Color Power for Cancer Management

A new staining method could offer a noninvasive alternative to cystoscopy when monitoring bladder cancer.

By Yael Glickman

With over 400,000 cases diagnosed annually, urinary bladder cancer is the most common malignancy of the urinary system. At any one time, there are 2.7 million people with a history of bladder cancer worldwide (1) – and with an up to 80 percent risk of recurrence, these people require lifelong surveillance, making bladder cancer one of the most expensive malignancies to manage (2).

As with most cancers, early detection is the key to improving outcomes – the five year survival rate decreases dramatically by over 95 percent for flat tumors, to five percent for distant ones (3). Today, cystoscopy remains the standard for diagnosis and monitoring, despite its invasiveness and high cost; patients with a history of bladder cancer undergo up to 14 cystoscopy exams in the five years following diagnosis. It therefore, goes without saying that there is a real demand for a noninvasive method, but current alternatives are less than ideal.

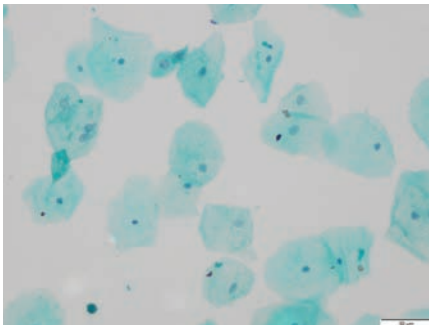
We have recently developed an approach which we believe enhances current noninvasive methods including urine cytology– that could greatly increase test sensitivity and offer a noninvasive alternative.

Urine cytology, 70 years on

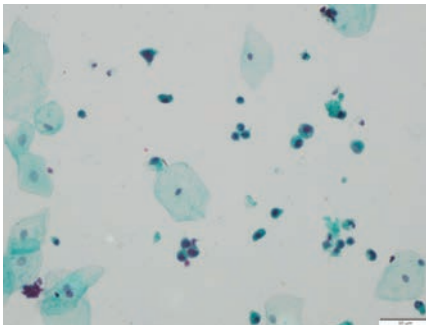
Currently, urine cytology is the established noninvasive method for detecting and monitoring bladder cancer. Following a report by Lambl et al. in 1856 describing the first use of exfoliative cytology for detection of cancer cells in urine, Papanicolaou and Marshall officially introduced urine cytology in the mid nineteen-forties. Since this landmark development, great advances in the preparation of urine specimens have been made, addressing the challenges caused by the small number of urothelial cells in the urine. Sedimentation flasks were rapidly replaced by centrifuges; cyto-centrifuges were later introduced, so too was the membrane-filter method, and more recently, liquid-based technology.

Many studies have reported the high specificity of urine cytology, and its significant clinical value when diagnosing high grade tumors. But the detection of low grade tumors remains an issue – the subtle morphological differences between reactive cell changes and low grade papillary carcinoma, among other factors, makes sensitivity a problem, and urine cytology has limited uses in patient management. This has led to the development of additional methods, including protein-based urinary markers, cytokeratin markers, and fluorescence in situ hybridization (FISH). However, none of these approaches have yet been widely integrated into routine patient management because of high cost, low accuracy, and/or high complexity. As

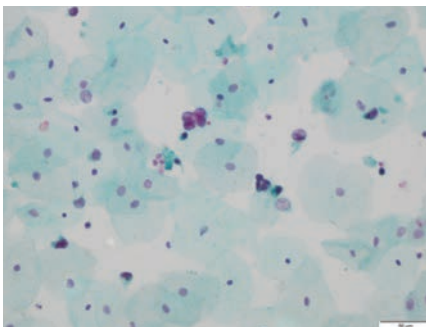
Figure 1. Different staining results found in non-cancerous and cancerous urothelial cells.



Urine, negative. 40x
Normal urothelial cells featuring green nuclei.



Urine, low grade. 40x
Low grade urothelial carcinoma cells with purple nuclei and high nucleus/cytoplasm ratio.



Urine, high grade. 40x
High grade urothelial carcinoma cells with purple nuclei and for which cytoplasm may not be observed.

At a Glance

- *Current detection methods for bladder cancer are either invasive, or lack sensitivity for low-grade tumors*
- *New biomarkers have been identified but these can be expensive, or require complex testing techniques*
- *I describe a new staining approach that could complement urine cytology by highlighting cancerous cells while preserving cell morphology*
- *Noninvasive diagnosis and management could make screening feasible, particularly when combined with digital pathology methods*

well as the implications for existing patients, this means that bladder cancer screening, which could be beneficial for high-risk populations, is not currently possible, due to the lack of accurate and cost-effective biomarkers. So despite major developments, Papanicolaou's staining procedure is still the most common technique for the microscopic examination of exfoliated tumor cells obtained from urine or bladder washes.

Sophisticated staining

I believe that the new staining platform developed by our team, in the form of a histochemical assay, could address the drawbacks of current methods.

How does it work? Using a proprietary plant extract and three generic dyes, the CellDetect stain colors the nuclei of neoplastic cells reddish-purple, while normal cells are counter-stained with green (Figure 1). The most likely theory is that this difference in color is caused by the change in energy metabolism found in cancer cells, which leads to a rise in cellular pH and a fall in extracellular pH. By also preserving the important morphological characteristics of cells, this technique can improve diagnostic performance and allow results to be obtained faster.

So far, this method has been validated for both cervical and bladder cancer diagnosis (4–6). Proof-of-concept has also been established for prostate and lung cancers, and circulating tumor cells. Using standard processes routinely used in pathology labs, the staining platform is applicable to both cyto-centrifugation

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and liquid-based technologies.

Tests of the staining method yielded promising results – an open-label study assessing the use of the stain found it showed superior sensitivity across all tumor grades when compared with standard urine cytology (4). A blinded, multicenter trial involving over 200 patients with a history of bladder cancer showed 84.7 percent sensitivity for early stage tumors: double the sensitivity of conventional staining (7). I believe this is because the method can further pinpoint suspicious cells, even at an early stage, and this can help cytopathologists to focus their morphological examination on these highlighted cells. As well as improving manual detection of early stage tumors, it is hoped that the staining platform could also have applications in digital pathology.

"The method can further pinpoint suspicious cells, even at an early stage"

Enhancing digital screening

Digital pathology is driven by a need for improved workflow efficiency and reduced costs, and it is now used for gaining second opinions, training, archiving and sharing (8). Recent advances in the implementation of Whole Slide Imaging (WSI), combined with the development of increasingly sophisticated analytical tools, have paved the way for automated quantitative scoring of immunohistochemistry slides – for example, a recent US survey of 174 pathologists and labs using digital pathology found that HER2 scoring was the first use of digitalization, ahead of education and consultation (9). I think this use of automated image-analysis for the quantification of breast cancer biomarkers clearly demonstrates the eagerness of the pathology community to embrace new tools to assist clinical diagnosis.

In the field of cytopathology, automated tools have also seen success, particularly for cervical cancer screening. Two main approaches are currently used: the "primary screening system", which triages negative slides and identifies those that do not require further review; and the "interactive screening system", which pre-selects suspicious fields of view for review by the cytotechnologist. The analytical tools used in both systems, which mainly rely on morphologic changes associated with malignancy, could benefit from additional features that enhance diagnostic power.

The development of robust and accurate algorithms combining color and morphology may also motivate the implementation of further screening platforms. National screening programs already exist for breast, cervical and bowel cancers, and more recently, lung cancer in the US. Since prevention is recognized by the World Health Organization as the most cost-effective long-term strategy for the control of cancer, the development of reliable automated tools may support the creation of more screening programs in the future.

Although the staining method described here could potentially have a role in future screening programs, perhaps its most immediate advantage is its potential to improve manual analysis of urothelial cells. It's clear that more is needed to improve the diagnosis of bladder cancer, and I believe a reliable, noninvasive method for detecting cancerous cells has the potential to become an important component of bladder cancer diagnosis and management.

After studying and training at the Weizmann Institute, Israel, and Tufts University, MA, USA, Yael embarked on a career in the field of medical devices, with a focus on the development of innovative technologies for cancer diagnosis. Recognizing the key role of pathology in cancer diagnosis and management, Yael recently joined the team at Micromedic Technologies, and is involved in the development of a cytopathology staining platform for early cancer detection.

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