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TOPIC PAPER

Can urinary biomarkers replace cystoscopy?

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Abstract

Purpose Diagnosis and follow-up in patients with non-muscle invasive bladder cancer (NMIBC) rely on cystoscopy and urine cytology. The aim of this review paper is to give an update on urinary biomarkers and their diagnosis and surveillance potential. Besides FDA-approved markers, recent approaches like DNA methylation assays, mRNA gene expression assays and cell-free DNA (cfDNA) are evaluated to assess whether replacing cystoscopy with urine markers is a potential scenario for the future.

Methods We performed a non-systematic review of current literature without time period restriction using the National Library of Medicine database (http://ww.pubmed.gov). The search included the following key words in different combinations: "urothelial carcinoma", "urinary marker", "hematuria", "cytology" and "bladder cancer". Further, references were extracted from identified articles. The results were evaluated regarding their clinical relevance and study quality.

Results Currently, replacing cystoscopy with available urine markers is not recommended by international guidelines. For FDA-approved markers, prospective randomized trials are lacking. Newer approaches focusing on molecular, genomic and transcriptomic aberrations are promising with good accuracies. Furthermore, these assays may provide additional molecular information to guide individualized surveillance strategies and therapy. Currently ongoing prospective trials will determine if cystoscopy reduction is feasible.

Conclusion Urinary markers represent a non-invasive approach for molecular characterization of the disease. Although fully replacing cystoscopy seems unrealistic in the near future, enhancing the current gold standard by additional molecular information is feasible. A reliable classification and differentiation between aggressive and nonaggressive tumors by applying DNA, mRNA, and cfDNA assays may change surveillance to help reduce cystoscopies.

Keywords Non-muscle invasive bladder cancer · Disease detection · Follow-up · cfDNA · Molecular urine markers · Liquid biopsy

Introduction and methods

Diagnosis and surveillance of bladder cancer (BC) are based mainly on cystoscopy, which is invasive, cost-intensive and associated with discomfort. Urine cytology is an important adjunct to cystoscopy. Despite the progress in technology (e.g., photodynamic diagnostic) and increased sensitivity, not all tumors are detected. For example, sensitivity of cytology for low-grade (LG) disease is poor. The clinical need for new cost-effective markers to improve diagnostic accuracy and reduce further testing, like cystoscopies, has been discussed before. Despite the availability of some FDA-approved assays, replacing cystoscopies for detection and monitoring BC is currently implausible. The aim of this article is to give an update on different molecular markers in urine, focusing on newer approaches with an outlook on whether next generation sequencing (NGS) of urinary biomarkers could replace cystoscopy, and how biomarkers may help enhance information provided by cystoscopy.

We reviewed current literature without time period restriction non-systematically using the National Library of Medicine database (http://ww.pubmed.gov). The search included the following key words in different combinations: "urothelial carcinoma", "urinary marker", "cytology", "hematuria" and "bladder cancer". Further, references were extracted from identified articles. The results were evaluated regarding their clinical relevance and study quality.

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Potential clinical scenarios

The potential benefit of urinary biomarkers varies depending on the clinical situation.

Scenario 1: Urinary biomarkers could be used prior to cystoscopy as the primary diagnostic approach; either by screening the population or only in symptomatic patients presenting with hematuria. The individual marker should provide a high negative predictive value and specificity to avoid false-positive results. However, using urine markers for screening purposes is currently not recommended because BC prevalence is too low and the available biomarkers do not adequately detect BC with high sensitivity or specificity [1]. Patients with hematuria should be categorized by gross and microscopy hematuria. Every patient with gross hematuria should receive a cystoscopy; however, urinary markers could be an important adjunct to nomograms leading to better evaluation of patients with microscopic hematuria [2].

Scenario 2: Following the detection of a suspicious lesion, EAU guidelines recommend a photodynamic-enhanced transurethral resection and deep resections containing detrusor muscle where a high-grade (HG) tumor or carcinoma in situ (CIS) is predicted [3]. A positive urine marker examination may increase the investigator's awareness for a more intense examination bladder. Moreover, a urine marker result indicating the presence of aggressive tumors should encourage the surgeon to take deep resections including detrusor muscle [4].

Scenario 3: Urinary markers might be helpful after transurethral tumor resection. Here, the potential use of urinary markers depends on tumor grading. In LG tumors, markers could be a surveillance tool reducing the frequency of cystoscopies. Progression in these cases is rare, so urinary markers and sonography may guide follow-up investigations. Although some authors describe a feasible marker-guided follow-up, (e.g., the ongoing UroFollow trial, comparing cystoscopy-based follow-up with non-invasive surveillance using cell-based assays) further evidence is needed [5]. Figure 1 shows the UroFollow trial design. It is unlikely that clinicians will renounce re-resection to rely only on biomarkers in HG tumors; in this context, however, urinary markers could monitor patients receiving intravesical Bacillus Calmette–Guerin (BCG) therapy to evaluate whether a patient should receive further instillations or cystectomy [6].

Finally, urinary markers could aid patients with muscle invasive bladder cancer (MIBC) receiving neoadjuvant therapy before cystectomy [7]. Currently, no reliable tools exists that can identify which patients would benefit from neoadjuvant chemotherapy [3]. In the future, genetic analyses of urine may help select these potential patients, in a non-invasive way. In adjunct with imaging, molecular markers [e.g., cell-free DNA (cfDNA)] in plasma or urine may indicate early progression during neoadjuvant therapy, even when no systemic progress is visible in the CT. Figure 2 illustrates potential clinical scenarios for using urine markers.

Several markers, investigated in the past, address the clinical needs outlined above. The most broadly used marker, urine cytology, can be interrogated by various tests for structural genomic aberrations [e.g., urovysion fluorescence

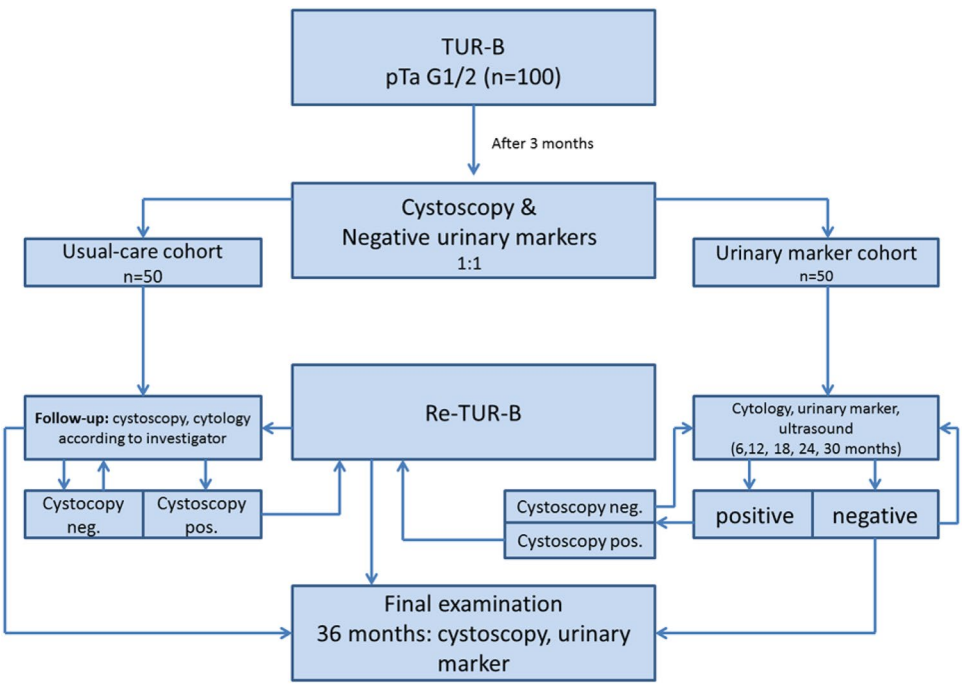

Fig. 1 Flowchart of the Urofollow trial. *PDD* photodynamic diagnostics, *TUR-BT* transurethral resection of a bladder tumor

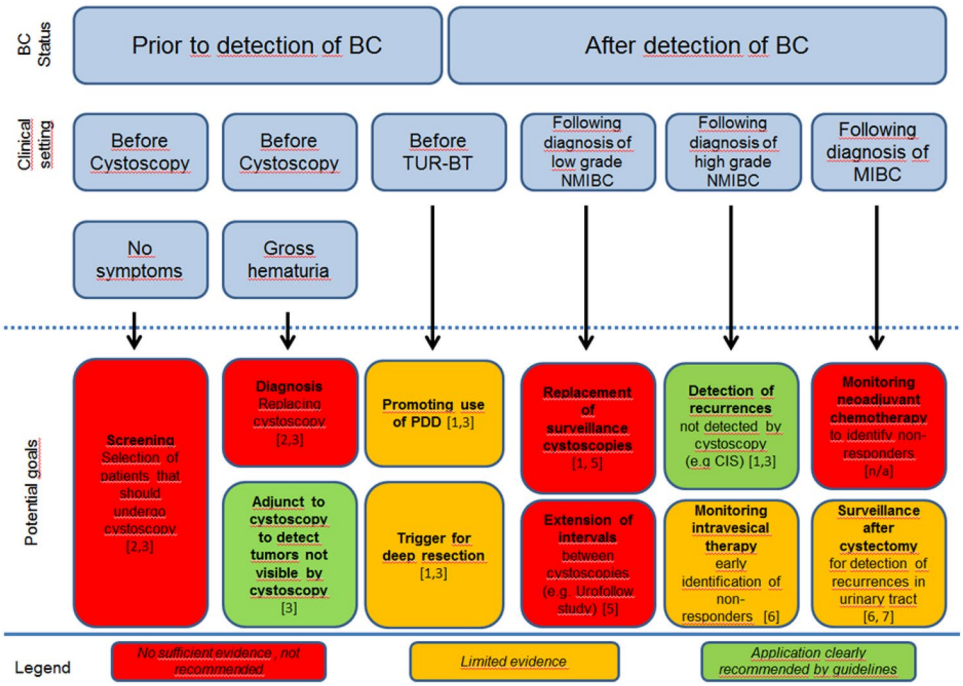

Fig. 2 Potential clinical scenarios for using urine markers. *BC* bladder cancer, *PDD* photodynamic diagnostics, *TUR-BT* transurethral resection of a bladder tumor

in situ hybridization (FISH) and protein alterations (e.g., NMP22)]. Table 1 provides a summary of available urine markers. While some assays have been approved, their application is not clearly recommended in current guidelines. Recently, specific assays analyzing gene mutations or methylation and tumor-associated mRNAs present in urine have been introduced. cfDNA release by tumor cells presents new options for assessing tumor-associated DNA in urine. The information about genetic aspects of malignant urothelial cells might help to incorporate molecular parameters in addition to morphological and clinical aspects into clinical decision making; urine-based analyses may be able to contribute to the analysis of new subtypes of bladder cancer that are based on genomic aberrations [8].

Urine cytology

Urine cytology remains an important part in the diagnosis and surveillance of BC. Urine cytology is more accurate for HG (specificity 83–99, 7% and sensitivity 38–84%) than LG (sensitivity 12–26%) [9]. Due to the discrepancy between cancer types, cytology should not be a stand-alone diagnostic or replacement of cystoscopy. However, it remains an important compliment, both in primary diagnosis and monitoring. In patients with HG or CIS with recent intravesical therapy, cytology should be used in recurrence monitoring. In patients with divergent results (i.e., positive cytology and negative cystoscopy) further diagnostic procedures should include imaging of the upper urinary tract and quadrant biopsies of the bladder to evaluate suspicious cell origin [3]. To reduce discomfort in patients receiving frequent cystoscopies, urine cytology in combination with ultrasound may be considered for LG nonaggressive tumors.

One limitation of cytology is the interobserver variability. A reliable assessment and interpretation of cytology scans remain challenging, in particular with recurrent inflammation or previous immunotherapy. To address this, the Paris system working group established a standardized reporting system which includes diagnostic categories and cytomorphologic criteria. This reporting system represents a significant step towards a more reproducible interpretation of atypical cytology [10].

FDA-approved urine markers

UroVysion (Vysis, Abott Molecular Inc., USA) is a FISH assay which detects chromosome copy number (aneuploidy of chromosomes 3,7,17 and 9p21 loss) aberrations. The assay is FDA approved for both diagnosis and follow-up. In trials and meta-analysis, the assay has shown a sensitivity (65–84%) superior (for LG) to cytology with a reasonable specificity (78–92%) [11]. Various authors have discussed a potential anticipatory feature proclaiming that patients with positive FISH tests are more likely to suffer a recurrence even without a visible tumor [12, 13]. However, further validation is needed.

FISH analyses are cost and labor intense with some challenges for implementation in daily clinical routines. Most

Table 1 Summary of current urine markers

Test & manufacturer	Detected marker, specimen	Assay type	FDA approval	Reported sensitivity	Reported specificity	References
Urine cytology	Atpyical exfoliated urothelial cells, urine	Microscopy		38–84% (HG) 12–26% (LG)	83–99%	Lotan et al. [9]
uCyt+/Immunocyt Scimedx, Inc., USA	Bladder tumor cell associated mucins/carcinoembryonic antigen, urine	Immunocytochemistry	Follow-up	85% (78–90%)	83% (77–87%)	Chou et al. [16]
UroVysion Abbott Vysis, USA	Alterations in chromosomes 3, 7, 17, and 9 p 21, urine	FISH	Diagnosis, follow-up	65–84%	78–92%	Mowatt et al. [11]
NMP22 Matritech, Inc., Alere, Germany	Nuclear mitotic apparatus proteins, urine	Sandwich ELISA or point-of-care test	Follow-up (ELISA), diagnosis, follow-up (p-o-c)	62–75%	70–83%	Chou et al. [16]
BTA trak. (ELISA) BTA stat. (p-o-c) Polymedco, Cortlandt, USA	Complement factor H-related protein and complement factor H, urine	Sandwich ELISA or immunoassay	Diagnosis, follow-up	58–69% (p-o-c) 54–75% (ELISA)	73–81% (p-o-c) 64–82% (ELISA)	Chou et al. [16]
UBC rapid (p-o-c) UBC ELISA (ELISA) IDL Biotech, Sweden	Cytoskeletal proteins CK8 and 18, urine	Sandwich ELISA or Point-of-care test	–	50–59.3% (p-o-c)	82–86% (p-o-c)	Schmitz-Dräger et al. [14]
CYFRA 21-1 Roche Diagnostics, Suisse	Cytoskeletal protein CK19, urine	Electrochemiluminescent immunoassay	–	70–90%	73–86%	Huang et al. [20]
BLCA 4 Eichrom Technologies, USA	Nuclear matrix protein, urine	Sandwich ELISA	–	93–96%	97–100%	Konety et al. [21] Cai et al. [22]
Survivin Fujirebio Diagnostics Inc., Japan	Inhibitor of apoptosis genes, urine	ELISA Bio-dot assay	–	64%	93%	Shariat et al. [23]
AssureMDx MDx Health, USA	Mutation analysis of FGFR3, TERT, and HRAS, methylation analysis of OTX1, ONECUT2, and TWIST1, urine	Mutation analysis	–	93–97%	83–86%	Van Kessel et al. [31]
CxBladder Assay Pacific Edge, NZ	Measurement of 5 mRNAs (IGFBP5, HOXA13, MDK, CDK1, CXCR2), urine	PCR	–	CxBladderDetect 82% CxBladderMonitor 93%	CxBladderDetect 85%	O'Sullivan et al. [34] Kavalieris et al. [35]
Xpert BC test Cephaid, USA	Detection of mRNA expression of 5-genes (CRH, IGF2, UPK1B, ANXA10, and ABL1), urine	PCR	–	73–84%	90–91%	Pichler et al. [37] Wallace et al. [36]
TaqMan Arrays ThermoFisher, USA	12 + 2 gene-set panel	PCR	–	98%	99%	Mengual et al. [38, 39]

Table 1 (continued)

p-o-c point of care, *HG* high grade, *LG* low grade

importantly, the correct interpretation of cell abnormalities caused by inflammation and other benign conditions (e.g., prostate enlargement, hematuria or urolithiasis) [14]. Using other interpretation algorithms may improve FISH accuracy. For instance, considering the potentially benign tetraploidic cells may help to increase specificity of the UroVysion assay [15]. Cost of the UroVysion assay may be reduced by improving automation and probe number. The aneuploidy of the probes is partially overlapping, indicating that a reduction of probes may be possible. These improvements may lead to easier and faster processing and reduce variability between observers.

The uCyt+ assay (Scimedx Inc., Denville, NJ, USA) is a combination of urine cytology and immunohistochemical staining with monoclonal antibodies (LDQ10, M344, 19A11). It is FDA approved for diagnosis and follow-up with a specificity 83% (77–87%) and sensitivity of 85% (78–90%) [16]. Its improved sensitivity for LG makes it a potential surveillance method in patients with low-risk BC. Combining urine cytology with uCyt+ could offer high sensitivity for both high- and low-grade tumors [17]. Limitations include false positives in benign conditions (e.g., inflammation or urolithiasis), the requirement of specialized laboratories and staff, and a long processing time. The manufacturer has terminated the production of uCyt+ ; therefore, no further development or research is expected.

The BTA (Polymedco, Cortlandt, NY, USA) and NMP22 assays (Alere, Waltham, MA, USA) are two protein-based tests approved by the FDA for diagnosis and follow-up. These tests have a qualitative point-of-care and a quantitative ELISA assay available.

The NMP22 assay (BladderChek) detects NMP22 released by apoptotic cells. The sensitivity of the quantitative approach is 62–75% and specificity is 70–83% [16]. Higher cell apoptosis results in higher levels of NMP22 increasing sensitivity in HG [18].

The BTA test identifies a complement h-related factor in urine (hCFHrp). The qualitative p-o-c BTA stat tests have a sensitivity of 58–69% and specificity of 73–81%, while the quantitative BTA Trak has sensitivity of 54–75% and specificity of 64–82% [16].

Both assays show a significant susceptibility for benign conditions such as hematuria, infections, ureteral or nephrostomy stents [19], limiting their use in screening and diagnosis. Potential false positives in tests may also be caused by previous BCG therapy, limiting their use as a surveillance tool after intravesical therapy. Combining the quantitative assay and cytology might be a powerful tool to stratify tumor aggressiveness. Todenhöfer et al. [18] have demonstrated that a positive CYT and NMP22 are associated with a 20-fold risk for G3/CIS.

Non-FDA-approved markers

Non-FDA-approved assays are predominantly based on malignant-cell expressed proteins. UBC rapid (point-of-care) and UBC ELISA detect cytokeratin 8 and 18, proteins which form part of the cascade for tumor invasion. The reported sensitivity and specificity (UBC rapid) are 50–59.3% and 82–86.1%, respectively [14]. CYFRA 21-1 is an ELISA assay detecting fragments of cytokeratin 19; the sensitivity and specificity are 70–90% and 73–86%, respectively [20]. Data for this assay are limited. BLCA-4 focusses on another member of the nuclear matrix protein family (sensitivity 93–96%, specificity 97–100%) [21, 22]. The survivin assay investigates a protein in a family of apoptosis inhibitors. Although Shariat et al. [23] reported a sensitivity of 64% and specificity of 93%, the potential of survivin is still unknown.

Further protein-based assays are available: soluble FAS (sFAS) is an antiapoptotic protein protecting the cancer cells from anti-tumor activity. ELISA tests are available for urine and serum. Although data of large prospective trials are still missing and a standardized procedure needs to be implemented, available data show an association between high levels of sFAS and tumor stage ≥ T1, higher NMP22 levels and positive urine cytology [24].

Hyaluronic acid (HA) is known from other cancer types; it plays an active role in cell adhesion and proliferation, and promotes tumor metastasis. The correlating hyaluronidase enzyme (HAase) and HA were evaluated in patients with NMIBC in a study by Lokeshwar et al. [25] (sensitivity 91%, specificity 70%).

An important part of genome stability are telomeres at the end of chromosomes and they are synthesized by telomerase. Hyperactivity of telomerase protects cancer cells chromosomes in various tumor entities. For NMIBC patients, Brems-Eskildsen et al. [26] found a positive association between high levels of telomerase and recurrence; furthermore, they showed that combination of telomerase and cytology led to an increased sensitivity.

Summing up, the mentioned non-FDA-approved marker systems were not able to replace established marker systems or cystoscopy [27].

Urinary biomarkers for BCG response prediction

BCG is an important treatment option in patients with high grade tumors or carcinoma in situ. However, patients with BCG failure need to be identified early to evaluate the need of an early cystectomy. Urovysion has been shown to be able to early identify non-responders to BCG (molecular non-responders) [1, 28].

BCG efficacy is depending on its ability to induce an immune response; as a result, cytokine levels are rising. Based on this, Kamat et al. [29] evaluated the role of 12 urinary cytokines to predict clinical response to BCG. They developed a—on nine cytokines based—normogram which was able to predict recurrence with 85.5% accuracy. However, further data to evaluate the potential of cytokines as early predictor of BCG failure are needed.

Assure MDX, CxBladder and Xpert bladder cancer tests

New platforms AssureMDx (MDx Health, USA), CxBladder Assay (Pacific Edge, NZ) or Xpert BC test (Cephaid, USA) are the product of recent progress in high-throughput technologies resulting in fast, comprehensive and simultaneous characterization of molecular, genomic and transcriptomic aberrations. Through variant analysis, these platforms are able to detect the presence of disease and to give an idea of which molecular changes are present in the tumor: an important step towards personalized therapy [30].

AssureMDx evaluates the DNA methylation of OTX1, ONECUT2 and TWIST and mutational load of FGFR3, TERT and HRAS in cell pellets from centrifuged urine samples. Van Kessel et al. [31] detect a sensitivity of 97% and specificity of 83% in 154 patients with gross hematuria. In a second, multicenter trial, the authors measure a sensitivity of 93% and a specificity of 86%. The authors see a potential of reducing unnecessary cystoscopies in patients with hematuria by 81.7%. This has led to an ongoing multicenter validation trial in 700 patients. Using a similar approach, the same group analyzed the methylation status of OTX1 and a mutation analysis of FGFR3 and TERT without accounting for other aberrations in 977 patients. The primary diagnosis sensitivity for LG is 81% and 94% for HG. During surveillance, the sensitivity for LG is 57% and 72% for HG (specificity LG 59%, HG 55%) [32]. Although these data seem to be promising and may lead to these assays being used in a surveillance protocol, further prospective studies are needed.

The CxBladder assay consists of three parts: CxBladderTriage, CxBladderDetect, and CxBladderMonitor using qPCR to measure 5 mRNAs (IGFBP5, HOXA13, MDK, CDK1, CXCR2). To evaluate whether a cystoscopy is appropriate in patients with hematuria, CxBladderTriage creates an individual risk profile by collecting clinical factors (e.g., age, sex, smoking, exposures, character of hematuria) along with the mRNA analysis. For this approach, Kavalieris et al. [33] report a 100% HG detection rate in 587 patients with gross hematuria. O'Sullivan et al. [34] evaluated CxBladderDetect in 485 patients with hematuria, with overall sensitivity of 82% (compared to 38% in NMP22 POC, 50% in NMP22 ELISA and 56% for cytology). Remarkably, sensitivity was significantly higher for LG as compared to cytology (pTa 68%). The reported specificity is 85%. The CxBladderMonitor test has been evaluated in a multicenter study in 803 patients with non-muscle invasive BC (NMIBC) history, separated into training (354) and validation cohorts (449). The authors measure a sensitivity of 93% outperforming all other evaluated tests across all stages and grades (sensitivity for LG 86%, HG 95%, NPV 97%) [35]. This marker has the potential to reduce the frequency of cystoscopies following NMIBC; however, further data are needed.

Lastly, Xpert Bladder cancer test uses a qRT-PCR to detect gene expression of CRH, IGF2, UPK1B, ANXA10 and ABL1. In a cohort of 450 patients with hematuria, Wallace et al. [36] measure an overall sensitivity of 73% and specificity of 90%. Compared to cystoscopy and cytology of NMIBC, Pichler et al. [37] report a significantly superior overall sensitivity and NPV than cytology (84% vs 33% and 93% vs 76%, respectively) even in LG and pTa tumors (sensitivity for LG 77% and pTa 82%) without reducing specificity (91% vs. 94%).

A 12 + 2 gene-set panel based on qRT-PCR (TaqMan® Arrays), developed by Mengual et al. [38], detects BC and predicts its aggressiveness. In their analysis of 341 urine probes from patients with BC and 235 controls, they find a sensitivity of 98% and specificity of 99% in the differentiation between the two. Prediction of tumor aggressiveness has a sensitivity of 79% and specificity of 92%. The accuracy of the gene set panel on the same cohort is 80% sensitivity and 86% specificity in discrimination between BC and controls and 75% for both measurements when predicting tumor aggressiveness [39]. The authors also designed four gene signatures which were applied in a prospective international multicenter cohort. These data indicate that the signature from two genes (GS_D2) is equal or better to cytology (sensitivity 81.48%, specificity 91.26%) [40]. For surveillance purposes, mutation and mRNA and gene methylation tests are promising approaches for the future and may reduce the frequency of cystoscopies; however, further data are required.

cfDNA

Analysis of cfDNA may be key in implementing individualized cancer therapies in the future [41]; by providing real-time insights of the tumor genome during all stages, making it valuable for risk assessment and therapy selection. cfDNA is detected in blood and urine with two analysis options; digital droplet PCR (ddPCR) has high sensitivity for low-abundance mutations while NGS spans multiple genes and new variants [42].

Birkenkamp-Demtöder et al. designed 377 ddPCR probes for cfDNA in 12 patients with recurrent/progressive BC to detect tumor DNA levels longitudinally. They demonstrate that cfDNA is detectable in patients with NMIBC in both urine and plasma, but not in healthy patients. Furthermore, patients with progressive disease have significantly higher cfDNA levels before disease than patients with recurrent disease. One patient had detectable tumor DNA 1 year before clinical progression indicating the importance of personalized assay of genomic variants in monitoring [43].

When personalized ddPCRs of genes with high mutation rates in BC (specifically FGFR3 and PIK3CA) are analyzed in two cohorts (NMIBC 363 and MIBC 403 patients (before cystectomy)), only 36% NMIBC (not all patients had detectable ctDNA) and 11% MIBC patients show one or both mutations in their initial tissue sample. In the MIBC cohort, ctDNA levels are correlated with recurrence and overall survival. Implementation of further mutations could help to improve the performance of the approach [44].

To evaluate the use of cfDNA for treatment efficacy and detection of relapse in 370 liquid biopsies (plasma and urine) of advanced disease, Birkenkamp-Demtröder and colleagues [45] use NGS from primary tumors and personalized ddPCR assays longitudinally to monitor for recurrence. They demonstrate that disease-free patients have significantly lower cfDNA levels compared to patients with metastatic relapse, indicating that cfDNA analysis detects recurrence earlier than imaging.

These studies are a proof of principle that cfDNA alterations can be used as marker for BC. This approach might also be valuable for risk stratification (clinical scenario 3): Detection of cfDNA in plasma or high levels in urine could indicate the need to switch from intravesical therapy to cystectomy [42].

cfDNA seems useful for initial disease diagnoses. cfDNA data for BC are still limited and larger studies are required [41, 46].

Conclusion

This review addresses whether urine markers could replace cystoscopy and which low-risk diagnostic approaches may reduce cystoscopies. In the past, studies have focused on the potential for individual markers to replace the gold standard (urine cytology and cystoscopy). However, the FDA-approved assays show a lack of evidence which would lead to the recommendation to replace the gold standard for diagnosis and surveillance of NMIBC.

Newer commercialized assays for assessing RNA or DNA variants (e.g., Assure MDx or CxBladder Assay) are attractive by providing additional information that cannot be achieved by standard practices. Due to their non-invasive approach in the molecular characterization of alterations, they might enable a reliable risk stratification of tumors because they identify individual molecular features. On the other hand, it has to be addressed that the none-commercialized marker assays are not yet ready for a broader clinical application outside academic settings.

Based on these data, urinary markers could change follow-up strategies:

In nonaggressive tumors with no mutations, the surveillance strategy could switch from cystoscopy-based to marker- and sonography-based. In aggressive tumors with a high number of mutations, urinary markers could indicate the need to switch from receiving intravesical therapy to an early cystectomy.

In diagnoses, urinary markers could raise the investigator's awareness during cystoscopy.

Next-generation biomarker assays are promising because they enhance the diagnostic quality by providing additional molecular information not yet achieved. Identification and follow-up of individual mutations might be the future of individualized care in BC.

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Compliance with ethical standards

Conflict of interest T Todenhöfer serves as a consultant for Ipsen and has scientific cooperations with IDL and Qiagen. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

1. Chang SS, Boorjian SA, Chou R, Clark PE, Daneshmand S, Konety BR, Pruthi R, Quale DZ, Ritch CR, Seigne JD, Skinner EC, Smith ND, McKiernan JM (2016) Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO

guideline. J Urol 196(4):1021–1029. https://doi.org/10.1016/j.juro.2016.06.049
2. Schmitz-Drager BJ, Kuckuck EC, Zuiverloon TC, Zwarthoff EC, Saltzman A, Srivastava A, Hudson MA, Seiler R, Todenhofer T, Vlahou A, Grossman HB, Schoenberg MP, Sanchez-Carbayo M, Brunn LA, van Rhijn BW, Goebell PJ, Kamat AM, Roupret M, Shariat SF, Kiemeney LA (2016) Microhematuria assessment an IBCN consensus-Based upon a critical review of current guidelines. Urol Oncol 34(10):437–451. https://doi.org/10.1016/j.urolonc.2016.05.030
3. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R (2017) EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 71(3):447–461. https://doi.org/10.1016/j.eururo.2016.05.041
4. van der Aa MN, Steyerberg EW, Bangma C, van Rhijn BW, Zwarthoff EC, van der Kwast TH (2010) Cystoscopy revisited as the gold standard for detecting bladder cancer recurrence: diagnostic review bias in the randomized, prospective CEFUB trial. J Urol 183(1):76–80. https://doi.org/10.1016/j.juro.2009.08.150
5. Schmitz-Drager C, Bonberg N, Pesch B, Todenhofer T, Sahin S, Behrens T, Bruning T, Schmitz-Drager BJ (2016) Replacing cystoscopy by urine markers in the follow-up of patients with low-risk non-muscle-invasive bladder cancer?—an International Bladder Cancer Network project. Urol Oncol 34(10):452–459. https://doi.org/10.1016/j.urolonc.2016.06.001
6. Kamat AM, Dickstein RJ, Messetti F, Anderson R, Pretzsch SM, Gonzalez GN, Katz RL, Khanna A, Zaidi T, Wu X, Grossman HB, Dinney CP (2012) Use of fluorescence in situ hybridization to predict response to bacillus Calmette-Guerin therapy for bladder cancer: results of a prospective trial. J Urol 187(3):862–867. https://doi.org/10.1016/j.juro.2011.10.144
7. Alfred Witjes J, Lebret T, Comperat EM, Cowan NC, De Santis M, Bruins HM, Hernandez V, Espinos EL, Dunn J, Rouanne M, Neuzillet Y, Veskimae E, van der Heijden AG, Gakis G, Ribal MJ (2017) Updated 2016 EAU guidelines on muscle-invasive and metastatic bladder cancer. Eur Urol 71(3):462–475. https://doi.org/10.1016/j.eururo.2016.06.020
8. Yousef PG, Gabril MY (2018) An update on the molecular pathology of urinary bladder tumors. Pathol Res Pract 214(1):1–6. https://doi.org/10.1016/j.prp.2017.11.003
9. Lotan Y, Roehrborn CG (2003) Sensitivity and specificity of commonly available bladder tumor markers versus cytology: results of a comprehensive literature review and meta-analyses. Urology 61(1):109–118 **(discussion 118)**
10. Barkan GA, Wojcik EM, Nayar R, Savic-Prince S, Quek ML, Kurtycz DF, Rosenthal DL (2016) The Paris system for reporting urinary cytology: the quest to develop a standardized terminology. Acta Cytol 60(3):185–197. https://doi.org/10.1159/000446270
11. Mowatt G, Zhu S, Kilonzo M, Boachie C, Fraser C, Griffiths TR, N'Dow J, Nabi G, Cook J, Vale L (2010) Systematic review of the clinical effectiveness and cost-effectiveness of photodynamic diagnosis and urine biomarkers (FISH, ImmunoCyt, NMP22) and cytology for the detection and follow-up of bladder cancer. Health technology assessment (Winchester, England). Health Technol Assess 14(4):1–331. https://doi.org/10.3310/hta14040 **(iii–iv)**
12. Todenhofer T, Hennenlotter J, Guttenberg P, Mohrhardt S, Kuehs U, Esser M, Aufderklamm S, Bier S, Harland N, Rausch S, Gakis G, Stenzl A, Schwentner C (2015) Prognostic relevance of positive urine markers in patients with negative cystoscopy during surveillance of bladder cancer. BMC Cancer 15:155. https://doi.org/10.1186/s12885-015-1089-0
13. Seideman C, Canter D, Kim P, Cordon B, Weizer A, Oliva I, Rao J, Inman BA, Posch M, Herr H, Lotan Y (2015) Multicenter evaluation of the role of UroVysion FISH assay in surveillance of patients with bladder cancer: does FISH positivity anticipate recurrence? World J Urol 33(9):1309–1313. https://doi.org/10.1007/s00345-014-1452-9
14. Schmitz-Drager BJ, Droller M, Lokeshwar VB, Lotan Y, Hudson MA, van Rhijn BW, Marberger MJ, Fradet Y, Hemstreet GP, Malmstrom PU, Ogawa O, Karakiewicz PI, Shariat SF (2015) Molecular markers for bladder cancer screening, early diagnosis, and surveillance: the WHO/ICUD consensus. Urol Int 94(1):1–24. https://doi.org/10.1159/000369357
15. Moatamed NA, Apple SK, Bennett CJ, Aronson WJ, Klisak I, Shirley BJ, Moatamed F (2013) Exclusion of the uniform tetraploid cells significantly improves specificity of the urine FISH assay. Diagn Cytopathol 41(3):218–225. https://doi.org/10.1002/dc.21831
16. Chou R, Gore JL, Buckley D, Fu R, Gustafson K, Griffin JC, Grusing S, Selph S (2015) Urinary biomarkers for diagnosis of bladder cancer: a systematic review and meta-analysis. Ann Intern Med 163(12):922–931. https://doi.org/10.7326/m15-0997
17. Comploj E, Mian C, Ambrosini-Spaltro A, Dechet C, Palermo S, Trenti E, Lodde M, Horninger W, Pycha A (2013) uCyt+/ImmunoCyt and cytology in the detection of urothelial carcinoma: an update on 7422 analyses. Cancer Cytopathol 121(7):392–397. https://doi.org/10.1002/cncy.21287
18. Todenhofer T, Hennenlotter J, Aufderklamm S, Kuhs U, Gakis G, Germann M, Stenzl A, Schwentner C (2013) Individual risk assessment in bladder cancer patients based on a multi-marker panel. J Cancer Res Clin Oncol 139(1):49–56. https://doi.org/10.1007/s00432-012-1297-9
19. Todenhofer T, Hennenlotter J, Kuhs U, Tews V, Gakis G, Aufderklamm S, Stenzl A, Schwentner C (2012) Influence of urinary tract instrumentation and inflammation on the performance of urine markers for the detection of bladder cancer. Urology 79(3):620–624. https://doi.org/10.1016/j.urology.2011.10.067
20. Huang YL, Chen J, Yan W, Zang D, Qin Q, Deng AM (2015) Diagnostic accuracy of cytokeratin-19 fragment (CYFRA 21-1) for bladder cancer: a systematic review and meta-analysis. Tumour Biol 36(5):3137–3145. https://doi.org/10.1007/s13277-015-3352-z
21. Konety BR, Nguyen TS, Dhir R, Day RS, Becich MJ, Stadler WM, Getzenberg RH (2000) Detection of bladder cancer using a novel nuclear matrix protein, BLCA-4. Clin Cancer Res 6(7):2618–2625
22. Cai Q, Wu Y, Guo Z, Gong R, Tang Y, Yang K, Li X, Guo X, Niu Y, Zhao Y (2015) Urine BLCA-4 exerts potential role in detecting patients with bladder cancers: a pooled analysis of individual studies. Oncotarget 6(35):37500–37510. https://doi.org/10.18632/oncotarget.6061
23. Shariat SF, Casella R, Khoddami SM, Hernandez G, Sulser T, Gasser TC, Lerner SP (2004) Urine detection of survivin is a sensitive marker for the noninvasive diagnosis of bladder cancer. J Urol 171(2 Pt 1):626–630. https://doi.org/10.1097/01.ju.0000107826.78479.90
24. Svatek RS, Herman MP, Lotan Y, Casella R, Hsieh JT, Sagalowsky AI, Shariat SF (2006) Soluble Fas—a promising novel urinary marker for the detection of recurrent superficial bladder cancer. Cancer 106(8):1701–1707. https://doi.org/10.1002/cncr.21795
25. Lokeshwar VB, Schroeder GL, Selzer MG, Hautmann SH, Posey JT, Duncan RC, Watson R, Rose L, Markowitz S, Soloway MS (2002) Bladder tumor markers for monitoring recurrence and screening comparison of hyaluronic acid-hyaluronidase and BTA-Stat tests. Cancer 95(1):61–72. https://doi.org/10.1002/cncr.10652
26. Brems-Eskildsen AS, Zieger K, Toldbod H, Holcomb C, Higuchi R, Mansilla F, Munksgaard PP, Borre M, Orntoft TF, Dyrskjot L (2010) Prediction and diagnosis of bladder cancer recurrence based on urinary content of hTERT, SENP1, PPP1CA,

and MCM5 transcripts. BMC Cancer 10:646. https://doi.org/10.1186/1471-2407-10-646
27. Soria F, Droller MJ, Lotan Y, Gontero P, D'Andrea D, Gust KM, Roupret M, Babjuk M, Palou J, Shariat SF (2018) An up-to-date catalog of available urinary biomarkers for the surveillance of non-muscle invasive bladder cancer. World J Urol. https://doi.org/10.1007/s00345-018-2380-x
28. Savic S, Zlobec I, Thalmann GN, Engeler D, Schmauss M, Lehmann K, Mattarelli G, Eichenberger T, Dalquen P, Spieler P, Schoenegg R, Gasser TC, Sulser T, Forster T, Zellweger T, Casella R, Bubendorf L (2009) The prognostic value of cytology and fluorescence in situ hybridization in the follow-up of nonmuscle-invasive bladder cancer after intravesical Bacillus Calmette-Guerin therapy. Int J Cancer 124(12):2899–2904. https://doi.org/10.1002/ijc.24258
29. Kamat AM, Briggman J, Urbauer DL, Svatek R, Nogueras Gonzalez GM, Anderson R, Grossman HB, Prat F, Dinney CP (2016) Cytokine Panel for Response to Intravesical Therapy (CyPRIT): nomogram of changes in urinary cytokine levels predicts patient response to bacillus Calmette-Guerin. Eur Urol 69(2):197–200. https://doi.org/10.1016/j.eururo.2015.06.023
30. Aly MS, Khaled HM, Emara M, Hussein TD (2012) Cytogenetic profile of locally advanced and metastatic schistosoma-related bladder cancer and response to chemotherapy. Cancer Genet 205(4):156–162. https://doi.org/10.1016/j.cancergen.2012.01.011
31. van Kessel KE, Beukers W, Lurkin I, Ziel-van der Made A, van der Keur KA, Boormans JL, Dyrskjot L, Marquez M, Orntoft TF, Real FX, Segersten U, Malats N, Malmstrom PU, Van Criekinge W, Zwarthoff EC (2017) Validation of a DNA methylation-mutation urine assay to select patients with hematuria for cystoscopy. J Urol 197(3 Pt 1):590–595. https://doi.org/10.1016/j.juro.2016.09.118
32. Beukers W, van der Keur KA, Kandimalla R, Vergouwe Y, Steyerberg EW, Boormans JL, Jensen JB, Lorente JA, Real FX, Segersten U, Orntoft TF, Malats N, Malmstrom PU, Dyrskjot L, Zwarthoff EC (2017) FGFR3, TERT and OTX1 as a urinary biomarker combination for surveillance of patients with bladder cancer in a large prospective multicenter study. J Urol 197(6):1410–1418. https://doi.org/10.1016/j.juro.2016.12.096
33. Kavalieris L, O'Sullivan PJ, Suttie JM, Pownall BK, Gilling PJ, Chemasle C, Darling DG (2015) A segregation index combining phenotypic (clinical characteristics) and genotypic (gene expression) biomarkers from a urine sample to triage out patients presenting with hematuria who have a low probability of urothelial carcinoma. BMC Urol 15:23. https://doi.org/10.1186/s12894-015-0018-5
34. O'Sullivan P, Sharples K, Dalphin M, Davidson P, Gilling P, Cambridge L, Harvey J, Toro T, Giles N, Luxmanan C, Alves CF, Yoon HS, Hinder V, Masters J, Kennedy-Smith A, Beaven T, Guilford PJ (2012) A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. J Urol 188(3):741–747. https://doi.org/10.1016/j.juro.2012.05.003
35. Kavalieris L, O'Sullivan P, Frampton C, Guilford P, Darling D, Jacobson E, Suttie J, Raman JD, Shariat SF, Lotan Y (2017) Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. J Urol 197(6):1419–1426. https://doi.org/10.1016/j.juro.2016.12.010
36. Wallace E, Higuchi R, Satya M, McCann L, Sin MLY, Bridge JA, Wei H, Zhang J, Wong E, Hiar A, Mach KE, Scherr D, Egerdie RB, Ohta S, Sexton WJ, Meng MV, Weizer AZ, Woods M, Jansz GK, Zadra J, Lotan Y, Goldfarb B, Liao JC (2017) Development of a 90-minute integrated noninvasive urinary assay for bladder cancer detection. J Urol. https://doi.org/10.1016/j.juro.2017.09.141
37. Pichler R, Fritz J, Tulchiner G, Klinglmair G, Soleiman A, Horninger W, Klocker H, Heidegger I (2018) Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance. BJU Int 121(1):29–37. https://doi.org/10.1111/bju.14019
38. Mengual L, Burset M, Ribal MJ, Ars E, Marin-Aguilera M, Fernandez M, Ingelmo-Torres M, Villavicencio H, Alcaraz A (2010) Gene expression signature in urine for diagnosing and assessing aggressiveness of bladder urothelial carcinoma. Clin Cancer Res 16(9):2624–2633. https://doi.org/10.1158/1078-0432.ccr-09-3373
39. Mengual L, Ribal MJ, Lozano JJ, Ingelmo-Torres M, Burset M, Fernandez PL, Alcaraz A (2014) Validation study of a noninvasive urine test for diagnosis and prognosis assessment of bladder cancer: evidence for improved models. J Urol 191(1):261–269. https://doi.org/10.1016/j.juro.2013.06.083
40. Ribal MJ, Mengual L, Lozano JJ, Ingelmo-Torres M, Palou J, Rodriguez-Faba O, Witjes JA, Van der Heijden AG, Medina R, Conde JM, Marberger M, Schmidbauer J, Fernandez PL, Alcaraz A (2016) Gene expression test for the non-invasive diagnosis of bladder cancer: a prospective, blinded, international and multicenter validation study. Eur J Cancer (Oxford, England, 1990) 54:131–138. https://doi.org/10.1016/j.ejca.2015.11.003
41. Olsson E, Winter C, George A, Chen Y, Howlin J, Tang MH, Dahlgren M, Schulz R, Grabau D, van Westen D, Ferno M, Ingvar C, Rose C, Bendahl PO, Ryden L, Borg A, Gruvberger-Saal SK, Jernstrom H, Saal LH (2015) Serial monitoring of circulating tumor DNA in patients with primary breast cancer for detection of occult metastatic disease. EMBO Mol Med 7(8):1034–1047. https://doi.org/10.15252/emmm.201404913
42. Todenhofer T, Struss WJ, Seiler R, Wyatt AW, Black PC (2018) Liquid biopsy-analysis of circulating tumor DNA (ctDNA) in bladder cancer. Bladder Cancer (Amsterdam, Netherlands) 4(1):19–29. https://doi.org/10.3233/blc-170140
43. Birkenkamp-Demtroder K, Nordentoft I, Christensen E, Hoyer S, Reinert T, Vang S, Borre M, Agerbaek M, Jensen JB, Orntoft TF, Dyrskjot L (2016) Genomic alterations in liquid biopsies from patients with bladder cancer. Eur Urol 70(1):75–82. https://doi.org/10.1016/j.eururo.2016.01.007
44. Christensen E, Birkenkamp-Demtroder K, Nordentoft I, Hoyer S, van der Keur K, van Kessel K, Zwarthoff E, Agerbaek M, Orntoft TF, Jensen JB, Dyrskjot L (2017) Liquid biopsy analysis of FGFR3 and PIK3CA hotspot mutations for disease surveillance in bladder cancer. Eur Urol 71(6):961–969. https://doi.org/10.1016/j.eururo.2016.12.016
45. Birkenkamp-Demtroder K, Christensen E, Nordentoft I, Knudsen M, Taber A, Hoyer S, Lamy P, Agerbaek M, Jensen JB, Dyrskjot L (2018) Monitoring treatment response and metastatic relapse in advanced bladder cancer by liquid biopsy analysis. Eur Urol 73(4):535–540. https://doi.org/10.1016/j.eururo.2017.09.011
46. Aravanis AM, Lee M, Klausner RD (2017) Next-generation sequencing of circulating tumor DNA for early cancer detection. Cell 168(4):571–574. https://doi.org/10.1016/j.cell.2017.01.030