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Seminar article

Urine markers for detection and surveillance of bladder cancer

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Abstract

Objectives: Bladder cancer detection and surveillance includes cystoscopy and cytology. Urinary cytology is limited by its low sensitivity for low-grade tumors. Urine markers have been extensively studied to help improve the diagnosis of bladder cancer with the goal of complementing or even replacing cystoscopy. However, to date, no marker has reached widespread use owing to insufficient evidence for clinical benefit.

Material and methods: Pubmed/Medline search was conducted to identify original articles, review articles, and editorials regarding urine-based biomarkers for screening, early detection, and surveillance of urothelial carcinoma of the bladder. Searches were limited to the English language, with a time frame of 2000 to 2013. Keywords included urothelial carcinoma, bladder cancer, transitional cell carcinoma, biomarker, marker, urine, diagnosis, recurrence, and progression.

Results: Although several urinary markers have shown higher sensitivity compared with cytology, it remains insufficient to replace cystoscopy. Moreover, most markers suffer from lower specificity than cytology. In this review, we aimed to summarize the current knowledge on commercially available and promising investigational urine markers for the detection and surveillance of bladder cancer.

Conclusions: Well-designed protocols and prospective, controlled trials are needed to provide the basis to determine whether integration of biomarkers into clinical decision making will be of value for bladder cancer detection and screening in the future. © 2014 Elsevier Inc. All rights reserved.

Keywords: Urothelial carcinoma; Bladder cancer; Molecular marker; Biomarker; Urine; Detection; Surveillance

Introduction

Bladder cancer (BC), a highly aggressive and heterogeneous disease, is the most common malignancy of the urinary tract [1]. The global incidence of BC was approximately 357,000 cases in 2012 [1]. Its high incidence, coupled with its high propensity to recur pose an enormous socioeconomic problem. At any point in time, it is estimated that 2.7 million people have the diagnosis of BC in Western countries [2]. Most BC (75%–85%) presents as non–muscle invasive BC (NMIBC) at first diagnosis (Ta, T1, and carcinoma in situ (CIS)) [3,4]. Among these NMIBCs, approximately 70% are Ta, 20% are T1, and 10% are CIS lesions [3,4]. Disease recurrence occurs in up to 80% of patients with NMIBC and is the main problem for patients with Ta NMIBC, whereas disease progression occurs in up to 30% of patients and is the main threat to patients with T1 or CIS [3,4]. NMIBC is particularly sensitive to nuances in care, and each intervention changes the biological and clinical behavior of the disease. Therefore, an in-depth understanding of risk factors and management is necessary to ensure optimal evidence-based clinical care for each patient with NMIBC.

Owing to the lack of disease-specific symptoms, diagnosis and follow-up of BC remain a challenge. Cystoscopy, the gold standard for the detection of BC, is invasive and relatively expensive, thus limiting its use. Although new
cystoscopy technologies, such as fluorescence or narrow-band imaging, are emerging, the invasiveness and added costs of these procedures further underscore the need for better, simpler, and cheaper diagnostic tests in the management of patients with BC [5–7]. Voided urine cytology is a highly specific, noninvasive adjunct to cystoscopy. It has good sensitivity for detecting high-grade BC, but its sensitivity for detection of low-grade tumors is only 4% to 31% [8,9]. Furthermore, the performance of cytology is dependent upon the level of expertise of the cytopathologist, it is relatively expensive and it is not readily available in all countries. Thus, a noninvasive, highly sensitive, and specific marker for detecting BC could decrease the morbidity associated with cystoscopy, improve patient quality of life, and decrease costs by substituting a less expensive, noninvasive test for the more expensive endoscopic procedure. The clinical scenarios in which such a test could play a role are in the early diagnosis (voiding symptoms, hematuria, and high-risk populations) of BC and the surveillance of patients with previous occurrence of BC.

In this review, we discuss first these 2 clinical scenarios and then report on the performance of the most known commercially available and investigational urinary markers subdivided into cell and protein markers.

Screening and early diagnosis

BC screening could be an indication for the use of a noninvasive diagnostic test [10]. Although the mortality/incidence ratio is higher for BC than for prostate cancer, the comparatively low incidence of BC in the general population, along with the low mortality from BC because of a high amount of cases with nonfatal tumors, has been an obstacle to the development of effective screening strategies for BC. Nevertheless, data from a few screening trials and theoretical considerations on cost-effectiveness issues have revitalized this discussion recently [11–13]. Screening of well-defined high-risk populations with a disease prevalence comparable to tumor entities accepted for screening (e.g. breast cancer or colorectal cancers) may offer a solution to this problem [14]. A recent study, which incorporated data from the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial used simple decision analytic techniques to identify the best candidates for a screening trial [15]. The authors showed that screening for BC can be optimized by restricting it to a subgroup of patients considered to be at an elevated risk. Using a risk stratification tool improved the detection rates when compared with general population (selected on age) and resulted in approximately 25% of the population being screened to prevent 57 invasive or high-grade BC per 100,000 population (while screening the entire population would prevent only an additional 38 cases). As of now, the main risk factors for BC remain age, gender, smoking history, and intensity, as well as some occupational exposures. Determining whether a population is at sufficient risk to justify screening is as important as developing a diagnostic test.

Surveillance

Surveillance of patients with a history of BC is a key area for the use of new markers. This is largely due to the high prevalence and recurrence rate of the disease [4]. Molecular markers may be able to detect BC before they are visually evident [16,17]. However, this causes a significant problem in defining negative tests. Currently, there is no reliable way of separating false-positive tests from true-positive tests when patients do not present with a visually detectable tumor. Theoretically, in the surveillance setting, a marker could both reduce the number of cystoscopies and detect disease recurrence or progression earlier than the traditional tests.

Protein-based urinary markers

NMP22

Nuclear matrix proteins (NMPs) are part of the structural framework of the nucleus and provide support for the nuclear shape. One member of this family, nuclear mitotic apparatus protein 22 (NMP22), is much more prevalent in malignant urothelial cells than in normal cells [18]. Apoptosis is accompanied with a release of NMP22 into the urine, and patients with BC have a significantly elevated concentration of NMP22 compared with their healthy counterparts [18]. The 2 marker tests for BC detecting NMP22 in urine are the original NMP22 BC test kit (Matritech Inc, Newton, MA), a laboratory-based, quantitative, sandwich-type, microplate, enzyme immunoassay, and the NMP22 BladderChek (Alere), a qualitative point-of-care test cartridge containing NMP22 detection and reporter antibodies. Both are Food and Drug Administration (FDA) approved for use in BC surveillance, and the NMP22 BladderChek test is also a approved diagnostic test for BC for individuals who have symptoms of or are at risk for BC.

The sensitivity of the original NMP22 immunoassay ranges from 47% to 100% and its specificity from 60% to 90%, depending on the cutoff value [18–27]. When compared with cytology, NMP22 has a significantly higher sensitivity for detecting BC. The improvement in sensitivity is primarily due to the detection of low-grade and stage BC [28–30]. Similarly to all markers, there is also a possible effect of marker sensitivity based on whether the marker is used for detection or surveillance. However, this may be related to the fact that tumors are larger at diagnosis and have a more advanced stage than those detected during surveillance [28]. Moreover, in the 2 large prospective multicenter studies, NMP22 BladderChek test had a
sensitivity of 56% and 50% for the detection and surveil-

lance of BC [31,32]. Although the sensitivity in the
surveillance setting was lower, NMP22 had a low sensi-
tivity in both clinical scenarios.

The main disadvantage of current markers is their lower
specificity compared with cytology. Because NMP22 pro-
tein is released from dead and dying urothelial cells, many
benign conditions of the urinary tract (such as stones,
infection, inflammation, and hematuria) carry these proteins
as well, and cystoscopy can cause a false-positive test
result. Indeed, up to 80% of false-positive test results were
due to benign inflammatory or infectious conditions, renal
or bladder calculi, recent history of a foreign body in the
urinary tract, bowel interposition segment, another genito-
urolaryngeal cancer, or an instrumented urinary sample [19].

Finally to evaluate the clinical benefit of NMP22 in the
surveillance of patients with NMIBC and negative cytology,
one recent study used a decision-curve analysis [26]. The
authors found that NMP22 could help in decision making
toward immediate versus delayed cystoscopy depending on
the clinician's risk threshold for conducting a cystoscopy.

**Bladder tumor antigens (BTA)**

Both BTA stat and TRAK assays detect human comple-
ment factor H-related protein and complement factor H.
Human complement factor H-related protein interrupts the
complement cascade and confers a selective growth advant-
age to cancer cells by allowing them to evade the host
immune system [33–36]. Both tests are noninvasive and
approved by the FDA as adjuncts to cystoscopy in the
detection of urothelial cancer, but not as primary diagnostic
tools [37,38]. The BTA stat is an inexpensive, office-based,
single-step, immunochromatographic assay usually per-
formed on voided fresh or refrigerated urine samples
producing results in 5 minutes with minimal training of
personnel [19,28,30,39–51]. The BTA TRAK is a sandwich
immunoassay method, which requires trained laboratory
technologists several hours to complete. In this assay,
antihuman complement factor H-related protein monoclonal
antibody coated onto a 96-well microtiter plate captures its
target in urine [36,37,52].

The reported overall sensitivity and specificity of the
BTA stat test are 57% to 83% and 60% to 92%, respectively
[34,36–38,53,54]. There are several clinical situations in
which either of the BTA tests could prove useful. The first
is as a diagnostic tool for the detection of primary urothelial
carcinoma in subjects with signs and symptoms of BC or in
screening of risk populations. In a meta-analysis of 1,160
subjects, the sensitivity of BTA stat test was 70% and the
specificity was 75%. The sensitivity and specificity of the
BTA TRAK test were 66% and 65%, respectively (n =
829). Thus, the current literature does not support the use of
either BTA test alone for the detection of urothelial
carcinoma.

The second clinical situation, monitoring of patients with
a history of BC for recurrence is the indication for which
the FDA has approved the BTA tests as an adjunct to
cystoscopy [38]. A systematic review reporting on 1,377
patients in whom the BTA stat test was performed and 360
subjects on whom the BTA TRAK test was performed in
the surveillance setting found that the sensitivity was higher
for BTA TRAK than stat (71% vs. 58%, respectively). The
specificity was higher (73%) for 2,084 BTA stat-tested
subjects than for 195 BTA TRAK-tested subjects (66%).
Subset analysis of recurrent tumor stratified by grade
showed lower sensitivities for grade 1 and 2 tumors for
both BTA stat and TRAK (grade 1 = 45% and 55%,
respectively; grade 2 = 60% and 59%, respectively) as
compared with grade 3 tumors (75% and 74%, respectively)
[36]. A trend toward increasing sensitivity and specificity
for overall tumor detection was also noted with advancing
tumor stages [36]. Furthermore, the BTA stat test has been
shown to have a lower sensitivity for detecting recurrent as
opposed to primary tumors; possibly related to the smaller
size of recurrent tumors, BTA TRAK had an increasing
sensitivity and specificity with higher tumor grades and
stages [34].

Both current forms of the BTA test are limited by
conditions producing false-positive results. Because com-
plement factor H is present at high concentrations in blood,
a positive BTA stat or TRAK test will occur when
hematuria is present, regardless of the presence or absence
of urothelial tumor [37,38]. Greater than 80% of false-
positive results in either form of BTA test occur in subjects
with hematuria, dysuria, incontinence, a history of intra-
vesical therapy, ureteral stents or nephrostomy tubes, renal
or bladder calculi, benign inflammatory disease (urinary
tract infections or prostatitis), bowel interpositions, or other
genitourinary cancers (renal or prostate) [19,37,38,40,44].
Although the use of exclusion criteria can improve the
performance of both BTA tests, the signs and symptoms of
benign inflammatory conditions overlap those seen in
subjects with BC. This limits the usefulness of these tests
for discriminating between malignant and nonmalignant
etiologies [19,44].

**UBC tests**

UBC-Rapid and UBC-enzyme-linked immunosorbent
assay (ELISA) tests are immunological assays available
from IDL (IDL Biotech, Borlange, Sweden). Both assays
detect cytokeratin 8 and 18 fragments in urine [55]. As
cytokeratins are intracellular proteins, their detection in
urine is possible only when they are released in urine
following cell death. The UBC-Rapid assay is a qualitative
point-of-care assay wherein cytokeratin 8 and 18 fragments
present in urine react with gold-labeled antibodies forming a
complex [56]. UBC-ELISA is a solid-phase 2-step sand-
wich assay. Levels of UBC in the urine specimens are
calculated from a standard curve. A suggested cutoff limit for UBC-ELISA is 12 µg/l. UBC-ELISA requires trained personnel to perform the ELISA. A meta-analysis of UBC-Rapid based on 3 studies reporting on 623 patients showed sensitivity and specificity of 59% and 86%, respectively. However, it is noteworthy that barring the initial study [56], the 2 other studies reported an overall sensitivity of less than 50% [49,50]. With regard to the UBC-ELISA test, different studies have used different cutoff limits with a range between 0.16 µg/l and 15 µg/l. For these reasons, a meta-analysis of UBC-ELISA results could not be performed. This marker needs extensive evaluation before entering clinical practice.

Survivin

Survivin is an antiapoptotic protein that is a member of the inhibition of apoptosis protein gene family. Survivin levels are elevated in BC, and has been suggested as a promising biomarker for BC [57–59]. The commercially available BioDot assay (Fujirebio Diagnostics, Inc.) for survivin is a technique that is routinely performed in any research laboratory to detect a variety of proteins. In survivin BioDot assay (a dot-blot assay), urine samples are blotted onto a nitrocellulose or Immobilon membrane, along with recombinant survivin protein blotted at various concentrations. Following sequential incubations with a rabbit polyclonal antisurvivin antibody and a horseradish peroxidise-conjugated secondary antibody, the dots are visualized by chemiluminescence. The intensity of the specimens and the standard dots is measured by densitometry, and the amount of survivin in specimens is determined from a standard curve. This assay, however, has not been used recently and the current assays reported in various articles are either quantitative or qualitative reverse transcription polymerase chain reaction assays. In the latter, survivin mRNA expression is detected by agarose gel electrophoresis, followed by ethidium bromide staining.

As the dot-blot assay detects survivin protein and the polymerase chain reaction (PCR) assays detect mRNA expression, the results reported in studies using the dot-blot and PCR assays cannot be used to perform a meta-analysis. Furthermore, in each study the PCR primers used were different, and therefore, no 2 PCR studies are alike. Urinary levels of survivin have been shown to have a high sensitivity (64%–83%) and specificity (88%–93%) for detection of BC [60,61]. Although the results are promising, the lack of assay standardization and cutoff value has to be resolved before possible clinical use.

BLCA-1 and BLCA-4

BLCA-1 and BLCA-4 are nuclear transcription factors present in BC. BLCA-1 is not expressed in nonmalignant urothelium [62], whereas BLCA-4 is expressed in both the tumor and adjacent benign areas of the bladder but not in nonmalignant bladders [63–65]. BLCA-4 is measured in the urine using an ELISA; its reported sensitivity ranges from 89% to 96% and its specificity reaches 100% [64,65]. Similarly, in a small study, BLCA-1 demonstrated good performance with 80% sensitivity and 87% specificity [62]. Tumor grade did not affect their expression. However, up to 19% of patients with spinal cord injuries have elevated BLCA-4 levels [66]. These markers need assay refinement and validation.

Cyfra 21-1

CYFRA 21-1 is a cytokeratin-based assay. Cytokeratins are intermediate filament proteins specific for epithelial cells. CYFRA 21-1 is an ELISA that detects fragments of CK19 with the help of 2 monoclonal antibodies (BM19.21 and KS19.1) in urine. Urinary stones, infection, and previous intravesical treatment with BCG can cause false-positive results [67]. Two studies reported on the added value of CYFRA 21.1 in the surveillance setting. The sensitivity and the specificity were 85% and 82%, respectively [29,68]. To date, the body of evidence for CYFRA 21-1 is limited. Thus, current information at this stage is insufficient for any definite statements on the clinical use of this marker in BC detection or follow-up.

Cell-based assays

uCyt+TM/ImmunocytTM

The uCyt+TM assay, formerly ImmunocytTM, is a commercially available immunocytological assay based upon microscopical detection of tumor-associated cellular antigens in urine-derived urothelial cells by immunofluorescence (Scimedx Inc., Denville, NJ). It combines cytology with an immunofluorescence assay [69]. It detects cellular markers for BC in exfoliated urothelial cells using fluorescent monoclonal antibodies for a high molecular weight form of carcinoembryonic antigen and 2 bladder tumor cell–associated mucins. After staining, the samples are studied for immunofluorescence, examining more than 500 nuclei. In most studies, specimens with ≥1 green or red urothelial cell are considered immunocytologically positive. Assay costs and requirements concerning laboratory equipment, time for specimen processing and reading as well as experience necessary for adequate interpretation of the staining must be considered and thereby restrict the use of this test. Reproducibility, i.e. interobserver variability, is reasonable provided that trained staff with high experience performs reading [70]. Immunocyt has a reported overall sensitivity of 50% to 100% [71–73]. Its specificity has been reported as 69% to 79%, with a higher false-positive rate in patients with benign prostatic hyperplasia or cystitis [72]. It has an improved sensitivity when compared with cytology, especially in low-grade tumors [74]. The test may be helpful as
an adjunct to cystoscopy and should be only used to monitor patients with BC. In general, the Immunocyt assay appears more sensitive but less specific as compared with urine cytology. Side-by-side comparisons with other assays have been reported. However, interpretation is difficult as adequate experience of the investigators is a prerequisite to obtain meaningful results for the Immunocyt assay but is not disclosed in these publications. A recent multicenter study showed that Immunocyt was a strong predictor of the presence of BC in patients with a history of BC who presented with painless hematuria \((n = 1182) [75]\). Moreover, incorporation of Immunocyt into predictive models improved their diagnostic accuracy (predictive accuracy of 90%). Decision-curve analysis revealed that models incorporating Immunocyt achieved the highest net benefit at all threshold probabilities [75].

### UroVysion

UroVysion (Abbott Molecular, Inc, Des Plaines, IL) is a multitarget fluorescence in situ hybridization (FISH) assay that detects aneuploidy in chromosomes 3, 7, and 17 as well as loss of the 9p21 locus of the P16 tumor-suppressor gene. The FDA both for the detection and surveillance settings has approved this test. The FISH test combines assessment of morphologic changes of conventional cytology with molecular DNA changes. Each probe is a fluorescently labeled, single-stranded DNA fragment complementary to specific target sequences of cellular DNA that are denatured to allow hybridization with the probe. A minimum of 25 cells are necessary to make a decision on the test result. If \( \geq 4 \) cells exhibit polysomy of 3, 7, or 17, or \( \geq 12 \) cells exhibit loss of 9p21, the case is considered positive for tumor. However, no uniform criteria exist for a positive UroVysion assay at this time [76]. The UroVysion assay also requires laboratory equipment, time for specimen processing and reading as well as experience for reading, thereby restricting its use to specialized laboratories. The UroVysion assay has been reported to be confounded by a variety of different urological conditions (other tumors, urolithiasis, or inflammatory conditions). It is noteworthy that a broad range of sensitivity and specificity of UroVysion has been reported and may not only reflect patient selection, study design, or tumor prevalence but also technical aspects such as cutoff definitions and experience of laboratory staff performing the FISH. However, in systematic reviews and meta-analyses, sensitivity has been found to exceed 70% and even approach 80% when omitting small and low-grade lesions, with a high specificity of 80% [76]. Moreover, the overall performance of UroVysion assay was better than that of cytology (area under the curve: 87% vs. 63%) [76]. This difference, however, was almost entirely attributable to the difference in diagnosing pTa patients. Side-by-side comparisons with other assays have been reported; however, interpretation is difficult as adequate experience of the investigators is prerequisite to obtain meaningful results for the UroVysion assay. Finally, there is a relatively high rate of false-positive results translating into a relatively low positive predictive value of the test, findings from several studies suggest that the low specificity in follow-up trials may be explained in part as an anticipatory positive result, in which a premalignant change precedes the discovery of a recurrent malignancy [17,77,78]. One study [78] found that 89% of the patients who had a false-positive test had a positive bladder biopsy within 12 months of the test, whereas another found that UroVysion assay preceded tumor recurrence in 85% of patients [77]. Nonetheless, the real role of an anticipatory positive result is still unclear as many patients with NMIBC eventually experience disease recurrence. Another potential role for UroVysion assay is as a reflex test for patients with atypical cytology or equivocal cystoscopic findings. Several prospective studies found a high positive predictive value for UroVysion assay in this setting [79,80].

### DD23

DD23 is a murine monoclonal antibody evaluated with quantitative fluorescence image analysis in exfoliated urothelial cells [81]. When used as a quantitative marker to detect BC, it has a sensitivity and specificity of 85% and 95%, respectively [81]. The DD23 assay test was subsequently developed using an avidin–biotin alkaline phosphatase immunocytochemical procedure [82,83], with a single positive cell considered as a positive test result. In 2 studies in the surveillance setting, sensitivity was 70% to 81% and specificity was 60% [82,83]. Moreover, DD23 was able to enhance the sensitivity of cytology, in particular for low-grade tumors [82,83]. To date, the body of evidence for DD23 is limited and does not allow definite conclusions on its clinical utility.

### Promising markers

Aurora kinase A (AURKA) is a gene encoding a key regulator of mitosis, AURKA is frequently amplified or overexpressed in cancer cells or both, and the level of AURKA amplification is associated with the level of aneuploidy. AURKA gene amplification has been investigated as a biomarker for the detection of BC [84]. The FISH test for the AURKA gene copy number yielded a specificity of 96.6% and sensitivity of 87% [84]. Moreover, a higher degree of gene amplification was associated with increasing tumor grade. These promising preliminary data need additional validation.

Cancer initiation and progression are driven by the accumulation of inherited or acquired DNA alterations. Epigenetic changes are defined as heritable changes in gene function, which do not involve changes in DNA sequence. Analysis of gene methylation has been shown to be feasible from voided urine [85–87]. Several panel of genes have been
investigated as potential biomarkers of BC presence in the diagnosis and surveillance setting [85–87]. Moreover, a panel of genes has been associated with outcomes in NMIBC [88,89]. However, methylation markers for BC diagnosis are still at an early stage compared with the FDA-approved markers. Most of the reported markers have been tested on cohorts that varied greatly between studies. In addition to this, many markers are lacking validation in independent prognostic experiments with predetermined cutoff values.

**Commercial availability**

The FDA-approved tests are as follows:

- The quantitative BTA TRAK and the qualitative point-of-care BTA (bladder tumor antigen) stat test (both by Polymedco Inc., Cortlandt Manor, NY).
- The quantitative immunoassay NMP22 and the qualitative, point-of-care test NMP22 BladderChek (Matritech Inc., Newton, MA).
- The UroVysion Bladder Cancer Kit (Vysis Inc., Downers Grove, IL), a multiple marker FISH test.
- The uCytTM test, an immunocytochemical assay (Scimedx Inc., Denville, NJ).
- With the exception of the uCyt test, which is only cleared for monitoring BC recurrence, all tests are FDA-approved as adjunctive tests for use in the initial diagnosis of BC and surveillance of patients with BC, in conjunction with standard procedures.

**Conclusions**

Urinary cytology is limited by its low sensitivity for low-grade tumors. Urine markers have been extensively studied; however, to date, no marker has reached widespread use. Although several urinary markers have shown higher sensitivity compared with cytology, most suffer from low specificity. Moreover, many of them are currently evaluated in prospective trials. Combination of different markers is promising concept and seems to represent the future. Another limitation is that each setting (screening/early detection and surveillance) suffers from different requirements. Finally, each marker has also to prove its cost-effectiveness.

**References**

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