Biomarkers for detection and surveillance of bladder cancer

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Abstract

Introduction: Bladder cancer is the fourth most common cancer in men and the ninth most common cancer in women in Canada. Early detection of tumours is essential for improved prognosis and long-term survival. The standard method for detection and surveillance is cystoscopy together with urine cytology. Cystoscopy is relatively sensitive but is expensive and invasive. Urinary cytology is a noninvasive method that has poor sensitivity but high specificity; it is relied on for the detection of carcinoma in situ. Currently, several urinary-based bladder tumour biomarkers with USFDA/Health Canada approval are available commercially, but none have been widely adopted by urologists despite their offering high sensitivity and/or specificity. We present here a review of recent studies evaluating 7 commercial biomarker assays for the detection and/or surveillance of bladder cancer.

Results: Sensitivity and specificity ranges, respectively, for each marker were reported as follows: BTA Stat (Polymedco), 52.5%–78.0% and 69.0%–87.1%; BTA Trak (Polymedco), 51%–100% and 73%–92.5%; cytology, 12.1%–84.6% and 78.0%–100%; hematuria dipstick, 47.0%–92.6% and 51.0%–84.0%; NMP22 Bladder Cancer Test (Matritech), 34.6%–100% and 60.0%–95.0%; NMP22 BladderChek (Matritech), 49.5%–65.0% and 40.0%–89.8%; ImmunoCyt/uCyt+ (DiagnoCure), 63.3%–84.9% and 62.0%–78.1%; ImmunoCyt/uCyt+ and cytology, 81.0%–89.3% and 61.0%–77.7%; and UroVysion (Abbott Molecular)/florescence in situ hybridization, 68.6%–100% and 65.0%–96.0%.

Conclusion: We find that no currently available bladder cancer urinary marker is sensitive enough to eliminate the need for cystoscopy. In addition, cytology remains integral to the detection of occult cancer. However, owing to their relatively high sensitivities, these markers may be used to extend the period between cystoscopies in the surveillance of patients with transitional cell carcinoma. Further study is required to determine which markers, alone or in panel, would best accomplish this.

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Bladder cancer is the fourth most common cancer in men and the ninth most common cancer in women in Canada.[1] The vast majority of patients with newly diagnosed bladder cancers have superficial, low-grade neoplasms that are associated with an excellent prognosis. However, these tumours have a 30% to 70% recurrence rate and may progress to invasive cancers in 10% to 30% of patients; progression greatly increases the risk of metastasis and subsequent mortality.[2–4] For this reason, the early detection of bladder tumours is essential for improved patient prognosis and long-term survival.

Cytology and cystoscopy have been used as detection tests for patients suspicious for bladder cancer or for the surveillance of patients at risk of tumour recurrence. Cystoscopy is highly sensitive for most tumours but has some practical limitations. It may fail to identify smaller, flat tumours such as carcinoma in situ. Also, despite the technical advances in cystoscopes, the procedure is often perceived as invasive and a source of patient anxiety.[5–7] There is also a significant financial cost related to frequent cystoscopic monitoring, in terms of health care resources and patient time. Conversely, urinary cytology is noninvasive and highly specific but has poor sensitivity for low-grade, well-differentiated lesions. Thus it cannot be used to replace (or prolong the intervals between) cystocopy and is used, rather, as an adjunct to help detect occult tumours.

Because cystoscopies are invasive and because cytology has poor sensitivity, noninvasive biomarkers have been sought as alternatives to cystoscopy and cytology for the detection and surveillance of bladder cancer. An ideal test for the detection of bladder tumours should be objective, accurate, rapid and easy to administer; moreover, it should offer high sensitivity and specificity. Whereas sensitivity is defined as the ability of a test to detect disease, specificity is defined as the ability to rule out disease. The positive and negative predictive values (PPV and NPV) are directly related to the sensitivity and specificity and the prevalence of the disease in the defined population (Box 1).

More than 20 known urine-based biomarkers with high sensitivity and/or specificity have been identified in the literature, and among these, 7 are available commercially with

Health Canada approval for the detection and/or monitoring of bladder cancer. Although none of these tests have proven to be powerful enough to replace cystoscopy, they are all more sensitive than cytology.[8–10] Their high sensitivity and high NPV suggest that they could be used as part of a surveillance regimen to increase the interval between cystoscopies. Despite this, urologists have been slow to adopt the use of these markers as an adjunct to existing surveillance and detection strategies. Reasons for this include the following: most of these markers are less specific than cytology, resulting in more false positives; some markers are unable to differentiate between urothelial malignancy and inflammation or other benign urologic conditions, again leading to false positives; large population studies have yet to be performed to evaluate these markers; and there are no standard protocols, cut-off values or scoring criteria for tests performed at different centres, meaning that studies by different groups cannot be readily compared.[11] Still, we present here a brief review of recent studies for each of the urinary biomarker tests that are currently commercially available in Canada.

Hematuria

One of the most common signs of bladder cancer is gross or microscopic hematuria. Hematuria can be easily measured in the office or at home by using a hematuria dipstick assay, a low-cost test strip that can reproducibly measure the presence of blood in urine. It has been shown that 85% of bladder cancer patients have gross or microscopic hematuria.[12,13] However, a wide range of benign inflammatory conditions not related to bladder cancer may also lead to detectable blood in the urine. Thus, although cancer without blood is rare, leading to high sensitivity, fewer than 5% of patients presenting with hematuria actually have bladder cancer, which translates to poor specificity and PPV.[14,15] False-positive results may also have technical or biochemical origins, including test strips exposed to light and/or air, use of expired strips or the presence of oxidizing contaminants such as hypochlorite. The test strips are also sensitive to hemoglobin and myoglobin, and in the case of female patients, a false-positive test may result from lingering menstrual blood.[16,17] Overall sensitivity values from recent studies using hematuria detection as a test for bladder cancer fall in the range of 47.0%–92.6%, and reported specificity ranges from 51.0%–84.0%[12,18–22] (Table 1).

Cytology

Urinary cytology is the direct microscopic investigation of shed urothelial cells. The cells are typically harvested from a fresh-voided urine specimen. Cytology is noninvasive and offers nearly perfect specificity but lacks sensitivity, especially for low-grade tumours. It can be a challenging test to perform and is highly dependent on the skills and experience of a trained cytopathologist. Interobserver variation as well as sample preparation and stability result in a wide range of reported sensitivities across the studies presently under review (12.1%–84.6%)[12,19–41] (Table 1). Halling and colleagues[45] noted that for grade 1, grade 2 and grade 3 bladder tumours, respectively, the grade-per-grade sensitivity of cytology before 1990 was 37%, 75% and 94% and that it decreased to 11%, 31% and 60% after 1990. The suspected reason for the drop in sensitivity is that, before 1990, studies were conducted by pathologists with great expertise in the field of urine cytology, whereas

Box 1. Defining the performance characteristics of urinary bladder cancer

Characteristic	Definition
Sensitivity	[True positve / (true positive + false negative)] × 100
Specificity	[True negative / (true negative + false positive)] × 100
Positive predictive value	[True Positve / (true positive + false positive)] × 100
Negative predictive value	[True negative / (true negative + false negative)] × 100

False negative = no. of patients with disease who scored a negative marker test; false positive = no. of patients without disease who scored a positive marker test; true negative = no. of patients without disease who scored a negative marker test; true positive = no. of patients with disease who scored a positive marker test.
Note: Patients with disease had a positive cystoscopy; patients without disease had a negative cystoscopy.

more recently, cytology has become one of many tests performed by general pathologists lacking direct expertise in urine cytology.[45] Still, cytology remains the gold standard for the detection of occult carcinoma in situ, which largely accounts for its continued widespread use.[31,46]

Table 1. Comparison of cytology and other urine-based markers for the detection and surveillance of bladder cancer

Type of marker and study	Total no. of tests with positive cystoscopy	Sensitivity, %	Total no. of tests with negative cystoscopy	Specificity, %
Cytology				
Abd El Gawad et al.[23]	46	54.3	40	100.0
Babjuk et al.[24]	78	33.3	140	100.0
Bhuiyan et al.[20]	67	40.0	58	95.0
Boman et al.[22]	87	42.0	61	97.0
Grossman et al.[25]	76	15.8	1211	99.2
Grossman et al.[26]	98	12.2	552	96.9
Hautmann et al.[27]	30	73.0	64	79.7
Krause et al.[28]	69	70.0	33	85.0
Kumar et al.[29]	46	41.3	85	96.4
Laudadio et al.[30]	44	34.0	55	93.0
Lodde et al.[31]	101	48.5	176	94.9
Messing et al.[32]	52	23.0	274	93.0
Mian et al.[33]	298	38.9	1588	99.4
Moonen et al.[34]	29	42.9	77	93.2
Parekattil et al.[19]	27	66.7	226	81.0
Saad et al.[21]	52	48.0	68	87.0
Sarosdy et al.[35]	51	38.0	N/A	N/A
Schroeder et al.[12]	34	70.6	58	81.0
Shariat et al.[36]	898	70.0	N/A	N/A
Sun Y et al.[37]	151	36.4	100	100.0
Tetu et al.[38]	136	29.0	734	98.0
Toma et al.[39]	42	84.6	84	80.0
Tritschler et al.[40]	40	44.0	60	78.0
Tsui et al.[41]	135	23.5	50	96.9
Hematuria Dipstick				
Bhuiyan et al.[20]	70	47.0	163	82.0
Boman et al.[22]	87	47.0	38	84.0
Halling et al.[18]	73	74.0	80	51.0
Parekattil et al.[19]	27	92.6	226	51.8
Saad et al.[21]	52	50.0	68	54.0
Schroeder et al.[12]	59	50.8	78	78.2
BTA Stat				
Babjuk et al.[24]	78	74.4	140	87.1
Bhuiyan et al.[20]	70	76.0	163	69.0
Boman et al.[22]	88	78.0	61	73.0
Halling et al.[18]	72	78.0	80	74.0
Saad et al.[21]	52	63.0	68	82.0
Schroeder et al.[12]	59	52.5	77	76.7
Sun Y et al.[37]	151	76.8	100	87.0
Toma et al.[39]	42	66.6	84	78.2

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Bladder tumour antigen test

The bladder tumour antigen test (BTA) is a test based on antibodies that detects elevated levels of the complement factor H–related protein (CFHrp) in voided urine (product insert). This protein is similar in structure and function to human complement factor H (FH) and is released by normal cells

Table 1. continued

Type of marker and study	Total no. of tests with positive cystoscopy	Sensitivity, %	Total no. of tests with negative cystoscopy	Specificity, %
BTA Trak				
Abd El Gawad et al.[23]	46	100.0	40	92.5
Babjuk et al.[24]	78	75.6	140	72.6
Tsui et al.[41]	135	51.0	50	73.0
ImmunoCyt / uCyt+				
Hautmann et al.[27]	30	63.3 / 83.3	64	75.0 / 85.9
Lodde et al.[31]	101	84.2 / 86.1	176	78.1 / 77.7
Messing et al.[32]	52	81.0 / 81.0	274	75.0 / 73.0
Mian et al.[33]	298	84.9 / 89.3	1588	72.5 / 72.5
Têtu et al.[38]	100	74.0 / 84.0	453	62.0 / 61.0
Toma et al.[39]	42	78.3 / 89.1	84	73.8 / 72.5
NMP22 Bladder Cancer Test				
Abd El Gawad et al.[23]	46	91.3	40	87.5
Bhuiyan et al.[20]	67	61.0	162	60.0
Boman et al.[22]	89	75.0	60	73.0
Chang et al.[42]	28	50.0	303	82.8
Parekattil et al.[19]	27	70.4	225	45.6
Saad et al.[21]	52	81.0	68	87.0
Shariat et al.[36]	898	73.3	N/A	N/A
Shariat et al.[11]	1045	73.5	1826	81.0
Sun Y et al.[37]	151	77.5	100	81.0
Toma et al.[39]	42	68.5	84	65.2
Tsui et al.[41]	135	77.5	50	73.5
NMP22 BladderChek				
Grossman et al.[25]	76	55.7	1252	85.7
Grossman et al.[26]	98	49.5	565	87.3
Kumar et al.[29]	46	84.8	85	77.6
Moonen et al.[34]	29	57.1	77	89.8
Tritschler et al.[40]	40	65.0	60	40.0
UroVysion/ florescence in situ hybridization				
Bollmann et al.[43]	13	100.0	34	N/A
Constantinou et al.[44]	25	96.0	N/A	N/A
Halling et al.[18]	73	81.0	80	96.0
Krause et al.[28]	69	83.0	33	71.0
Laudadio et al.[30]	44	73.0	55	65.0
Sarosdy et al.[35]	51	68.6	422	77.7
Toma et al.[39]	42	68.8	84	89.1

to protect them from being targeted by the body's own immune system. CFHrp has been shown to be released by tumour cells in culture, and may play an in vivo role in helping tumour cells to evade attack by the host's immune defenses.[47] There are 2 BTA-based tests currently available, the qualitative, point-of-care BTA Stat (Polymedco) and BTA Trak (Polymedco), which is an enzyme-linked immunosorbent type of qualitative assay. Reported sensitivity ranges for these tests are 52.5%–78.0% for BTA Stat and 51.0%–100% for BTA Trak (Table 1). BTA Stat specificity has been reported in the range of 69.0%–87.1%, and the range for BTA Trak is 73.0%–92.5%.[12,18,20–24,37,39] Both tests are more sensitive but less specific than cytology, owing to the fact that they are affected by benign inflammatory conditions, calculi, foreign bodies, recent instrumentation, bowel interposition segment or another genitourinary cancer, which can lead to false-positive results.[48] Past bacille Calmette-Guérin (BCG) therapy, recent intravesical instillation and factors including nicotine, caffeine, acetylsalicylic acid and acetaminophen may also result in false-positive BTA scores.[49,50] Both BTA tests are approved for the surveillance of recurrent bladder cancer.

Nuclear matrix protein 22

Nuclear matrix proteins (NMPs) are part of the structural framework of the nucleus and provide support for the nuclear shape.[51] These proteins have also been attributed roles in DNA replication, in ribonucleic acid transcription and in the regulation of gene expression.[52,53] One member of this family, nuclear mitotic apparatus protein (NMP22), is much more prevalent in malignant urothelial cells than in their normal counterparts. Apoptosis is accompanied with a release of NMP22 into the urine, and patients with bladder cancer have a significantly elevated concentration of NMP22, reported to be as high as 25 times that of normal concentrations.[51,54] There are 2 marker tests for bladder cancer that rely on detecting NMP22 in voided urine: the original NMP22 Bladder Cancer Test (Matritech), which is a quantitative, sandwich-type immunoassay, and the NMP22 BladderChek (Matritech), which is a qualitative point-of-care test cartridge containing the NMP22 detecting and reporter antibodies.

Reported sensitivity ranges are 34.6%–100%, and 49.5%–65.0%, for the Bladder Cancer Test and BladderChek assays, respectively (Table 1). Specificity of the Bladder Cancer Test is reported to be 60.0%–95.0%, and specificity of the NMP22 BladderChek is reportedly in the range of 40.0%–89.8%.[11,19–23,25,26,32,34,36,37,39–42] The NMP22 tests are based on the release of NMPs into the urine following apoptosis of the urothelial cells, a process that can also occur as a result of benign conditions. As is the case with the BTA tests, NMP22 levels are raised in patients with urinary tract infections, concurrent urolithiasis, history of bladder interposition, other malignancies, intravesical therapies and even cystoscopy, all of which may contribute to false-positive results.[11,42] Some studies have excluded patients with these confounding factors, which improves the specificity and PPV and contributes to the large variation of reported performance characteristics.

Another consideration relates to the selected cut-off value for test positivity, which varies widely across reported studies for the quantitative assays for both NMP22 and BTA. Although the manufacturer recommends a cut-off of 10 U/mL for the NMP22 Bladder Cancer Test, cut-offs ranging from 3.6 to 27 U/mL have been reported. A high cut-off will lead to fewer false-positive scores at a cost of lower sensitivity. Conversely, sensitivity can be improved by lowering the cut-off, but specificity will be reduced. Some researchers suggest that the test cut-off should be selected according to the needs of the patient population being tested.[11] Although flexibility in the cut-off may allow tailored use of these tests, it makes comparison between studies more difficult. Cut-off variability is not an issue for the point-of-care NMP22 BladderChek and BTA Stat tests, where a fixed cut-off is universally applied. However, Shariat and colleagues[11] suggest that the reduction of all cancer-related factors to a single, arbitrary cut-off value could lead to "spectrum bias." Spectrum bias refers to the fact that, for an arbitrary cut-off such as 10 U/mL, a score of 9.9 U/mL would be classified as a negative test result when such a level may in fact be clinically relevant and indicative of disease. To emphasize this point, an example considered only patients with measured NMP22 levels between 9 and 11 U/mL, with 10 U/mL as cut-off; of 83 patients scoring below 10 U/mL, 19 (23%) had cancer, whereas of 71 patients scoring above 10 U/mL, 37 (52%) did not have

cancer.[11] The fixed cut-off values of the point-of-care tests may lead to easier comparison through universality, but these tests are affected to a greater extent by spectrum bias than their quantitative counterparts. The NMP22 tests have been approved for both the detection of new cancers and the surveillance of recurrent tumours, and the NMP22 BladderChek is the only point-of-care test to receive this distinction.

ImmunoCyt/uCyt+

Whereas the hematuria dipstick, BTA and NMP22 assays detect molecules present in urine, other assays are based on changes occurring at the cellular level. The ImmunoCyt/uCyt+ test (Diagnocure) is an immunocytological fluorescence assay designed to improve the sensitivity of cytology. A cocktail of 3 monoclonal antibodies is used to detect antigens originating specifically from tumours of transitional epithelial cells. The M344 and LDQ10 antibodies are labelled with fluorescein, a green fluorescence, and will recognize a mucin-like antigen located in the urine on exfoliated tumour cells. The 19A211 antibody will recognize the presence of a high molecular weight glycosylated form of carcinoembrionic antigen and is labelled with Texas Red.[55] Cells collected from urine are fixed to a slide and analyzed by a pathologist using a fluorescent microscope equipped with appropriate filters. A test is scored as positive when a single red or green cell is observed, although the manufacturer recommends all positive cells be correlated to morphology. A slide must contain a minimum of 500 cells for a negative score to be valid.[56]

Unlike other urinary markers, ImmunoCyt/uCyt+ is not approved as a stand-alone test but, rather, is only approved for use as a surveillance test in conjunction with cytology, which makes direct comparison with other markers difficult.[26] Overall sensitivity of the combined ImmunoCyt/uCyt+ and cytology assay is reportedly in the range of 81.0%–89.3%, which is an improvement over either test on its own (Table 1). Specificity of the combined assay reaches 61.0%–77.7%, which is less than that offered by cytology alone.[27,31–33,38,39] The ImmunoCyt/uCyt+ assay requires technical expertise, extensive sample handling and preparation and specialized equipment. However, a person with minimal cytology training and experience can perform the test. In fact, smaller laboratories with the necessary equipment have reported results equivalent to those of larger, dedicated laboratory facilities.[31] In general, studies suggest that ImmunoCyt/uCyt+ has superior sensitivity over cytology for pathological stage Ta-T2 and grade 1–2 tumours and similar or better sensitivity for grade 3 tumours and carcinoma in situ.[31,32,38] Unlike the molecular-based tests, ImmunoCyt/uCyt+ is relatively unaffected by benign conditions and instillation therapy.[57] Still, this test, like cytology, remains subjective and depends in part on the technician. Observer experience, specimen stability and handling and differences in sample size may explain the variation in reported ImmunoCyt/uCyt+ sensitivity.[27]

UroVysion

Another fluorescence-based cellular assay is the UroVysion assay (Abbott Molecular), which uses fluorescence in situ hybridization (FISH) to visually inspect chromosome copy numbers and specific DNA sequences directly in the cell nucleus.[28,58] Genetic alterations resulting in bladder cancer are most frequently found in chromosomes 1, 3, 5, 7, 9, 11 and 17.[28,59–61] The UroVysion assay uses a multitarget set of probes that hybridize to the centromeres of chromosomes 3, 7 and 17 and to the 9p21 locus of chromosome 9. The loss of the 9p21 locus, site of the p16 tumour suppressor gene, is the earliest and most frequent genetic aberration in bladder cancer.[62]

Recent published series on the FISH assay have demonstrated results in the range of 68.6%–100% and 65.0%–96.0% for sensitivity and specificity, respectively[18,28,30,35,39,43,44,58,62,63] (Table 1). Thus the UroVysion assay, which has received FDA approval for the detection of recurrent bladder cancer, is more sensitive than urinary cytology but provides a similar, or slightly lower, specificity. In all studies, sensitivity increased with higher cancer grade. The FISH method examines changes at the nuclear level of the cell and is therefore unaffected by any benign conditions of the patient; it has been approved for use in patients with hematuria.[30,35] Kipp and colleagues[64] demonstrated that the UroVysion assay might be used to monitor the effects of BCG and other intravesicle therapies where a positive FISH assay results in a high likelihood for the progression to muscle-invasive disease.[64] Variation in scoring criteria, the use of

voided urine as opposed to bladder-wash urine, observer experience and sample stability and handling contribute to the variation of reported performance. Criticism of this method typically focuses on the high cost of the probes and the need for expensive equipment (fluorescent microscopes, specific filters, etc.) and trained technical personnel.[28] The test is not point-of-care, and is time-consuming and not suited to high-throughput screening.

There is an important consideration with regard to false-positive results with the FISH test. Several studies have reported the detection of recurrent tumours within weeks or months after a false-positive test result. Although it is always possible that these tumours were originally overlooked during the original cystoscopy, others suggest that the assays are sensitive enough to detect changes occurring at the cellular level before the development of endoscopically visible lesions. Halling and colleagues[18] have termed the latter phenomenon the "anticipatory positive" result; these authors reported recurrences in 7 of 13 patients within 3–12 months after a false-positive FISH score. Studies with other marker tests have also addressed the predictive value of false-positive results[29,40,65] and include a study by Pode and colleagues,[66] who discussed false-positive results with the BTA Stat test. However, Friedrich and colleagues[67] have argued that false-positive BTA Stat tests can be expected, owing to the influence of many benign conditions, and that cystoscopy will often fail to detect small lesions and carcinoma in situ. Whereas other reports focused strictly on false-positive results, Friedrich and colleagues compared recurrences after false-positive and true-negative scores and determined that there was no significant difference among these groups with use of the BTA Stat, NMP22 Bladder Cancer Test or ImmunoCyt/uCyt+.[67] Nevertheless, it remains advisable to closely monitor patients in the weeks and months after a false-positive test result.[30]

The current surveillance protocol for bladder cancer patients calls for cystoscopy with cytology every 3 months for the first 1–2 years, then every 6 months for 1–5 years and then yearly afterwards, provided no recurrent tumours are detected. This is a very costly endeavour and does not take into consideration the fact that not all patients are at equal risk for tumour recurrence.[31] Although not powerful enough to replace cystoscopy, urinary biomarkers may be useful in extending the time between cystoscopic examinations because of their relatively high sensitivity and high NPV (especially for higher-grade lesions). In a recent study evaluating the ImmunoCyt/uCyt+ test, Lodde and colleagues[31] ranked patients as low-, intermediate- or high-risk for tumour recurrence according to tumour size and initial stage and grade of the malignancy. Although 30 of 84 low-risk patients developed recurrent tumours within 3–96 months, all lesions were stage pTaG1, and there were no cases of progression into the muscle. These authors concluded that after resection and an initial cystoscopy at 3 months, a follow-up protocol of yearly cystoscopy and testing with ImmunoCyt/uCyt+ and cytology every 6 months could result in a significant cost savings without placing the patient at a greater risk for disease progression.[31]

Other recent studies have considered the economic impact of the use of urinary biomarkers, particularly in the screening of patients for bladder cancer. Such considerations are directly dependent on the incidence of the disease as well as on the sensitivity and specificity of the marker, in addition to the overall costs of each test.[7] Lotan and colleagues[6] reported that screening of all men aged 55 years or older was significantly more costly on a per-cancer basis than screening only in a high-risk group (more than US$400 000 v. $3130). Svatek and colleagues[7] suggest that marker screening of a high-risk group could be cost effective, although additional studies in an asymptomatic cohort are required before screening of a general population is recommended.

Although no ideal marker currently exists, a distinction should be made between the usefulness of a marker for the detection of *de novo* bladder tumours as compared with the monitoring of recurrent tumours in bladder cancer patients. For the detection of new tumours (e.g., in a screening population), a marker must have a high sensitivity for all tumours, even at the expense of a lower specificity. In the surveillance or monitoring setting, delayed diagnosis of a low-grade recurrent lesion will be unlikely to affect the patient's prognosis, and as such, the sensitivity for high-grade lesions would be more important. A test with a high sensitivity for high-grade tumours would result in a high NPV (for high-grade tumours), and this would be useful in prolonging the time interval between cystoscopies.

Future directions

In addition to the currently available markers, the search for more sensitive and specific biomarkers of cancer is ongoing. Promising results have been reported for markers that include telomerase, survivin, aurora-A and others, all of which remain at the experimental stage. While one or more of these may yet prove to be an improvement over the currently available markers, many believe that no single marker will be powerful enough to replace cystoscopy. Instead, the desired prognostic or diagnostic test may involve the use of several less sensitive and specific markers combined together in a panel.[68,69]

The creation of these biomarker panels has entered the realm of possibility as a result of the completion of the Human Genome Project, advances in high-throughput technology and the development and integration of computational biology.[69] Although it was initially believed that microarray technology would help identify overexpressed genes as biomarkers, it now seems apparent that a better approach is through analysis at the level of the proteome.[68] Several proteomics-based methods have been described for biomarker discovery, including 2-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and various mass spectrometry (MS) techniques. Several studies used 2D-PAGE to discover 4 proteins, A-FABP, GST-μ, PGDH and keratin 13, which are expressed in normal urothelium and low-grade transitional cell carcinoma (TCC), but not in high-grade TCC.[70–72] Surface-enhanced laser desorption ionization time-of-flight (SELDI-TOF) MS is a low-resolution technique that can provide reproducible protein profiles generated from crude biological fluids such as serum and urine.[73] Despite some recent criticism regarding certain technological aspects, the SELDI-TOF method has been applied toward biomarker discovery in prostate, ovarian, breast and other cancers.[68,74] Using SELDI-TOF MS to analyze urine protein profiles from patients with TCC, Vlahou and colleagues[75] discovered a combination of protein biomarkers and clusters with 77% sensitivity and 66% specificity for bladder cancer. More recently, advances in liquid chromatography-tandem MS technologies, which permit direct peptide sequencing, are beginning to emerge as the premiere method for biomarker identification and validation.

In conclusion, none of the currently available bladder cancer urinary markers are sensitive enough to eliminate the need for cystoscopy. In addition, cytology remains integral in the detection of occult cancer. However, owing to their relatively high sensitivities, these markers may be used to extend the period between cystoscopies in the surveillance of patients with TCC. Further study is required to determine which markers, alone, or in panel, would best accomplish this.

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