that may be used in the anatomic pathology/cytology laboratory, including digital image analysis, cytochemical and immunocytochemical stains, and FISH. Excluded are clinical laboratory methods such as metabolomics, microRNA and DNA assays, deep sequencing, and serum-based methods of soluble biomarkers.

**Digital Image Analysis and Morphometry**

Digital image analysis was superior to flow cytometry for DNA ploidy analysis, with sensitivities of 83% to 91% and 71%, respectively, and was even higher in carcinoma in situ, grade 3 cancer, and stages T2 and T3 cancer (all nearly 100%). Digital image analysis was superior to cytologic examination for prediction of tumor recurrence after negative findings by cystoscopic examination and was equivalent to cytology for detection of high-grade cancer. Muralidaran et al. demonstrated the utility of an artificial neural network for urine cytology diagnosis based on nuclear area, diameter, perimeter, standard deviation of nuclear area, and integrated gray density.

Apoptotic index was found to be diagnostically useful. Excluding ileal conduit specimens, the finding of a high apoptotic index...
with the presence of pyknosis or karyorrhexis in the urine correlated with high-grade cancer.\textsuperscript{193}

Quantitative phase imaging is a new method that measures the nuclear mass and entropy of cells, and showed significant differences between “negative” and “positive.”\textsuperscript{194} There was a progressive increase in patients with “negative” diagnosis compared with those with “atypical,” “suspicious,” and “positive” cytologic diagnoses that was predictive of subsequent biopsy results.

Nuclear/nucleolar volume ratio creates an index for discrimination of benign and malignant urothelial cells with sensitivity of 56%, specificity of 89%, positive predictive value of 85%, and negative predictive value of 64% (cut point of 1.5%).\textsuperscript{195}

Digital image telepathology is feasible. Specificity and sensitivity regarding categorized diagnoses were 83% to 92% and 85% to 93%, respectively; overall accuracy rate was 88% to 90%.\textsuperscript{196} Interobserver agreement was substantial ($\kappa = 0.791$). The lowest rate of concordance was with the identification of benign lesions.

\textbf{Cytochemical Stains}

\textit{Acid Hematoxylin Stain}

Addition of nuclear staining such as the acid hematoxylin stain to Papanicolaou staining increased sensitivity in one laboratory by about 28% by eliminating background debris and improving detection of mitotic figures and other changes in chromatin that may be obscured.\textsuperscript{197} The combination of Papanicolaou staining with nuclear staining and image analysis achieved 90% sensitivity for diagnosis of urothelial carcinoma in one report.\textsuperscript{198} DNA ploidy analysis was equivalent with the two methods. Both stains were superior to Papanicolaou stain for examination of nuclear chromatin texture and content. These findings indicate that acid hematoxylin is a satisfactory substitute for Feulgen staining in cytologic preparation for DNA ploidy studies and provides additional technical advantages.

In addition to Papanicolaou stain, we routinely use acid hematoxylin stain for urine cytology. This inexpensive, nontoxic, and easily performed stain enhances nuclear chromatin pattern (surrogate Feulgen stain), removes unwanted background debris, and optimizes cellular adherence to the slide. Acid hematoxylin complements Papanicolaou stain for diagnosis of malignant cells (Fig. 7.37A and B) and is superior to Papanicolaou stain for differentiating “decoy” cells from malignant cells (Fig. 7.38A and B).\textsuperscript{79}

Acid hematoxylin stain can also be used for DNA ploidy analysis with the aid of an image analyzer.\textsuperscript{199,200} The increased accuracy of diagnosis is cost effective, obviating the need for unnecessary cystoscopies and other invasive and expensive techniques. Acid hematoxylin provided superior cellular yield compared with Feulgen stains, as well as more consistent staining and better preservation of nuclear size (Fig. 7.39A and B).\textsuperscript{79}

\textbf{CellDetect}

CellDetect is a unique histochemical panel consisting of a proprietary plant extract and three dyes that enables color discrimination between benign (green) and malignant (red) cells based on specific metabolic alterations exclusive to the latter. It was superior to standard cytology (sensitivity of 94% versus 46%, respectively, and specificity of 89% for both), particularly for sensitivity with low-grade tumors (88% versus 17%, respectively).\textsuperscript{201,202}

\textbf{Immunocytochemical Stains}

\textit{Telomerase}

The ribonucleoprotein telomerase is a reverse transcriptase enzyme that adds repeat sequences to the 3’ end of telomeres, a region of repetitive sequences at each end of eukaryotic chromosomes. Telomeres protect the ends of the chromosomes from DNA damage or alteration. Telomerase is active in normal stem cells and most cancer cells but is normally absent from, or at very low levels in, most somatic cells. Expression of the hTERT protein has also been analyzed by immunocytochemistry using anti-hTERT antibodies.

The telomeric repeat amplification protocol polymerase chain reaction assay has been most widely used to assay telomerase activity, but it creates false-positive results in the presence of inflammation and nonbladder epithelial cells. The availability of polyclonal and monoclonal antibodies for nuclear and cytoplasmic hTERT protein expression allows cell-specific microscopic visualization of different cell components in urine and other tissues, minimizing the influence of false-positive results (Fig. 7.40).\textsuperscript{203-205} Inflammatory cells serve as internal positive controls.

\textit{HER2 and Cytokeratins}

Immunocytologic expression of HER and high-molecular-weight cytokeratin in cells from voided urine predicted bladder cancer recurrence.\textsuperscript{206} HER was expressed in 7% of cases without recurrence.
compared with 85% of those with recurrence; results for high-molecular-weight cytokeratin were 43% versus 64%, respectively.\textsuperscript{206}

Cytokeratin 20 expression in nonumbrella cells is a robust marker of urothelial carcinoma, and it was confirmatory of low-grade urothelial malignancy in “atypical” voided specimens, as well as excluding cancer in those with reactive changes caused by calculus disease.\textsuperscript{207,208} Aberrant staining may be observed in cases of cystitis. The combination of CK20 and p53 immunostaining revealed positivity in 90% of urothelial cancers, 50% of “atypical” cases, and 25% of “negative” cases. Accuracy for cytology versus cytology combined with the two immunostains showed sensitivity of 73% versus 91%, specificity 100% versus 74.3%, positive predictive value 100% versus 89%, and negative predictive value 63% versus 79%.\textsuperscript{209} It appears that combined immunocytochemical staining for CK20 and p53 is easy to perform and evaluate, improves sensitivity, helps in establishing the diagnosis of malignancy, and may be of value as a triage tool to select patients who require cystoscopy during clinical follow-up.

**Vimentin**

Vimentin immunocytochemical staining may be useful as an ancillary method for evaluation of exfoliated atypical reactive/repair renal tubular cells in select urinary specimens. This may avoid unnecessary diagnostic procedures for evaluation of urothelial carcinoma in vimentin-positive cases, suggesting further diagnostic workup for evaluation of renal disease.\textsuperscript{210}

**ImmuNoCyt/uCyt ImmunocytoLOGY**

ImmuNoCyt/uCyt consists of a targeted panel of antibodies, including fluorescently labeled M344, LDQ10, 19a, and glycosylated high-molecular-weight carcinoembryonic antigen. A minimum of 500 epithelial cells is required, and identification of one or more fluorescent cells is positive. Sensitivity is 62% (47% for low-grade and 83% for high-grade cancer); overall specificity was 79%.\textsuperscript{211} Metaanalysis revealed that ImmunoCyt had a higher sensitivity (73%) than urine cytology test, but the specificity, positive likelihood ratio (LR), negative LR, diagnostic odds ratio (DOR), AUC, and Q index were lower.\textsuperscript{212} The combination of ImmunoCyt and cytology provided sensitivity, specificity, positive LR, negative LR, DOR, AUC, and Q index of 83%, 64%, 2.80, 0.23, 13.50, 0.86, and 0.79, respectively.

**ProExC**

ProExC is a commercially available immunocytochemical panel biomarker directed against topoisomerase II α and minichromosome maintenance 2 proteins, both of which are involved in DNA replication and overexpressed in dysplastic and malignant tissues. ProExC is positive when nuclear staining identifies at least
one morphologically atypical urothelial cell. Sensitivity and specificity were 85% and 69%, respectively, compared with 85% and 31% for ImmunoCyt, and 93% and 23% for the combination of tests; prediction of high-grade urothelial carcinoma had sensitivity of 92% for ProExC, 86% for ImmunoCyt, and 92% for the combination. ProExC was superior to FISH for prediction of cancer, with sensitivity of 89% and 56%, specificity of 78% and 44%, positive predictive value of 89% and 67%, and negative predictive value of 78% and 33%, respectively.

Other Immunostains
Ubiquilin-2, a ubiquitin-related protein, is expressed in the nuclei of urothelial carcinoma cells, but not benign cells. Overall sensitivity was 88%, specificity was 99%, positive predictive value was 98%, and negative predictive value was 93% for detection of carcinoma.

ERG immunocytochemistry has sensitivity and specificity for prostate cancer in urine of 23% and 100%, respectively; it should be noted that up to half of prostate cancers are ERG− in prostatic tissues, so the expected sensitivity in urine will be accordingly limited.

WT1 antibody recognizes a podocyte marker that can distinguish normal and nonrenal urinary tract disease from kidney disease. WT1+ cells were found in 50% of voided urine samples, whereas no positive cells were found in patients with lower urinary tract disease or in healthy volunteers.

Immunostains for p53 or Ki-67 (optimal cutoffs were 5% for p53 and 3% for Ki-67) in combination with cytology increased specificity without penalizing sensitivity for detection of carcinoma; sensitivity and specificity for the detection of all cancers were 86% and 77% for cytology alone, 81% and 93% for cytology and p53, 76% and 88% for cytology and Ki-67, and 69% and 98% for the full combination of cytology, p53, and Ki-67, respectively.

PAX8 is a useful immunostain to diagnose nephrogenic adenoma in voided specimens; however, care must be taken to avoid misinterpretation of positive staining in lymphocyte nuclei.

Epidermal growth factor receptor positivity was an independent risk factor for recurrence after intravesical chemotherapy.

Polyomavirus-infected cells were categorized as SV40+/S100P+ and SV40+/S100P− by immunostains.

Calreticulin, annexin A2, and annexin A3 were overexpressed in upper tract carcinoma.

In patients with bladder cancer independent of stage, TERT mutations were found in 55%, FGFR3 mutations in 30%, PIK3CA in 14%, and TP53 mutations in 12%, with 70% sensitivity and 97% specificity.

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![Fig. 7.40 Telomerase Immunocytology. (A) Benign urine nuclei have scant fine dusty staining of nuclei, easily distinguished from (B to D) intense but variable nuclear immunoreactivity in high-grade carcinoma.](image)
Fluorescence In Situ Hybridization

Multitarget FISH using probes for chromosomes 3, 7, 17, and 9p21 has high sensitivity and specificity for detecting urothelial carcinoma (Fig. 7.41A to E). A positive result is defined as polysomy in four or more cells, which also includes tetrasomy. The sensitivity of FISH for detecting cancer was superior to cytology, despite similar specificity, irrespective of cancer grade and stage: overall, 81% versus 33%; low grade, 76% versus 12%; high grade, 85% versus 50%; nonmuscle invasive, 81% versus 28%; and muscle invasive, 80% versus 45%, respectively. Sensitivity ranged from 55% to 98% and specificity from 55% to 100%. 9p21 loss (>12%) was an independent prognostic factor for recurrence.

About 27% of patients under surveillance for recurrent bladder cancer with no immediate clinical evidence of recurrence had positive FISH, and about 65% of these anticipatory positive patients had recurrent cancer within 29 months.

FISH may be useful in decreasing the rate of atypia, but it is limited by high false-positive incidence. Carcinoma was diagnosed more frequently in patients with positive than in those with negative FISH results (49% versus 9%, respectively). Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of FISH were 45%, 82%, 47%, 80%, and 72% for all cancers and 48%, 79%, 28%, 90%, and 74% for high-grade carcinoma, respectively. FISH showed a high false-positive rate (53%)

The Problem of Hematuria

Hematuria is present in about 21% of Americans, including up to 2% of children. It is most commonly microscopic, usually discovered incidentally during laboratory studies accompanying physical examination, or gross and typically apparent to patient. Only 1 mL of red blood cells per liter is sufficient to noticeably change the color of urine. Microscopic hematuria is defined as at least three red blood cells per hpf in freshly voided centrifuged urine, preferably documented on three separate occasions. Up to 3% of adults normally excrete small numbers of red blood cells

Fig. 7.41 Fluorescence in situ hybridization (FISH) in voided urine cytology. (A) Normal urothelium showing two copies of chromosome 3 (red), two copies of chromosome 7 (green), two copies of chromosome 17 (aqua), and two copies of LSI 9p21 (gold) by FISH. (B) Aneuusomic urothelial cell showing five copies of chromosome 3 (red), three copies of chromosome 7 (green), five copies of chromosome 17 (aqua), and no copies of LSI 9p21 (gold) by FISH. (C) Aneuusomic urothelial cell showing five copies of chromosome 3 (red), seven copies of chromosome 7 (green), and four copies of chromosome 17 (aqua) by FISH. (D) Aneuusomic urothelial cell showing four copies of chromosome 3 (red), two copies of chromosome 7 (green), two copies of chromosome 17 (aqua), and two copies of LSI 9p21 (gold) by FISH. (E) Urinary carcinoma cell showing gains of chromosomes 3 (red), 7 (green), and 17 (aqua) by FISH.
(up to two red blood cells per hpf, or the equivalent of 1000 red blood cells/mL), so it is important in such cases to avoid overdiagnosis. Gross hematuria is the visible discoloration of urine secondary to blood.

Hematuria may be symptomatic or asymptomatic, transient or persistent, but is often accompanied by other physical findings including proteinuria, edema, and hypertension. A thorough history and physical examination is mandatory. Current guidelines around the world recommend against the use of routine urine cytology for evaluation of asymptomatic microscopic hematuria.\(^{247,248}\) Metaanalysis revealed an overall pooled urinary tract cancer rate for hematuria of 3.3%.\(^ {249}\)

Hematuria may originate anywhere in the urinary tract, from the glomeruli to the distal urethra. Causes of hematuria are numerous and may be benign or malignant (Table 7.14 through Table 7.16). “Atypical urothelial cells” are present in 33% of cases of gross or microscopic hematuria, with 10% of these predictive of carcinoma (3% of all cases of hematuria).\(^ {250}\) The commonest causes of gross hematuria in adults are urinary tract infection (33%), malignancy (23%), and urolithiasis (11%). The most common causes of hematuria in adults are idiopathic (43% of cases of microscopic hematuria) and urinary tract infection (33% of cases of gross hematuria (Table 7.14)).\(^ {251}\) Among children the most common causes are idiopathic (80% of cases of microscopic hematuria) and renal disease (34% of cases of gross hematuria).\(^ {245}\) It is critical that the source of persistent hematuria be identified, according to the Best Practice Policy of the American Urologic Association.\(^ {251}\) Such patients should be referred for appropriate urologic workup.

### Routine Laboratory Investigation of Hematuria

Routine urinalysis combines the macroscopic reagent strip (dipstick) test with microscopic examination of the sediment to detect disorders of the urinary tract. Dipsticks detect 0.05 to 0.3 mg of

<table>
<thead>
<tr>
<th>TABLE 7.14</th>
<th>Causes of Hematuria in Children and Adults (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Causes</strong></td>
<td><strong>CHILDREN</strong>(^ {245})</td>
</tr>
<tr>
<td></td>
<td>Microscopic Hematuria</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>38</td>
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<tr>
<td>Malignancy</td>
<td>0</td>
</tr>
<tr>
<td>Calculi</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>0</td>
</tr>
<tr>
<td>Renal disease</td>
<td>3</td>
</tr>
<tr>
<td>Hypercalciuria</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 7.15</th>
<th>Site-Specific Causes of Hematuria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lower Urinary Tract Bleeding</strong></td>
<td><strong>Upper Urinary Tract (Renal) Bleeding</strong></td>
</tr>
<tr>
<td>Tumors (urethra, bladder, prostate, ureters, renal pelvis)</td>
<td><strong>Primary glomerulopathies</strong></td>
</tr>
<tr>
<td>Obstructive uropathy</td>
<td>Immunoglobulin A nephropathy</td>
</tr>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>Postinfectious glomerulonephritis</td>
</tr>
<tr>
<td>Lithiasis (stones)</td>
<td>Membranoproliferative glomerulonephritis</td>
</tr>
<tr>
<td>Infections (cystitis, prostatitis, schistosomiasis, tuberculosis, condyloma acuminatum)</td>
<td>Focal glomerular sclerosis</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td><strong>Secondary glomerulopathies</strong></td>
</tr>
<tr>
<td>Trauma</td>
<td>Lupus nephritis</td>
</tr>
<tr>
<td>Radiation therapy</td>
<td>Henoch-Schönlein syndrome</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>Vasculitis (polyarteritis nodosa)</td>
</tr>
<tr>
<td>Vigorous exercise</td>
<td>Wegener granulomatosis</td>
</tr>
<tr>
<td>Menstrual contamination</td>
<td>Hemolytic-uremic syndrome</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>Essential mixed cryoglobulinemia</td>
</tr>
<tr>
<td><strong>Familial conditions</strong></td>
<td>Intertstitial nephritis</td>
</tr>
<tr>
<td>Hereditary nephritis (Alport syndrome)</td>
<td><strong>Infections</strong></td>
</tr>
<tr>
<td>Hemoglobinopathies</td>
<td>Pyelonephritis (acute or chronic)</td>
</tr>
<tr>
<td>Metabolic disorders (hypercalciuria)</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>Polycystic kidney</td>
<td>Cytomegalovirus</td>
</tr>
<tr>
<td><strong>Nephrolithiasis</strong></td>
<td>Polymavirus</td>
</tr>
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<td><strong>Light chain deposition</strong></td>
<td><strong>Amyloid</strong></td>
</tr>
<tr>
<td><strong>Diabetic nephropathy</strong></td>
<td><strong>Renal tumors (renal cell carcinoma)</strong></td>
</tr>
</tbody>
</table>
hemoglobin per deciliter of urine. The sensitivity of dipstick examination to detect three or more red blood cells per hpf is around 90%. Myoglobin is also detected by screening reagent strips, so confirmatory testing by an appropriate reagent strip and microscopic examination is necessary. The degree of hematuria bears no relation to the severity of underlying disease and should be considered evidence of disease until proven otherwise.

**Dysmorphic Red Blood Cells Indicate Glomerular Disease**

Red blood cell morphology is useful for determining the site of origin (glomerular or nonglomerular) of hematuria. Dysmorphic red blood cells are associated with hematuria caused by glomerular injury. Two distinct types of dysmorphic red blood cells have been described: (1) target cells containing a distinct central inclusion of heme pigment surrounded by a clear zone, and (2) cells with multiple cytoplasmic blebs (Fig. 7.42A to D). How dysmorphic red blood cells are formed has not been definitively elucidated, although some suggest that causes include mechanical damage to the red blood cell within the glomerulus followed by osmotic injury. Although some suggest that causes include mechanical damage to multiple cytoplasmic blebs. How dysmorphic red blood cells are formed has not been definitively elucidated, although some suggest that causes include mechanical damage to the red blood cell within the glomerulus followed by osmotic injury. How dysmorphic red blood cells are formed has not been definitively elucidated, although some suggest that causes include mechanical damage to the red blood cell within the glomerulus followed by osmotic injury.

We analyzed 146 patients (Table 7.17) who had diagnostic renal biopsies and prior cytologic examination of urine sediment, including detailed analysis of red blood cell morphology (unpublished data). Of 108 patients with histologically confirmed glomerulopathy, 67 had dysmorphic red blood cells in urine. One of 38 patients with no glomerulopathy diagnosed at renal biopsy had dysmorphic red blood cells. Using histologically confirmed glomerulopathy as the end point for analysis of dysmorphic red blood cells and glomerulopathy, sensitivity was 70%, specificity was 97%, positive predictive value was 99%, and the negative predictive value was 54%.

Red blood cell morphology alone is not sufficient to categorize patients with hematuria. Although the specificity of dysmorphic red blood cells is high, their absence does not entirely exclude the possibility of glomerulopathy, nor does their presence guarantee renal disease. The evaluation of red cell morphology is most useful when accompanied by examination of all components of urine sediment, including urothelial cell morphology, urinalysis with urine chemistry, and serum chemistries.

Studies have evaluated various percentages of dysmorphic red blood cells in patients with known glomerular disease. Increasing the percent required for the diagnosis of glomerular bleeding increases the specificity of the test. Among patients exhibiting ≥40 dysmorphic red blood cells, 34% had urologic diseases and 29% had glomerular diseases. Urologic diseases included 27% with malignancies and 52% with conditions requiring immediate treatment. For predicting glomerular disease, the presence of proteinuria was more accurate than the number of dysmorphic cells.

**Comprehensive Analysis of Urine for Evaluation of Hematuria**

Comprehensive quantitative evaluation of the sediment, also known as optimal cytodiagnostics analysis, is useful to help distinguish inflammatory, infectious, degenerative, or neoplastic conditions of the kidney and the lower urinary tract, and can often discriminate glomerular and tubular injury. Enhanced cytologic preparation improves cell recovery to maximize microscopic visualization and quantitative assessment of dysmorphic and isomorphic red blood cells, inflammatory cells, renal casts, and renal tubular elements. Diagnostic findings are correlated with serum findings, providing the clinician with the full spectrum of chemical and morphologic abnormalities, and allowing triage of patients to the appropriate specialist (e.g., nephrologist, urologist). There are six components in comprehensive analysis of urine for evaluation of hematuria:

1. Patient history
2. Physical examination of the urine sample, including color, character, and specific gravity
3. Chemical examination, consisting of multiparameter reagent dipstick testing and confirmatory tests; albumin, β₂ microglobulin, and protein are reported quantitatively
4. Microscopic urine sediment examination using standardized sediment recovery and high-contrast Papanicolaou stain
5. Quantitative microscopic examination of the sediment entities and 10 specific morphologic categories: background, cellularity, epithelial fragments, inclusion-bearing cells, red blood cells, neutrophils, eosinophils, lymphocytes, renal tubular cells, and casts
6. Diagnostic interpretation

There is a high level of intraobserver and interobserver agreement in determination of origin of renal cells (glomerular, tubular, interstitial, or vascular cells). Similarly, the correlation is high with biopsy findings: 89% correlation in native kidneys and 77% in transplant kidneys. Sensitivity and specificity for glomerular lesions alone in native and transplant kidneys was 91% and...
85%, respectively. Severity scores showed good correlation between optimal cytodiagnostic urinalysis results and renal biopsy in native and transplanted kidneys, and correlated well with increased creatinine concentration. In cases with biopsy-proven glomerular lesions, more severe changes were found by optimal cytodiagnostic urinalysis when the biopsy showed a proliferative lesion than when only normal glomeruli were found by light microscopy. Optimal cytodiagnostic urinalysis has an advantage over renal biopsy in that it can be repeated as often as necessary, thereby providing data regarding a renal lesion over time.258

A study of 201 patients demonstrated that the presence of more than five lymphocytes/hpf correlated with a 4.3 increased odds ratio of acute rejection among patients after antithymocyte globulin induction; the association was lost, however, with alemtuzumab induction. In addition, the study showed that a demonstration of polyomavirus infection was associated with polyomavirus nephropathy.259

Data from quantitative measurement of marker proteins (e.g., albumin, transferrin, IgG, α1-microglobulin, retinol binding protein, α2-macroglobulin, Bence Jones proteins) have challenged the dominant role of microscopy. Renal biopsy abnormalities were identified in all cases by marker protein excretion, but in only 41% of cases by sediment.260

References are available at expertconsult.com

**TABLE 7.17**

<table>
<thead>
<tr>
<th>Glomerulopathy</th>
<th>Negative for Glomerulopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysmorphic red blood cells (n)</td>
<td>76</td>
</tr>
<tr>
<td>Isomorphic red blood cells (n)</td>
<td>32</td>
</tr>
</tbody>
</table>

**Fig. 7.42** (A) Isomorphic red blood cells. Note the smooth membranes and uniform amount of hemoglobin. Compare with (B), dysmorphic red blood cells, “target cell type.” Note the central inclusion of heme pigment. (C) Dysmorphic red blood cells with prominent “blebs” or protrusions of red cell membrane. (D) Dysmorphic red blood cell “target cell type” with cytoplasmic bleb.
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