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Urothelial bladder cancer urinary biomarkers

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ABSTRACT

Urothelial bladder cancer is the fourth most prevalent male malignancy in the United States and also one of the ten most lethal. Superficial or non-muscle-invasive bladder cancer has a high rate of recurrence and can progress to muscle invasive disease. Conventional surveillance requires regular cystoscopy and is used often with urinary cytology. Unfortunately, the cystoscopy procedure is invasive for patients and costly for health care providers. Urinary biomarkers have the potential to improve bladder cancer diagnosis, the efficiency and also the cost-effectiveness of follow up. It may also be possible for urinary biomarkers to help prognosticate particularly for patients with high-grade bladder cancer who may want enhanced assessment of their risk of disease progression. In this review the important historical urinary biomarkers and the newly emerging biomarkers are discussed. As will be presented, although many of the tests have good performance characteristics, unfortunately no single test can fulfill all the roles currently provided by cystoscopy and cytology. It is likely that in the future, urinary biomarker testing will be used selectively in a personalized manner to try and improve prognostication or reduce the necessity for invasive cystoscopy in patients understanding the limits of the test.

Introduction

Urothelial Bladder Cancer (UBC) is the 4th most common malignancy affecting American males and the 8th most lethal(1). Urinary biomarkers have been the subject of great interest in the field of UBC as urinary sediment may contain exfoliated (intact) or fragments of UBC cells, potentially allowing clinicians to screen, diagnose, prognosticate and follow up patients with this disease. In order to understand how biomarkers may fulfill these different roles it is necessary to understand the natural history of this disease. UBC presents as two clinically distinct groups as assessed by stage. the first group (70% of new cases) is non-muscle-invasive bladder cancer (NMIBC) where the disease is limited to the urothelium and submucosa but has not invaded the muscularis propria (detrusor muscle) and the second group (30% of new cases) is muscle invasive bladder cancer (MIBC) where the disease has invaded into the detrusor muscle. Approximately 50% of patients with MIBC will harbour occult metastatic disease and therefore despite radical treatments (cystectomy or radiotherapy), will die of their disease(2). Early detection (screening of asymptomatic) is desirable especially for individuals at risk (smokers and workers in industry with carcinogenic exposure(3)), and this is one role that urinary biomarkers may fulfill.

Patients that present with symptoms or signs suggestive of UBC (macro or microscopic hematuria), the standard evaluation involves an office cystoscopy and cytological examination of the urine for malignant exfoliated cells in addition to upper tract imaging (as urothelial carcinoma can be found in the renal pelvis or ureter). There is interest in using urinary biomarkers to enhance the detection of UBC in this setting.

For patients treated for NMIBC approximately 50% may have a recurrence of their disease and 10 – 15% of patients may progress to MIBC. The majority of cases are of low grade with very low potential of progression to life threatening MIBC. Here the goal is to detect disease recurrence and this is currently being performed by using a combination of cystoscopy and urinary cytology(4). The cost of performing this follow up protocol is high and is responsible for UBC being labelled as the most expensive cancer for health care providers to treat (4). As urine is in contact with the entire urothelium, including the upper-tract, which cannot be reviewed by a cystoscope, a urine-based biomarker for detecting recurrence would be desirable, especially if it could obviate the need for cytology and / or cystoscopy in a cost effective manner.

Patients with “high risk” NMIBC (stage pT1, carcinoma-in-situ, or high grade disease) currently provide the greatest challenge, as clinicians need to follow them to ensure that their disease has not progressed or recurred. Unlike in patients with low-risk NMIBC, where the risk of progression is very low, a urinary biomarker in this setting must have excellent sensitivity, as this disease can be lethal if missed. Within the high grade NMIBC group there is also the option for utilizing a urinary biomarker

that can help define which patients may progress (and therefore be recommended to undergo early cystectomy or more vigorous follow up) from those that will not. The ability of urinary biomarkers to prognosticate disease has been evaluated and will be discussed.

Urinary UBC biomarkers have been reviewed previously (5, 6). The focus of this review is to highlight laboratory based urinary biomarker tests that can help clinicians to screen, diagnose, survey and prognosticate UBCs. Some tests described have been approved by the FDA and are established in clinical practice. In addition to established tests the surge in genomic evaluation in UBC has identified a number of candidate genes and gene regulatory networks that may have a role in UBC biology. Some of the more promising genes will also be discussed.

Protein Assays

Bladder tumour antigen (BTA)

Bladder tumour antigen (BTA) assays aim to detect the human complement factor H-related protein (enables UBC to evade host immune responses) in urine (7). There are two commercially available assays for BTA; BTA STAT™ which is a qualitative point of care test and BTA TRAK™ which is a quantitative sandwich immunoassay reference laboratory test (POLYMEDCO, Washington, US) (8). BTA STAT™ has a reported sensitivity of 57% to 83% and specificity of 60% – 92% (9-13). Thomas *et al*, used ROC analysis and calculated that the optimum BTA TRAK™ level was 14 kilounits/L (14), they used this cut off level and calculated a sensitivity of 66% for diagnosing UBC in a population suspected of harbouring UBC. Including the paper by Thomas *et al*, the reported overall sensitivity ranges from 62% to 91% (14-21). Recent publications have further demonstrated that the presence of hematuria can lead to false positive results with the BTA STAT™ and BTA TRAK™ assays(22-24), given these limitations the FDA have only approved BTA assays in combination with cystoscopy.

Nuclear matrix protein 22

There are two commercially available detection methods for identifying the presence of urinary nuclear mitotic apparatus protein 22 (NMP22), which is more abundant in the urine of patients harbouring UBC. The first test is NMP22® (Alere™, Scarborough, Maine, USA) a laboratory-based, quantitative, sandwich-type, enzyme immunoassay and the second is a qualitative point-of-care test called BladderChek® (Alere™, Scarborough, Maine, USA). Historically the NMP22® has been shown to have a variable sensitivity (47% - 100%) and specificity (60% – 90%) depending on the value assigned for a positive result(9-11, 20, 25-29). False positive results can be seen in any urinary condition that can cause cell death and release of NMP22 such as benign inflammatory conditions, infection or urolithiasis(10) and also in concentrated urine(30). NMP22® and NMP22 BladderChek® have been compared in 100 urine samples collected from patients prior to Trans-Urethral Resection of Bladder

Tumour (TURBT) and 100 normal controls, using the manufacturer ELISA cut-off of 10U/ml. The NMP22® had a sensitivity of 40% and a specificity of 99% where as the BladderChek® was found to have a superior sensitivity of 59% and a specificity of 93%(31). NMP22® has been used to screen an "at risk" population that have had industrial exposure to aromatic amines but the authors did not feel that this test could be used alone for screening this specific population(32). NMP22® has been shown to be a cost effective alternative to urine cytology in the diagnosis of UBC using cystoscopy(33), and it is possible that in certain patients, the NMP22® could be used as an alternative to cystoscopy(34) although the FDA has only approved the test to be used along with cystoscopy.

Urinary UBC test

Fragments of cytokeratins 8 and 18 are the markers targeted by three commercially available tests; two quantitative assays - UBC® ELISA, UBC® IRMA (Immunoradiometric Assay) and UBC® Rapid (one stop qualitative point of care assay) all manufactured by IDL Biotech, Borlange, Sweden (35, 36). Hakenberg *et al*, evaluated the UBC® Elisa (cut off of 12µg/L), UBC® Rapid and cytology prospectively in 181 patients (117 pre TURBT, 43 post TURBT & 47 controls) and found the respective sensitivity and specificity to be 64.4% & 63.6% for UBC® rapid, 46.6% & 86.3% for UBC® ELISA and 70.5% & 79.5% for cytology(37). The UBC® Rapid test was also used to evaluate 180 urine samples from patients with symptoms suggestive of UBC or being followed up post TURBT. In this study 53 patients were found to harbour UBC and the sensitivity of the UBC Rapid test was 66% and specificity was 90%, which outperformed the BTA Stat™ test (sensitivity 53% and specificity 90%)(36). Babjuk *et al*, evaluated cytology, BTA TRAK™ and UBC IRMA® in the urine of patients with a history of low grade NMIBC undergoing surveillance cystoscopy, the reported sensitivities were 19.8%, 53.8% & 12.1% respectively with specificities of 99%, 83.9% & 97.2%(16). UBC does not have the desirable performance characteristics to replace cystoscopy or cytology.

BLCA-1 and BLCA-4

BLCA-1 and BLCA-4 are nuclear matrix proteins. BLCA-1 is not expressed in normal urothelium, while BLCA-4 is expressed in both the tumour and adjacent benign areas of the bladder but not in normal (no history of UBC) bladders(38). BLCA-1 has been evaluated (ELISA) in the urine of patients with UBC and found to have 80% sensitivity and 87% specificity(39). Similarly BCLA-4 has been measured (ELISA cut off value 13 units) in the urine of pre TURBT patients (N=54) and normal controls (N=51) and found to have a sensitivity of 96.4% and a specificity of 100%(40). In the same paper 38 of 202 urine samples from the spinally injured were positive at the BLCA-4 ELISA cut-off. These two markers require validation by another group (in fact a later publication, by the same group evaluating BLCA-4 functionality has recently been retracted(41)) and the lack of published work on these markers in the

last few years, suggests that by themselves, these are unlikely to become established diagnostic tests in their own right.

Cell Based

uCyt+™ / ImmunoCyt™

Urinary cytology is an established adjunct to cystoscopy, which involves examination of exfoliated bladder cancer cells by a trained cytopathologist. Cytology has been shown to have a high sensitivity for detecting high grade UBC, especially carcinoma-in-situ. To try and increase detection of low grade UBC the immunoCyt™ test was developed (42). This commercially available laboratory test, uCyt+™ (formerly ImmunoCyt™) ((Scimedx Corp., Denville, NJ) combines standard urine cytology with immunofluorescence detection of three monoclonal antibodies (M344, LDQ10, and 19A211) which target carcinoembryonic antigen and two tumour associated mucins (43). The test requires the urine to contain a minimum number of exfoliated cells and is dependent on a trained cytopathologist analysing the sample and is therefore expensive. Comploj *et al* (42), reported the results of 7,422 consecutive urine cytology and uCyt+™/ImmunoCyt™ tests, they found a sensitivity of 34.5%, 68.1% & 72.8% for cytology alone, uCyt+™/ImmunoCyt™ and both test combined respectively. The specificity was 97.9%, 72.3% & 71.9% for the same test combinations. It is known that false positive results can be seen in men with benign prostatic hyperplasia or cystitis(44). The sensitivity of uCyt+™/ ImmunoCyt™ is currently too low for this test to replace cystoscopy, however it may have a role for equivocal standard cytology tests(45).

UroVysion

UroVysion® (Abbott Molecular, Inc., Des Plaines, IL) employs fluorescence in-situ hybridization to detect gains in copy number of chromosomes 3,7, and 17 as well as loss of the 9p21, which contains the P16 gene(46). UroVysion® is designed to enhance the normal morphological assessment provided by cytology by assessing molecular changes. A 2008 published meta-analysis comparing UroVysion® to cytology found the sensitivity and specificity of all studies evaluating UroVysion® were 72% (69%-75%) and 83% (82%-85%) respectively, and for cytology the overall sensitivity and specificity was 42% (38%-45%) and 96% (95%-97%)(47). The meta-analysis demonstrated that the superiority of UroVysion® to urine cytology was based on the former tests superior sensitivity for superficial low grade UBC. The cost of UroVysion® is greater than that of urine cytology and requires specialised laboratory testing, it is therefore unlikely to be a cost effective alternative for units that employ cytology. UroVysion® may be able to help in the cases of equivocal urine cytology(48, 49), however what to do in the event of a positive UroVysion® in the absence of cystoscopic or radiological validation poses other clinical dilemmas(50-52).

Genes / DNA tests

TERT

TERT or hTERT is the abbreviated form of human Telomerase reverse transcriptase and is a catalytic subunit of the telomerase complex. Mutations in the promoter of TERT can lead to increased expression of telomerase enabling malignant cells to continue to renew telomeres and avoid end replication problems. It has been reported that TERT promoter mutations are the most common genetic lesion reported to date in NMIBC, seen in 65%, 68% & 86% (pTA LG, pTa HG & C.I.S respectively) of cases(53). Alloy *et al*, screened two different tumour cohorts and found the TERT promoter mutations present in 70% of tumours of all stages and in a second cohort 80% in NMIBC and 79% in MIBC, using a SNapShot® assay (Applied Biosystems®)(54). From the same paper, the sensitivity of urine samples analysed by SNapShot® assay had a sensitivity of 62% for detecting new tumours and 42% for recurrent samples. It also been shown that the TERT promoter mutations can be detected from urine using PCR amplification and miSEQ. In fact using this technique, Kindle *et al*(53), found that 8/15 patients with TERT promoter mutations, detected in urine following TURBT (at time of follow up cystoscopy), all 8 (100%) had subsequent recurrence discovered. Using a SNapShot®, Hurst *et al*, also demonstrated detection of 51/53 positive samples from the urine of patients prior to TURBT(55).

FGFR3 mutations

Fibroblast growth factor receptor 3 mutation is a frequent genetic event, particularly in low-grade tumours (56). Van Oers *et al*, developed and tested a SNapSHot® assay targeting nine FGFR-3 mutations. In this study 64 urine samples were analysed (29 from bladder harbouring a FGFR3 mutant tumour and 35 FGFR3 wildtype tumours), and the calculated sensitivity was 62% and specificity 89%(56). This SNapShot® assay panel was evaluated in the follow up setting of 200 patients known to have low grade NMIBC (67% of tumours were mutant FGFR3), the sensitivity of the assay was found to be 58% for concomitant disease. An FGFR3 positive urine sample was found to have a 3.8-fold higher risk of recurrence versus a negative sample(57). As FGFR3 is associated with low grade / stage tumours it has been combined with other markers to try and improve diagnostic performance, and featured as one of the markers for a bladder cancer screening study(58). FGFR3 in combination with TERT promoter mutations has been shown to have a sensitivity of 70% and a specificity of 71% in detecting new UBC tumours(54). Kandimalla *et al* (59), combined FGFR3 with a DNA hypermethylation assay and found a sensitivity of 52% for FGFR3 alone but 80% when in combination with the methylation assay. It is very unlikely that FGFR3 will have utility as a urinary biomarker in its own right as tumours with FGFR3 mutation can still progress to MIBC(57) and so cystoscopy could not be obviated in cases of urine positive for FGFR3 mutation.

STAG2

STAG2 ("stromal antigen" gene 2) is located on the X chromosome and encodes for a subunit of the cohesion molecule that is important for regulating sister chromatid cohesion during cell division and also regulates gene expression through DNA looping and interactions with transcription factors(60). There has been recent interest in this gene as three papers, performing genomic analysis of UBC tumours, all found STAG2 to be frequently mutated (inactivated) or deleted(60-62). The significance of STAG2 mutations has yet to be truly elucidated, with the published papers not agreeing on the prognostic implications or the exact mechanism of action of STAG2(63). To date no researcher as provided details of urinary evaluation of STAG2 mutation, but the frequency of mutation may make it an attractive future marker.

AURKA

The Aurora kinase A (*AURKA*) gene encodes a serine/threonine kinase that has a role in chromosomal segregation and centromere separation and overexpression of AURKA has been shown to drive oncogenesis(64). Park *et al*, used a FISH test to assess the urine from 100 patients with bladder cancer and 148 controls patients; they reported a sensitivity of 87.0% and a specificity of 96.6% (64). The AURKA has not been the subject of any other published study since 2008, however the recent development of an AURKA inhibitor, may renew interest in this urinary marker(65).

Survivin

Survivin is an important protein involved in the inhibition of apoptosis and tumour cell invasiveness, survivin mRNA has been identified in urine using an immunoassay (66). Smith *et al*, evaluated urinary survivin in UBC samples, survivin protein and mRNA were detected in all of 46 patients with bladder cancer, but in only 3 of 35 patients with negative cystoscopic evaluation (67). Shariat *et al*, (using a Bio-Dot microfiltration detection system (Bio-Rad, Hercules, California)) evaluated urinary survivin in 117 UBC cases and 92 controls and described a sensitivity of 64% and specificity of 93% (68). Horstmann *et al*, used PCR measurement of urinary survivin mRNA in 50 patients with suspicion of new or recurrent bladder cancer prior to transurethral resection (69) to yield sensitivity of 83% & 35% (for HG and LG UBC respectively) and specificity of 88%. The UroScreen study group performed a large prospective screening study on 1,540 chemical workers and analysed 5,716 samples for Survivin mRNA using rt-PCR(70). The study was limited by a very low number of tumours being detected (18) however Survivin demonstrated a sensitivity of 21.1% for all tumours and 36.4% for high grade tumours, surviving had a very low false positive rate and the authors concluded the test may be useful in a multi-marker panel.

RNA species

miRNA

MicroRNAs (miRNA) are small (less than 20bp) noncoding RNAs that post-transcriptionally regulate gene expression (71). Hanke *et al*, screened urinary sediment from UBC patients and controls for 157 different miRNAs using PCR. In this study miR-126, 182 and 199a were significantly increased in UBC patients urine compared to controls and the ratio of miR126:152 had a sensitivity of 72% for detecting UBC at a set specificity of 82% (72). Puerta-Gil *et al,* evaluated three miRs (143, 222, & 452 (in the urine of patients harbouring UBC using PCR), they found the diagnostic accuracy of miR-222 to be 77% and for miR-452 to be 85% (73). Miah *et al*, tested 121 urine samples (68 from UBC patients and 53 symptomatic controls attending cystoscopy clinic) for the presence of 15 miRs(using qPCR) known to be differentially expressed or associated with epigenetic hotspots in UBC. As a result of this analysis they found that a combination of miRs-135b/15b/1224-3p could detect bladder cancer with 94.1% sensitivity and 51% specificity (74). Recently, a study by Snowden *et al*, found urinary miR-125b to have an average 10.42-fold decrease ($p<0.01$) and miR-126 showing an average 2.70-fold increase ($p=0.30$) in UBC samples compared to controls (75). Shimizu *et al,* evaluated four methylated miRNAs (miR-137, miR-124-2, miR-124-3, and miR-9-3) in the urine of patients harbouring UBC. In this study the panel of four miRNAs was able to detect all UBC with an 81% sensitivity and 89% specificity and stage pTa and low – grade tumours (sensitivity 0.68, specificity 0.89), unlike conventional cytology.

mRNA markers

Messenger RNA (mRNA) based multi-gene commercial assays uRNA® (mRNAs = CDC2, HOXA13, MDK, and IGFBP5) and its derivative (has the additional marker CXCR2) Cxbladder™ (Pacific Edge Ltd, New Zealand) have demonstrated increased sensitivity in detection of UBC in comparison to NMP22 assay and cytology in patients with hematuria (76). In this 485 patient study, uRNA® had a sensitivity of 62.1% compared to NMP22 50% with a set specificity of 85% for the investigational assays. Cxbladder™ assay distinguished low-grade Ta tumours with a sensitivity of 91% and specificity of 90%. Mengual *et al*(77), have constructed a 12+2 gene expression signature for BC diagnosis and prediction of tumour aggressiveness on urine samples using qPCR assays. The twelve genes comprise: ANXA10, AHNAK2, CTSE, CRH, IGF2, KLF9, KRT20, MAGEA3, POSTN, PPP1R14D, SLC1A6, and TERT. The additional two genes, ASAM and MCM10, can help differentiate between LG and HG tumors. Overall, this gene set panel had 98% sensitivity and 99% specificity in discriminating between cases and controls samples and 79% sensitivity and 92% specificity in predicting tumour aggressiveness (high grade). They have tested the efficacy of this 12+2 gene set in voided urine and observed sensitivities and specificities of 89% and 95%, respectively and of 79% and 91%, respectively for predicting tumour aggressiveness.

Table 1. Summary of urinary biomarkers described in the text.
ELISA = Enzyme linked immunosorbent assay, FISH = Fluorescence in situ hybridization.

BIOMARKER	COMMERCIAL NAME	ASSAY TYPE	PERFORMANCE	REFERENCES	COMMENTS
PROTEIN					
Human complement factor H-related protein	BTA STAT™	Qualitative point of care	Sensitivity 57% – 83% Specificity = 60% - 92%	9 - 13	False Positive results with haematuria FDA approved only in combination with cystoscopy
	BTA TRAK™	ELISA	Sensitivity 62% – 91%	14 - 21	
Nuclear mitotic apparatus protein 22	NMP22®	ELISA	Sensitivity 47 - 100% Specificity = 60 - 90%	9 – 11, 20, 25 - 29	False positives with any cause of cell death e.g. benign inflammatory conditions, infection or urolithiasis
	BladderChek®	Qualitative point of care	Sensitivity = 59% Specificity = 93%	31	
Cytokeratins 8 & 18	UBC® ELISA	ELISA	Sensitivity = 64.4% Specificity = 63.6%	37	
	UBC® Rapid	Qualitative point of care	Sensitivity = 64.4 - 66% Specificity = 63.6% - 90%	36, 37	
	UBC® IRMA	Immunoradiometric Assay	Sensitivity = 12.1% Specificity = 97.2%	16	
BLCA-1	-	ELISA	Sensitivity = 80% Specificity = 87%	39	Results require validation
BLCA-4	-	ELISA	Sensitivity = 96.4% Specificity = 100%	40	
CELL BASED					
CEA & Tumour Mucins	uCyt+™	Immunofluorescence	Sensitivity = 72.8% Specificity = 71.9%	42	Requires minimum number of exfoliated cells and trained cytopathologist

(Table1 continued on next page)

(Table 1 cont'd) **Table 1. Summary of urinary biomarkers described in the text. ELISA = Enzyme linked immunosorbent assay, FISH = Fluorescence in situ hybridization.**

BIOMARKER	COMMERCIAL NAME	ASSAY TYPE	PERFORMANCE	REFERENCES	COMMENTS
Chromosomes 3,7,17 & 9p21	UroVysion®	FISH	Sensitivity = 72% Specificity = 83%	47	More expensive than cytology
mRNA / DNA					
TERT promoter mutations	-	SNapShot	Sensitivity = 42% - 62% Specificity = 73% - 90%	54	
FGFR3 mutation	-	SNapShot	Sensitivity = 58 - 62% Specificity = 89%	56	
AURKA		FISH	Sensitivity = 87% Specificity = 96%	64	
Survivin		Bio-Dot	Sensitivity = 35% - 83% Specificity = 88% - 93%	68, 69	

Epigenetic urinary markers

Analysis of gene methylation has been performed on voided urine (78, 79). Friedrich *et al,* analysed the methylation status of different markers in urine samples of patients with UBC and found that methylation of DAPK, BCL2, and TERT (see earlier) was detected in the majority of samples (78%), whereas they were unmethylated in the urine sediment from age-matched cancer-free individuals(79). Renard *et al*, identified TWIST1 and NID2 to be frequently methylated in urine samples collected from UBC patients and reported a sensitivity and specificity for this two-gene panel >90% (80). Scher *et al*, developed a small urine volume nested methylation-specific PCR assay for the detection of UBC based on methylation of BCL2, CDKN2A, and NID2 with a sensitivity of 80.9% and 86.4% (81). Chung et al used methylation markers (MYO3A, CA10, SOX11, NKX6-2, PENK, and DBC1) to screen urine for UBC with a 81-85% sensitivity and 95-97% specificity (82). Zuiverloon et al (83), using a methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay for 4 genes (APC, TERT_A, TERT_B & EDNRB), and found a sensitivity of 72.3% and specificity of 55.2% for detecting NMIBC recurrence.

Conclusion

UBC is a challenging disease for clinicians to manage and demands new methods of performing diagnosis and disease surveillance. Urinary biomarkers have the potential to enhance current diagnostic strategies and perhaps in the future, even replace existing techniques. As UBC is a disease

of the entire urothelium (field change effect), urinary biomarkers may have the potential to inform clinicians of the pathogenicity of the urothelium in the absence of visible carcinoma. As more information emerges about the different genetic pathways in low and high grade BC, it is likely that a much more genetic level based surveillance will be used to guide the success of the bladder sparing treatment protocols (BCG, Chemotherapy, Radiation).

As described, urinary biomarkers from the chromosome down to specific gene mutations have all been evaluated, not only for diagnosis of symptomatic patients but also for screening at risk populations and for predicting and detecting disease recurrence. Although many markers have shown superior test performance to urine cytology, there is a paucity of well-designed prospective trials (with cost-effectiveness) that will be needed to justify a new markers use. It seems more likely that markers will need to be used in combination to enhance the effectiveness of the test. Clinicians may also use these new biomarker tests selectively for example in patients with high grade disease that are pursuing bladder sparing treatments, or possibly for patients with very low risk of disease progression that do not want to undergo routine cystoscopy. Urinary biomarkers will undoubtedly find a role in the future of UBC management but at present, which test or test strategy has yet to be elucidated. Clinicians and patients desire a rapid bedside test particularly as in many health care scenarios UBC is assessed in “one stop” clinics. However the complexity and possibly the number of tests that will be required to be performed will still necessitate in the first instance, laboratory analysis, possibly in designated specialized centers.

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