

## UroVysion: A New Tool for Improving Surveillance of Urothelial Carcinoma Patients

*Michael de Tar, MD, Laboratory Medical Director, InCyte Pathology*

Cancer of the urothelial mucosa (urinary bladder, ureters, renal pelvis and urethra) represents a significant cause of cancer-related morbidity and mortality in this country, with more than 54,000 new cases diagnosed annually and 10,000 cases of mortality per year due to the disease. When in its early stages, cancer therapy is effective with relatively low morbidity. However, when the tumors extend beyond the surface of the mucosa and invade the underlying supporting structures of the urinary tract, more aggressive therapy is required, and the morbidity and mortality rates increase significantly.

Monitoring strategies of patients with early-stage cancer of the urinary tract have traditionally included a combination of urine cytology and a regular program of cystoscopic (direct visualization) examination of the bladder. Unfortunately, even with the combination of these two modalities, a certain subset of patients go on to develop invasion of bladder muscle and die from their disease. Despite the best attempts to improve the morphologic criteria for the detection of malignant cells in the urine by cytopathologists, even in the best of hands, the sensitivity of urine cytology is in the range of 60%, meaning four out of every ten patients who have malignancy will go undetected by cytology. While it is true that the lower grade tumors are even more difficult to diagnose by urine cytology (sensitivity of testing as low as 20%), these tumors are much less likely to progress to invasive disease.

Similarly, while the higher grade lesions are easier to diagnose in cytology (sensitivity up to about 70%), these are the tumors that are most likely to progress.

Enhancing the diagnostic sensitivity of urine cytology without losing specificity in testing has been the long-sought ideal for pathologists and urologists alike. Since the overwhelming majority of higher-grade urothelial carcinomas show genetic alterations with increased chromosome copy number ("aneuploidy"), genetic testing for

these abnormalities has held great promise. Up until recently, however, genetic testing relied upon technologies that were either too expensive or technically too demanding to be feasible in the general laboratory.

The introduction of fluorescence in-situ hybridization (FISH) has solved many of these problems in that testing can be performed on routinely collected specimens and has been standardized to the point of making it feasible to offer in the community setting. Many studies have

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demonstrated significantly enhanced sensitivity in detecting malignant cells using the FISH test, with an overall sensitivity around 80% (compared to overall urine cytology sensitivity of 60%). Of note, however, is that in the higher grade tumors, FISH outperforms cytology with a sensitivity of 97% in grade 3 tumors (versus 78% for cytology) and 76% for grade 2 tumors (versus 53% for cytology). It is in this population of patients that the enhanced sensitivity is most important because of the more aggressive biologic potential of the lesions.

InCyte Pathology is pleased to be able to offer FISH testing for patients who are in a surveillance program for previously diagnosed urothelial carcinoma. The UroVysion™ test is an FDA-approved FISH test designed to help monitor disease progression in this population of patients and is a very useful testing adjunct in this

setting. In the laboratory at InCyte, a board certified cytopathologist conducts a review of the routinely stained urine cytology specimen in parallel with the FISH test to combine the morphologic findings of the case with the genetic findings. This enhances the precision of diagnosis and, we are convinced, contributes significantly to high-quality patient care.

Are there other applications for the FISH test? While the test has been approved by the FDA for monitoring patients with biopsy-proven urothelial malignancy, a powerful and valuable application of the FISH test will be in assisting the cytopathologist in resolving diagnostically difficult cases. It is not uncommon to encounter atypical cells in a patient who is having initial screening cytology performed for hematuria or other urinary complaints. Sources of non-specific atypia include urolithiasis (stones), infections and inflammation, and degenerative changes in benign cells that mimic dysplasia. While clinically significant dysplasia will show aneuploidy over 90% of the time, benign mimics are virtually always diploid (normal).

The practice protocol we currently follow is to conduct the testing "per office request." We strongly recommend, however, the use of urine FISH testing in monitoring all urothelial carcinoma patients. For further information, please contact Drs. de Tar, Bassler or Hoak at InCyte Pathology. Our marketing representative, Molly Preston, is also available to provide you with more information. ▲

### References

- Halling KC King W Sokolova I Meyer RG. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *J Urol* 2000, 164: 1768-1775.
- Skacel M Fahmy M Brainard JA et al. Multitarget fluorescence in situ hybridization assay detects transitional cell carcinoma in the majority of patients with bladder cancer and atypical or negative urine cytology. *J Urol* 2003, 169: 2101-2105.



## PROFILE

### Geraldine Peterdy, MD

Dr. Geraldine Peterdy joined InCyte Pathology in February, 2003.

Born in Stockport, England, Dr. Peterdy received her MD from University College Cork in Cork, Ireland, and trained at Cork University Hospital in Cork and at Orlando Regional Medical Center in Orlando, Florida. She completed surgical pathology and dermatopathology fellowships in St. Louis, Missouri, at the Washington University Medical Center. Dr. Peterdy is board certified in anatomic pathology and in September 2003, received board certification in dermatopathology.

Dr. Peterdy and her husband are parents of four children Anna (5), Nicola (4), Kilian (2) and Owen (10 weeks). Although having a large young family takes up most of Dr. Peterdy's time, she enjoys skiing, traveling and housework!

InCyte is pleased to have Dr. Peterdy on staff to provide expertise in dermatopathology to our clients and patients.



# InCyte Pathology Now Provides Bladder Cancer Markers for Diagnosis on Bladder Biopsies

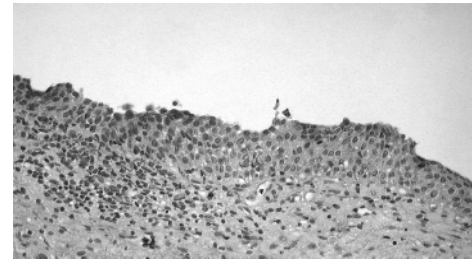
By David Hoak, MD

After malignant cells are identified by urine cytology and sometimes by aneuploidy evaluation (UroVysion™), cystoscopy with biopsy is performed. If the lesion is papillary, it can be relatively easy to locate and biopsy. If the lesion is flat, i.e., transitional cell carcinoma in situ, it can be difficult to tell where to biopsy, and subsequent biopsies may be random in nature. If the biopsy is negative for carcinoma, then additional biopsies or repeat cytology must be performed. Sometimes in bladder biopsies it can be difficult to distinguish carcinoma in situ (CIS) from reactive atypia, particularly in the presence of cystitis. Malignant urothelium can frequently shed cells, leaving only few atypical cells on the surface of the biopsies (denuding cystitis). InCyte Pathology now provides adjunctive immunohistochemical markers for bladder biopsies that aid in the diagnosis and prognosis of transitional cell carcinoma.

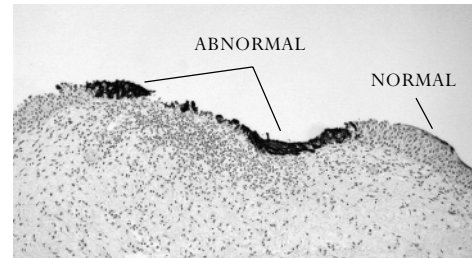
**Ki-67 (MiB-1)** Ki-67 is a monoclonal antibody to an antigen expressed only in proliferating or dividing cells. In normal urothelium, less than 5% of urothelial cells show weak to mild expression of Ki-67. In carcinoma, greater than 10% and usually greater than 50% of nuclei are positive for Ki-67.

**P53** P53 has been called the “guardian of the genome” and plays a central role in regulating growth and proliferation. Since it acts to prevent abnormal proliferation, it acts as an “emergency brake” in the cell cycle, allowing damaged DNA to be repaired. Thus, it is an important tumor preventor or suppressor. Over 50% of human tumors have mutations in P53 and most bladder cancers have P53 mutations. Normally, P53 is rapidly broken down in the cell so that it is hard to show by immunohistochemistry. However, when there is a mutation of P53, its degradation is delayed and it accumulates in the nucleus and can be seen by immunohistochemistry. In normal bladder epithelium, only a few cells will express P53, but in carcinoma, most cells strongly express P53. If greater than 20% of nuclei express P53, this is considered abnormal.

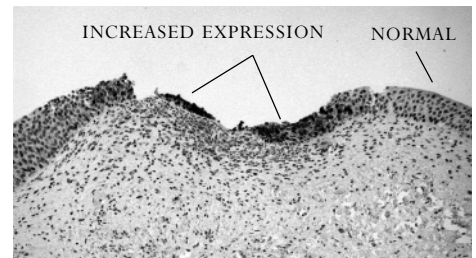
**Cytokeratin 20 (CK 20)** Cytokeratin 20 is a structural protein within cells. It is normally expressed in colon mucosa and the superficial umbrella cells of the bladder. However, in dysplastic or malignant bladder epithelium it is strongly expressed by all cells. ▲



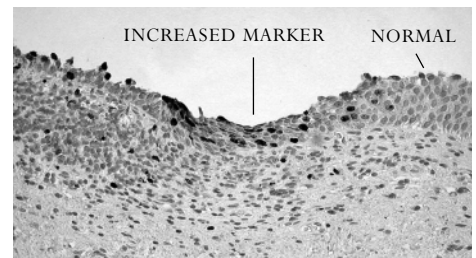
Bladder biopsy from a patient with suspicious urine cytology



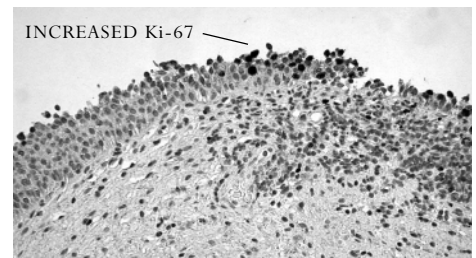
CK-20 abnormal full thickness positivity



P53 increased expression in area of dysplasia



Ki-67 proliferation marker increased in area of dysplasia



Ki-67 higher magnification

# Urine Cytology is Complementary to Cystoscopy

By Thomas J. Bassler, MD

Urine cytology is a common source of confusion and frustration to both urologists and pathologists. It is often viewed as insensitive and non-specific and, thus, unreliable. One perceived problem for urine cytology is that it is not uncommon to be negative and apparently normal in the face of obvious papillary lesions on cystoscopy. On the other hand, urine cytology may be abnormal (positive or suspicious) when there are no visible lesions on cystoscopy.

I believe this confusion is secondary to a fundamental misunderstanding of urine cytology. This misunderstanding can create an improper usage and unrealistic expectations of cytology. To understand this, one must review the basic histological classification of urothelial neoplasms.

The two types of urothelial neoplasms, papillary and flat, have distinct biology and clinical implications. Papillary neoplasms are usually low-grade and are less aggressive, having a 75% rate of recurrence and less than a 5% rate of progression to a higher-grade papillary neoplasm. In contrast, flat carcinoma in situ (CIS) is always high-grade, also has a 75% risk of recurrence, but the risk of progression is up to 50%.

Flat low-grade lesions and nodular low-grade lesions do not exist. Low-grade papillary lesions must be papillary in configuration to be recognized as neoplastic. Neoplastic papillae are lined by near-normal appearing cells with little to no atypia. The amount of atypia is well within the range of benign reactive changes. Since they show minimal cytologic changes by definition, they should not be found in a test which relies on cytologic features for diagnosis. In other words, urine cytology should actually be expected to be negative in a patient with a low-grade lesion. For this reason, unfortunately, cytology has a reputation of being unreliable in finding bladder cancer, but it is really only the low-grade lesions that cytology does not detect.

If a pathologist strives to diagnose low-grade lesions, she/he will end up calling many benign reactive urine cytologies as “positive” or “suspicious.” As discussed, the low-grade level of atypia in a papillary lesion is well within the spectrum of benign reactive changes. Consequently, the pathologist will falsely call many benign reactive urines abnormal, resulting in the complaint that urine cytology has many false positive diagnoses and is, therefore, not adequately specific. Making matters worse, in spite of all the false positives, most of the low-grade papillary lesions will still be missed.

## *Urine cytology and cystoscopy are complementary tests.*

When used appropriately, cytology complements cystoscopy. Cystoscopy and cytology target two different types of lesions – cystoscopy finds papillary lesions while cytology finds flat lesions. In cases of a negative cytology followed by a cystoscopy showing a papillary lesion, the cystoscopist can anticipate that the papillary lesion is most likely a low-grade lesion. On the other hand, in cases where a positive cytology is followed by a negative cystoscopy, a careful examination, where the cystoscopist takes random biopsies evaluating for CIS and the possibility of lesions in the ureters or renal pelvis, should be considered. Importantly, if the cytology is positive for a high-grade lesion and the biopsy shows a low-grade papillary lesion, a concomitant CIS lesion or high-grade tumor in the upper urinary tract should be ruled out.

Voided urine is an excellent diagnostic medium for identifying high-grade tumors, notably CIS of the bladder, ureters, and renal pelvis. This

method is also very efficient in the identification of invasive tumors. As reported,<sup>2</sup> three sequentially voided urine morning samples, appropriately processed and interpreted, will identify virtually all CIS and other high-grade tumors, including invasive cancers. The high-grade tumor that will not be identified with cytology usually sheds only necrotic debris that obscures the presence of cancer cells in the cytologic evaluation.

To summarize, cytology is an excellent tool for detecting high-grade neoplasms but not for low-grade tumors. Aggressive neoplasms can be and are detected by urine cytology. On the other hand, CIS may not be identified with cystoscopy. If only cystoscopy is used to investigate patients with signs and symptoms of bladder cancer, some cases of CIS may be missed. Urine cytology and cystoscopy are, therefore, complementary tests and both should be performed when there are signs or symptoms of a bladder neoplasm. ▲

## References

1. Koss, L.G.: Diagnostic Cytology of the Urinary Tract with Histopathologic and Clinical Correlations. Lippincott-Raven, 1996, 125.
2. Koss, L.G., et al, Diagnostic value of cytology of voided urine. Acta Cytol. 29, 810-816, 1985.

**Providing correct patient demographics, clinical history, and billing information saves office staff time and helps ensure accurate, reliable, and timely results.**

## Correlation of Cystoscopic and Cytologic Findings<sup>1</sup>

Cystoscopic Findings	Cytologic Diagnosis	Correlation
Visible papillary lesion	Negative	Low-grade papillary neoplasm
Visible papillary lesion	Positive or suspicious	a) High-grade tumor with or without CIS b) Low-grade tumor with CIS
No visible tumor	Suspicious or positive	CIS or tumor in upper urinary tract

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# PATHWAYS

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**PathWays** has items of interest for office personnel and assistants as well as for physicians, nurse practitioners, nurses and physician assistants. We recommend that, upon completion of circulation, your copy of **PathWays** be filed in the InCyte Pathology *Anatomic Pathology Services Manual* for future reference.

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