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Cytology and Urinary Markers for the Diagnosis of Bladder Cancer

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Abstract

Primary detection and follow-up of patients with non–muscle-invasive (NMI) bladder cancer (BC) is done by urethro-cystoscopy (UCS) and, in most cases, cytology. Many urine-based tests have been developed, and in general, these tests have a higher sensitivity than cytology but a lower specificity. In this review, we assessed the value of urine tests for screening, primary detection, and surveillance of NMIBC. Considering the frequency of UCS for follow-up, having markers for recurrent BC would be especially useful. Therefore, we updated our systematic review to include five commonly studied urine markers (BTA stat, NMP22, uCyt + / Immunocyt, FISH UroVysion, and microsatellite analysis) and cytology for surveillance. The sensitivity and/or specificity of cytology and these five markers were more than 5% lower for patients under surveillance compared to the numbers reported in other reviews, confirming that the performance of urine markers and cytology is lower for the detection of recurrent BC than is UCS. Recent data from the first randomized trial to investigate the possibility of lowering UCS frequency with urinary microsatellite analysis showed substantial underestimation of sensitivity and specificity if the urologist was not aware of the urine test outcome. These results question but do not replace UCS as the gold standard for NMIBC surveillance. In conclusion, cytology is still important as an adjunct for the evaluation of patients with hematuria and the surveillance of patients with high-risk NMIBC. Urine markers other than cytology may play a role in future screening studies and the follow-up of patients with low-grade (G1–2) NMIBC.

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1. Introduction

Approximately 380 000 cases of bladder cancer (BC) occur around the world each year [1]. In the western world, BC is the fourth most common cancer in men. Most BCs (75–85%) are non–muscle invasive (NMI) at first diagnosis (pTa, pT1, carcinoma in situ [CIS]) [2]. In NMIBC, around 70% of cases present as pTa, 20% as pT1, and 10% as CIS lesions [3]. Generally, the prognosis for patients diagnosed with NMIBC is good, although 30–80% of cancers will recur, and 1–45% will progress to muscle invasion within 5 yr [2,4]. In other words, NMIBC is a chronic disease with varying oncological outcomes requiring frequent follow-up and repeated treatments, making the cost per patient from diagnosis to death the highest of all cancers [5]. At any point in time, 2.7 million people in the world have a history of BC [6].

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The current standard of care for the primary detection and follow-up of NMIBC consists of urethro-cystoscopy (UCS) as the gold standard and urine cytology as an adjunct, every 3–4 mo for the first 2 yr and at a longer intervals in subsequent years. Imaging of the upper urinary tract is done at primary evaluation and for follow-up purposes in high-risk cases [2]. This approach is invasive and uncomfortable. Even for flexible UCS, the risk of patients' developing a urinary tract infection is around 10%, and the procedure causes discomfort and pain in about one-third of the patients under surveillance [7,8]. A reliable urine marker has the potential to replace and/or complement UCS. For the urological practice, in terms of cost reduction and convenience for patients, markers to detect recurrent disease would be particularly useful. However, urine cytology is hampered by operator dependency and low sensitivity, particularly for low-grade lesions [9,10]. Many new urine-based tests for UC have been developed [10–17]; among these, BTA stat, NMP22, uCyt + /ImmunoCyt, and FISH UroVysion have been approved by the U.S. Food and Drug Administration.

In this educational review, we discuss the definitions used to assess the performance of urine tests (ie, sensitivity, specificity, and positive and negative predictive values). We also discuss the value of urine markers and cytology in three clinical situations, specifying screening, primary detection of BC, and follow-up of NMIBC as separate clinical categories. For the last category, an earlier systematic review [10] on the value of urine markers for recurrent BC is updated for five well-known urine markers (BTA stat, NMP22, uCyt + /Immunocyt, FISH UroVysion, and microsatellite analysis [MA]) and for cytology.

2. Sensitivity, specificity, and predictive values

Sensitivity, specificity, and positive and negative predictive value are the instruments used to assess the value of a given urine test against the gold standard (ie, UCS, confirmed by biopsy in most studies).

The sensitivity of a urine test is defined as the percentage of patients with a positive UCS for whom the urine test is positive (ie, the "true positives," those who tested positive on the urine test divided by those with evidence of disease at UCS). Specificity is defined as the percentage of patients with a negative UCS in whom the test is also negative (ie, the "true negatives," those who tested negative on the urine test divided by those with no evidence of disease at UCS). Positive predictive value (PPV) is defined as the percentage of patients for whom the urine test is positive and the disease is present (ie, true positives divided by those with a positive urine test). Negative predictive value (NPV) is defined as the percentage of patients for whom the test is negative and the disease is absent (ie, true negatives divided by those with a negative urine test). Table 1a describes these four definitions. By definition, sensitivity and specificity remain constant between populations with different numbers of positive and negative events, but predictive values vary between two populations with different incidences of BC. Table 1b shows that NPV is higher and PPV is lower in a population with a low tumor incidence compared to a population with a high tumor incidence. This example illustrates that a given test may be of value only in a certain population and that predictive values are less useful for comparison between studies because tumor incidences in a given population are usually variable [10,11]. For instance, tumor incidence will be much lower in a screen setting than in a setting involving patients under surveillance for a previously diagnosed NMIBC. Comparing studies becomes even more problematic if we consider the fact that many urine tests have different sensitivity and specificity values for different tumor grades [10,11]. To decrease the heterogeneity of urine test outcomes, screening, primary detection of BC, and follow-up of NMIBC should be considered as three separate clinical

Table 1a – Definitions for sensitivity, specificity, and the positive and negative predictive value of a urine test against the gold standard (cystoscopy)

		Urine test result		Sensitivity = A/(A + B)
		Positive	Negative	Specificity = D/(C + D)
Gold standard	Tumor	A	B	PPV = A/(A + C)
	No tumor	C	D	NPV = D/(B + D)

Table 1b – Influence of different tumor incidences on positive and negative predictive values for a urine test with a given sensitivity (75%) and specificity (83%); modified from van der Poel and Debruyne [11]

	Urine test positive	Urine test negative	PPV	NPV
Incidence of tumor (40%)				
Tumor	30	10	30/40 (75%)	50/60 (83%)
No tumor	10	50		
Incidence of tumor (4%)				
Tumor	3	1	3/19 (16%)	80/81 (99%)
No tumor	16	80		

PPV = positive predictive value; NPV = negative predictive value.

categories, and sensitivity and specificity should be used to compare studies on urine tests.

3. Urine markers and screening for bladder cancer

Population-based screening for BC using urine markers has great appeal, and hematuria screening for BC has been shown to be feasible [18,19]. Moreover, hematuria screening may reduce mortality from BC compared to BC diagnosed at standard clinical presentation; study results have suggested that this screening is able to detect high-grade tumors before they become muscle invasive [20]. The design of the two pilot studies on BC screening consisted of home-based repeated hematuria testing; if tests were positive, a urological evaluation including cystoscopy was performed. These studies were performed in male participants aged >60 (Leeds, UK) and >50 (Wisconsin, USA) and included 2356 and 1575 men, respectively [18,19]. Because 16–20% of the participants tested positive for hematuria and only 5–8% of these actually had BC, a very large number of UCSs had to be done, rendering this approach unsuitable for population-based screening [18–20]. A urine test with a higher BC specificity than hematuria testing could be used to precede a UCS and could reduce the number of UCSs needed for effective screening. Such a urine marker would also have to have a high sensitivity, as it would be carried out before UCS. To increase the sensitivity, a good strategy may be to use a combination of markers for a study on screening. Another strategy that could increase the percentage of screen-detected BC would be to select asymptomatic subjects with a history of heavy smoking [15,21,22]. Lotan et al. [21] evaluated the NMP22 point-of-care test in 1502 high-risk subjects. Only 5.7% of the subjects tested positive, and 2 out of 69 (2.9%) UCSs detected BC. In the 1309 followed participants with negative NMP22, 2 developed low-grade NMIBC. Because of the low prevalence of BC, an assessment of the intervention efficacy was not possible [21]. Steiner et al. [22] screened 183 heavy smokers using a hematuria dipstick, NMP22, FISH UroVysion, and cytology. Of these individuals, 75 had at least one positive test and were evaluated. Interestingly, Steiner et al found signs of malignancy in 18 cases, 12 of which were premalignant (showing dysplasia) [22]. The high rate of dysplasia may be explained by the photodynamic (tumor) resection done in this cohort. Follow-up will show if these patients develop BC. In addition, other BC screening studies are ongoing [15,23]. In one of them, urine markers were able to reduce the number of UCSs for screening by 62% (see www.bluproject.org) [23].

Overall, screening for BC with urine markers is in an early phase. Further studies may show if population-based screening is feasible. Using a combination of urine markers will probably be the best strategy. However, *a priori*, two major concerns with regard to the feasibility of BC screening are the relatively low incidence of BC and its short asymptomatic period (BC is virtually never recognized as an incidental finding on autopsy [24]) compared to, for example, prostate cancer.

4. Urine markers in the primary detection of bladder cancer

The clinical value of using a urine marker other than urine cytology seems limited in patients who present with hematuria or other symptoms suggestive of BC. A UCS is an integrated part of the work-up used to detect possible primary BC in these patients. Consequently, cytology or a urine marker is used as an adjunct to UCS and upper tract evaluation, particularly to detect "invisible" CIS. Hence, using a marker with a high specificity is important in preventing unnecessary additional urological diagnostics. Urine cytology is a good example of a urine marker with a high specificity (usually >90%) and a reasonable sensitivity (>60%) for high-grade and CIS lesions [2,12,13].

5. Urine markers in the surveillance of non–muscle-invasive bladder cancer

Considering the large number of follow-up visits often involved in BC cases, markers to detect recurrent disease are particularly useful. A reliable urine marker has the potential to lower the frequency of UCS. We updated our previous systematic review of the literature on the performance (related to both sensitivity and specificity) of urine markers used for surveillance of BC patients [10]. We chose five commonly studied urine markers for this update. Table 2 shows the sensitivity and specificity of these markers and cytology for patients under surveillance. We also performed

Table 2 – Median sensitivity and specificity and number of studies, institutions, and patients for five common urine markers and cytology (data apply to patients under surveillance for bladder cancer)

Marker	Studies/institute (*n*/*n*)	No. pts.	Sensitivity	Range (min–max)	No. pts.	Specificity	Range (min–max)
BTA stat	17/14	1377	58	29–74	2084	73	56–86
NMP22 Elisa	16/14	1018	69	47–100	1325	73	55–98
NMP22 POC	2/2	159	62	50–86	654	86	77–87
uCyt + /ImmunoCyt	8/6	626	77	52–100	2109	74	62–82
FISH UroVysion	6/7	256	65	30–86	311	83	66–95
Microsatellite	7/4	192	73	58–92	884	76	73–100
Cytology	26/20	2213	35	13–75	3322	94	85–100

Pts. = patients; POC = point-of-care.

Table 3 – Median sensitivity per grade (G1-3, WHO 1973) and specificity of the urine markers for patients under surveillance (the same studies as reported in Table 2 were considered)

Marker (number of studies)	No. pts./sensitivity			No. pts./specificity
	G 1	G 2	G 3	
BTA stat (7)	228/45	206/60	208/75	972/79
NMP22 Elisa (4)	111/43	139/58	144/82	357/64
NMP22 POC (1)	38/32	16/44	32/75	565/87
uCyt + /ImmunoCyt (3)	172/79	108/86	113/90	1509/72
FISH UroVysion (3)	52/38	28/51	38/82	169/75
Microsatellite (6)	69/61	53/63	40/92	869/77
Cytology (10)	239/17	274/34	201/58	861/95

Pts. = patients; POC = point-of-care.

a subanalysis on the sensitivity of the urine tests per pathological grade (World Health Organization 1973, G1-3). Table 3 shows the results of this subanalysis. In general, for patients under surveillance, the sensitivity of urine markers is higher but the specificity is lower than urine cytology. The sensitivity increased for every marker by higher grade. Moreover, sensitivity and/or specificity (see Table 2) were more than 5% lower for patients under surveillance compared to the numbers reported in nine reviews [10]. This finding shows that the performance of urine markers and cytology are lower for the detection of recurrent BC compared to studies that do not use this selection criterion. A short overview per urine marker is given below.

5.1. *BTA stat and NMP22 assays*

BTA stat (Alidex Inc., Redmond, WA, USA) is a qualitative assay that can be performed in a few minutes, even before cystoscopy, as a point-of-care (POC) test. It measures the human complement factor H-related protein in urine. NMP22 (Matritech Inc., Newton, MA, USA) is a nuclear matrix protein that is responsible for chromatid regulation and cell separation during replication. It is available as a quantitative enzyme-linked immunosorbent assay (ELISA) or, recently, as a POC test (BladderCheck) with 10 IU/ml as the threshold for a positive result. Compared to the other urine markers, the BTA stat and NMP22 tests have been extensively studied. The update included no new studies on BTA stat, one on the NMP22 ELISA [25], and two on the NMP22 BladderCheck [26,27] (Table 2). Shariat et al. [25] and others found that a lower than 10 IU/ml threshold was better for NMIBC surveillance [10,17,25]. This may also explain the somewhat lower sensitivity and higher specificity of the NMP22 BladderCheck compared to the NMP22 ELISA reported in Table 3. The sensitivity of BTA stat and NMP22 was higher than that of cytology, but the specificity was lower. Benign inflammatory conditions such as hematuria and pyuria may give false positive results for BTA stat and NMP22 in more than 25% of cases [10,15,17]. Although the POC tests give an immediate result and are inexpensive compared to the more labor-intensive tests discussed below [15], many false negative cases for G1–2 (Table 3) will impede their applicability in the reduction of the number of follow-up UCSs.

5.2. *uCyt + /ImmunoCyt, microsatellite analysis, and FISH UroVysion*

The uCyt + /ImmunoCyt test (Diagnocure Inc., Québec, Canada) is an example of immunocytology. uCyt + /ImmunoCyt uses three fluorescent monoclonal antibodies: 19A211, M344, and LDQ10. Our earlier review reported controversial findings on this urine test [10]. A high interobserver variability and a 16% lower sensitivity for recurrent BC were reported [10], based on ±900 cases. Since then, two large studies with more than 1750 tests have been published [28,29]. Both studies revealed a higher sensitivity (81% and 85%) and equal specificity (75% in both) for detection of recurrent BC than reported before (sensitivity: 67%) [10]. The uCyt + /ImmunoCyt test was highly sensitive in G1 (79%) and G2 (86%) lesions (Table 3). In addition, a false positive result predicted short-term recurrence [28]. The results of these two studies suggest that the uCyt + /ImmunoCyt test may be more sensitive for recurrent BC than initially reported. A learning effect may be the reason for these observations. Further validation by other groups is required.

Microsatellites are tiny, highly polymorphic DNA fragments that are frequently found throughout the genome. BC is characterized by frequent loss of heterozygosity (LOH) at several chromosomal locations. MA combines 15 to 20 markers from regions with a high percentage of LOH. This polymerase chain reaction–based method proved very sensitive for low- and high-grade lesions with sensitivities of 67%, 86%, and 93% for recurrent G1, G2, and G3 lesions, respectively, and a specificity of 88% [10]. However, a multicenter prospective clinical trial in low/intermediate (pTa,1; G1,2) NMIBC reported a much lower sensitivity and specificity of 58% and 73%, respectively [30]. Furthermore, 19% of samples failed as a result of technical problems or prolonged storage of urine [30]. Nevertheless, MA could predict a recurrence before cystoscopical evidence in all four studies with extended follow-up [10,30]. Although MA still holds considerable promise, the prospective study showed that MA is not ready for widespread application.

Fluorescence in situ hybridization (FISH) with four multitarget probes to the centromeres of the chromosomes 3, 7, and 17 and to the 9p21 band form the basis of the FISH UroVysion (Vysis/Abbott, Downers Grove, IL, USA) assay. Polysomy or identification of 9p21 loss is considered a BC indicator. The main issue with the assay was the relatively

low specificity of 70% for patients under surveillance [10]. Nonetheless, FISH was capable of predicting an early recurrence in patients with a negative UCS; hence, specificity may have been underestimated in the context of surveillance. Anticipatory-positive urine test results were also found for uCyt + /ImmunoCyt and MA [10]. However, two subsequent studies reported very low sensitivities of 30% [31] and 39% [32] and a specificity >90% compared to urine cytology. The advantages of FISH may be its use in atypical or suspicious cytology [15] and the fact that results of the test are not affected by intravesical BCG therapy [15–17,33]. Further study in patients under surveillance is still indicated for this test.

5.3. *Surveillance of low-risk versus high-risk non–muscle-invasive bladder cancer*

When considering the follow-up of NMIBC, patients with low-grade (G1-2) lesions should be distinguished from patients with high-grade (G3/CIS) disease. High-grade tumors should be detected early in follow-up, and the percentage of tumors missed should be as low as possible. Therefore, the best surveillance strategy for these patients will continue to include frequent UCS and cytology as an adjunct to detect "invisible" disease and as the primary way of detecting BC in patients presenting with hematuria. Specificity is more important than sensitivity in this subset of patients, because the urine marker is used as an adjunct to UCS.

In contrast, for patients with low or intermediate risk of progression [2,4], it may be acceptable to postpone the diagnosis of recurrent low-grade tumors. The first target for a urine marker in this group is the reduction of the number of UCSs needed for follow-up by lowering the frequency of UCS with a urine test. In such an approach, the sensitivity and specificity of a urine marker are both important: a low sensitivity will eventually miss too many tumors, and a low specificity may lead to unnecessary invasive procedures being used to rule out upper urinary tract cancer. The sensitivity of cytology is too low for this purpose [10,26]. Determining what is the best alternative is difficult. As discussed in this review, all the urine markers have advantages and disadvantages. For example, some markers have been investigated in many patients and/or give instant test results (eg, BTAstat, NMP22) but are associated with lower sensitivity and specificity. Other markers (eg, uCyt + /ImmunoCyt, MA, and FISH) have a higher sensitivity and specificity but require more extensive studies for validation and are more expensive and labor intensive.

Recently, the first results of a multicenter, randomized prospective study (ClinicalTrials.gov/NCT-00126958) became available. This Dutch study is the first to investigate the possibility of lowering UCS frequency in a randomized fashion for low- and intermediate-risk NMIBC [30]. In 2 yr of surveillance, five UCSs (at 6, 9, 15, 18, and 21 mo after first resection) were replaced by MA, and only in the case of a positive MA was a UCS performed. The control arm had a UCS every 3 mo. As reported above, overall sensitivity (58%) was disappointing. Interestingly, this study found a sensitivity of 70% in the intervention arm, where the urologist was aware of the MA result, as compared to a much lower sensitivity (29%) in the control arm, where the urologist was not aware of the MA result [30]. In 131 UCSs performed with knowledge of a positive urine test, 42 recurrences were detected. Only 6 recurrences were found for the 120 UCSs without this information ($p < 0.001$). There was also no difference in detection of recurrences when urine test results were negative [34]. It seems that the urologist performing a UCS examines the bladder more carefully if the MA is positive. These data question but do not replace UCS as the gold standard for surveillance. However, the impact of these findings on the performance of urine testing for recurrent BC is profound, and it may explain the wide variation in sensitivity and specificity among different studies assessing urine markers.

Moreover, false positive results of uCyt + /Immunocyt, MA, and FISH tests were not really false positives, as recurrence was found in many cases within 6 mo [10]. This finding further questions the value of UCS as the gold standard. Photodynamic diagnosis by blue-light UCS may detect more BCs and may give a more accurate sensitivity and specificity of urine tests. Recently, Karl et al. [35] investigated 348 patients with a negative white-light UCS and found BC by blue-light UCS in 63 of 77 patients (82%) with positive cytology as opposed to 43 of 271 patients (16%) with negative cytology [35]. The next step may be to test urine markers other than cytology by white- and blue-light UCS.

6. Conclusions

Noninvasive tests for BC have many potential applications. A urine marker may help decrease bother for patients and the cost of follow-up. If we pursue 100% sensitivity and specificity for a urine test, the discussion will be short, because such a marker does not and will not exist. Furthermore, UCS, the gold standard, is far from 100% sensitive and specific. Nevertheless, UCS continues to be the mainstay in BC detection and follow-up. As cytology is highly specific, it is still important as an adjunct for the evaluation of patients with hematuria (in the detection of primary BC) and the surveillance of patients with high-grade NMIBC. Urine markers other than cytology may play a role in future screening studies and in the follow-up of patients with low-grade (G1–2) NMIBC. Recent data from the first randomized trial to investigate the possibility of lowering UCS frequency with urinary MA showed a profound impact of the awareness of the test outcome prior to UCS. The urologist performing a UCS examined the bladder more carefully if he knew the MA was positive, and more recurrent BC was found in this group. Hence, substantial underestimation of sensitivity and specificity occurred if the urologist was not aware of the urine test outcome. These results question but do not replace UCS as the gold standard for NMIBC surveillance.

Conflicts of interest

The authors have nothing to disclose.

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