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Bladder Cancer

Evaluation of Cxbladder and Adjudication of Atypical Cytology and Equivocal Cystoscopy

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Abstract

Background: Cxbladder diagnostic tests combine genomic information from urinary mRNA with phenotypic information to either rule out low-risk individuals or identify patients at a high risk of urothelial carcinoma (UC).

Objective: To evaluate the performance of Cxbladder and urine cytology, and Cxbladder's adjudication of atypical cytology and equivocal cystoscopy.

Design, setting, and participants: This is a retrospective analysis of pooled data from three prospective Cxbladder clinical trials and one real-world clinical study. Physicians were blinded to Cxbladder results, and Cxbladder providers were blinded to clinical results. This study analyzed diverse urology practices in the USA, Australia, and New Zealand. A total of 1784 consecutive, prospectively recruited patients with hematuria or previously diagnosed UC provided 852 samples with both local cytology and Cxbladder results; 153 had atypical cytologies and 14 had both atypical cytology and equivocal cystoscopy.

Outcome measurements and statistical analysis: Negative predictive value (NPV) and proportion of tumors missed for Cxbladder and local cytology, and evaluation of Cxbladder for adjudicating atypical cytology and equivocal cystoscopy.

Results and limitations: Cxbladder ruled out 35% of patients and NPV 97% (95% confidence interval [CI] 94–98%) compared with 93% (95% CI 91–94%) for cytology; Cxbladder missed 8.5% and cytology missed 63% of tumors. UC was diagnosed in 26/153 cases of atypical cytology (17%). Cxbladder correctly adjudicated all these patients including those with both atypical cytology and equivocal cystoscopy; these patients had a positive Cxbladder result and were diagnosed with UC by pathology. The incidence of patients with both atypical cytology and equivocal cystoscopy is low.

Conclusions: Cxbladder correctly adjudicated all patients diagnosed with UC among those with atypical cytology and equivocal cystoscopy, and outperformed cytology for accurately identifying patients who do not have UC.

Patient summary: Cxbladder accurately rules out patients who do not have cancer, and adjudicates cytology and cystoscopy with inconclusive results, minimizing the need for patients to undergo further unnecessary tests and procedures.

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1. Introduction

The American Urological Association estimates the incidence of hematuria in the adult population to be 2.4–31.1% [1]. Even when benign causes of hematuria are excluded, the number of individuals requiring investigation for urothelial carcinoma (UC) remains substantial, but approximately 80% of macrohematuria patients and 98% of microhematuria patients who have a workup for UC do not have cancer [2,3].

Cystoscopy, the current gold standard for bladder cancer diagnosis [4], is an invasive endoscopic procedure requiring a local or systemic anesthetic [2], equipment, resources, and technical expertise, while exposing the patient to discomfort, procedural risk, and time commitment in the clinic.

Cytology is commonly used as a companion test to provide clinical resolution for equivocal or false-negative cystoscopy. Urine cytology can have high specificity but very low sensitivity [4–6]; thus, cases of bladder cancer (including high-grade and muscle-invasive disease) may be missed due to false-negative results [6]. Therefore, any advantage of high specificity is significantly reduced by low sensitivity. A clinically effective test to rule out UC requires high sensitivity and a high negative predictive value (NPV) with the clinical goal to have no, or very few, false negatives in the rule-out fraction. The diagnostic value of cytology is highest when it is performed by experienced cytopathologists [7,8], with local cytopathology showing considerable interobserver variability [9–11].

A high proportion of cytology samples are categorized as “atypical” [12]; these inconclusive cytology results present a diagnostic dilemma for physicians with extra procedures, highlighting the need for a biomarker test to improve hematuria diagnostic evaluation and identify patients who may not need cystoscopy, thereby avoiding unnecessary expense and potential patient morbidity [13].

Cxbladder (Pacific Edge Ltd, Dunedin, New Zealand) is a clinically validated multiplex mRNA test (quantitative reverse transcription polymerase chain reaction) to measure concentrations of five genes in unfractionated urine. Biomarkers are used to calculate a Cxbladder score and allocate patients to either an “at-risk” or a “low-risk” category [14–16]. Specific Cxbladder tests have been developed and validated for use at several decision points in the diagnostic and evaluation process for UC (Supplementary Table 1).

The current study was designed to investigate the diagnostic performance of Cxbladder in comparison with urine cytology, and to investigate the utility of Cxbladder for adjudicating the status of cytology samples classified as “atypical” cytology and cystoscopy diagnosed as equivocal.

2. Methods

2.1. Design

This study retrospectively analyzed the results of urine samples collected prospectively from 1784 consecutively recruited eligible patients during prior clinical studies with Cxbladder in diverse practices in the USA, Australia, and New Zealand. Cxbladder-Triage (CxbT) and Cxbladder-Detect

(CxbD) were performed in patients with hematuria [16], and Cxbladder-Monitor (CxbM) was performed in patients with previously diagnosed UC [14]; in addition, CxbT was performed in patients undergoing Cxbladder testing during a real-world clinical utility output study (unpublished data). The sample size of the current analysis was defined by the inclusion of all patients who have both local cytology and Cxbladder in each of the studies (Supplementary material). Details of these are described in the Supplementary material, and Supplementary Tables 1 and 2.

Cxbladder test results were not provided to the treating physicians, and no clinical results were available to those generating Cxbladder test results. All studies were conducted with Independent Review Board approval, and with appropriate approval from local or national ethics committees. The studies were performed in accordance with the principles of the Declaration of Helsinki and adhered to International Conference on Harmonization Good Clinical Practice guidelines.

2.2. Patients

Patients either had a recent history of hematuria requiring investigation for possible UC, but no previous history of urinary tract malignancy, or were undergoing cystoscopy to monitor for recurrence after a prior diagnosis of UC during the past 2 yr. Patient eligibility criteria are described in the Supplementary material and Supplementary Table 2. To be eligible for this analysis, patients were required to have both local cytology and Cxbladder results. Patients with incomplete data for either cytology or Cxbladder were excluded (see Supplementary Figs. 1–4 for details).

2.3. Assessments

All patients provided midstream voided urine samples for Cxbladder testing and cytology assessment (same urine sample). Samples for Cxbladder testing were analyzed in a central laboratory [14–16] within 5 d of sample collection and subject to commercial quality assurance. Local cytology but not Cxbladder result was available to physicians for diagnostic purposes. Performers/readers of the Cxbladder results had no access to clinical results (cytology and/or cystoscopy). Clinical assessments for UC were made by conventional evaluation, usually including cystoscopy with or without a computerized tomography (CT) scan with contrast imaging, and confirmed by histopathological examination [14]. Disease stage was classified according to tumor-node metastasis criteria. Grade was classified according to local pathology practice using the 1973 WHO grading criteria or the 1998 WHO/International Society of Urological Pathology consensus classification.

All samples with both urine cytology and Cxbladder (CxbT or CxbM) results were analyzed ($n = 852$; Supplementary Table 3). This study used local cytology results from each site in order to replicate real-world clinical practice. Cytology results identified as positive or suspicious were classified as positive for this analysis, and those identified as atypical or negative were classified as negative. Atypical cytology and equivocal cystoscopy results were also evaluated for adjudication by Cxbladder.

2.4. Combination of CxbT and CxbD compared with cytology

An analysis was undertaken of hematuria patients (excluding those with recurrent UC) with both local cytology and CxbT + CxbD results. In this evaluation, a CxbT result was used first, to rule out patients at a low risk of UC who would not require further workup, second; samples from patients testing positive for CxbT were tested using CxbD for further segregation by UC risk. CxbD provides two components of utility: an additional segregation of patients who have a low risk of having UC and a complementary segregation of patients who have a high risk of having UC. This study evaluates only the former.

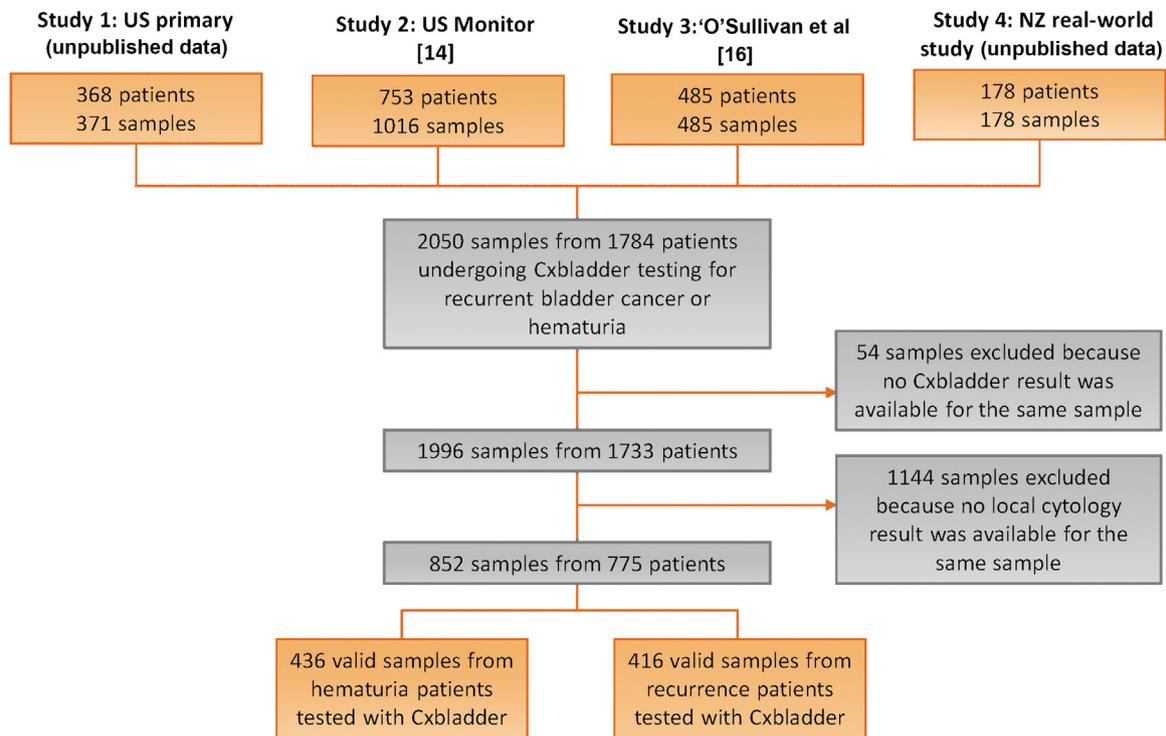


Fig. 1 – Samples included in the analysis. CxbD = Cxbladder Detect; CxbM = Cxbladder-Monitor; CxbT = Cxbladder-Triage.

2.5. Adjudication of atypia

Analysis of Cxbladder findings was also undertaken in a subgroup of 153 samples with atypical cytology results from 146 patients, including 14 who had both atypical cytology and equivocal cystoscopy results.

2.6. Statistical analysis

The comparative performance of the Cxbladder tests and cytology was analyzed by calculating the NPV, and tumors missed compared with biopsy-confirmed UC diagnoses. Sensitivity and specificity were also calculated for comparative purposes. These performance metrics and their 95% confidence intervals (CIs) were calculated by standard methods [17,18] using R-3.5.1 software.

3. Results

3.1. Patients

A total of 852 samples from 775 patients had both local cytology and Cxbladder results. Of these, 436 samples were from hematuria patients and 326 from patients being assessed for recurrent UC (Fig. 1 and Supplementary Figs. 1–4). Overall, 66% of samples were from US patients and 34% from New Zealand and Australia. Demographic and clinical characteristics for all patients are shown in Supplementary Table 3. Samples from patients with hematuria (not recurrent UC) were analyzed by both CxbT and CxbD. Of the 852 samples, 153 were with atypical cytology results with 26 were confirmed by pathology as positive for UC. Of the 153 samples, 118 had corresponding cystoscopy results

with 14 had both atypical cytology and equivocal cystoscopy. Two of the 14 were confirmed to be positive for UC by pathology.

3.2. Comparison with cytology

Within the 852 samples, cytology reported 801 (94%) as negative and missed 59 tumors (63%), while Cxbladder reported 302 (35%) samples as negative and missed eight tumors (8.5%; Table 1 and Supplementary Tables 4–6). The NPV of Cxbladder was 97.4% compared with 92.6% for cytology (Table 1).

In the cohort of 436 hematuria patients, 411 cytology results (94.2%) were negative, missing 25 tumors (56%). CxbT results were negative in 169 (39%) patients and four tumors (8.9%) were missed, while CxbT combined with

Table 1 – Number and percentage of samples with negative cytology and Cxbladder results, number and percentage of tumors missed in samples with a negative result, and negative predictive value in the 852-sample data set

N = 852 samples ^a	Cxbladder ^b	Cytology
Negative predictive value (%)	97.4	92.6
Samples with a negative result, n (%)	302 (35)	801 (94)
Tumors missed in those samples with a negative result, n (%)	8 (8.5)	59 (63)

^a Total tumor patients = 94. See also Supplementary Table 4.

^b A positive Cxbladder-Triage or Cxbladder-Monitor test result was the absence of a rule-out result.

Table 2 – Number and percentage of patients with negative cytology, Cxbladder-Triage and Cxbladder-Triage + Cxbladder-Detect results, number and percentage of tumors missed in samples with a negative result, and negative predictive value in the 436-sample data set of patients with hematuria

N = 436 hematuria patients	CxbT	CxbT + CxbD	Cytology
Negative predictive value (%)	97.6	97.8	93.9
Patients with a negative result, n (%)	169 (39)	320 (73)	411 (94.2)
Tumors missed in those with a negative result, n (%)	4 (8.9)	7 (16)	25 (56)

CxbD = Cxbladder-Detect; CxbT = Cxbladder-Triage.

Table 3 – Urothelial carcinoma diagnoses according to Cxbladder results in samples with atypical cytology results

	All samples with atypical cytology (total = 153)	
	UC negative	UC positive
Cxb negative	47	0
Cxb positive	80	26

Cxb = Cxbladder; UC = urothelial carcinoma.

CxbD was negative for 320 patients (73%) and missed seven tumors (15%; [Table 2](#) and [Supplementary Table 7](#)).

Cytology identified 91 more patients than CxbT as negative; however, 18 more tumors were missed. The combination of CxbT and CxbD increased the number of patients testing negative by 151 compared with CxbT alone, with three additional tumors missed ([Supplementary Table 8](#)).

3.3. Atypia adjudication

Local cytology results were classified as atypical in 153 of the 852 samples (18%), and 26 were confirmed to be positive for UC by pathology. Cxbladder correctly identified all 26 UC cases ([Supplementary Tables 9 and 10](#)) among those with atypical cytology. The Cxbladder result was positive in 106/153 samples and negative in 47/153 ([Table 3](#) and [Supplementary Table 11](#)). None of the 47 samples with a negative Cxbladder result were in patients with confirmed UC. One patient had a positive CxbT result, and a negative CxbD result had a low-grade UC. Two of the 14 patients where cytology results were atypical and cystoscopy was

equivocal had UC-positive pathology (one high grade \geq T1 and one carcinoma in situ [Cis]). Both were positive for Cxbladder.

3.4. Cystoscopy results

Cystoscopy results were available for 118 of the 153 samples with atypical cytology findings. Of these 118 samples 81 had negative cystoscopy results, 14 had equivocal results, and 23 had a papillary or nodular lesion. Nineteen of the 118 samples had a confirmed UC diagnosis, 17 had cystoscopy findings with papillary or nodular lesions, and two had equivocal cytology results (one Cis and one high grade \geq T1). All 19 of the confirmed UC diagnoses had a positive Cxbladder result ([Table 4](#)).

4. Discussion

This study demonstrates that Cxbladder outperformed cytology in the segregation of cancer patients with a low risk of UC. Cxbladder ruled out 302 of the 852 samples, sparing 35% from having to undergo cystoscopy, with eight false-negative results. To eliminate the false negatives, physicians would need to perform 302 cystoscopies. On average one extra tumor would be found for every 38 cystoscopies performed. Cxbladder also correctly adjudicated all the atypical cytology results that were subsequently diagnosed as UC. Using Cxbladder as a rule-out test, 47 of the 153 atypical cytology samples (31%) were correctly ruled out as negative for UC. Cxbladder had an improved NPV compared with urine cytology, which, when coupled with high sensitivity, provides reliable rule out of patients without UC.

Table 4 – Diagnosis of UC among patients with atypical cytology, stratified by cystoscopy findings and Cxbladder results

	Total, n	UC, n (%)	LG \geq Ta, n	HGTa, n	HG \geq T1, n	Cis, n	Negative biopsy, n
Papillary/nodular lesion							
Cxbladder negative	1	0	0	0	0	0	0
Cxbladder positive ^a	22	17 (77)	10	4	3	0	0
Equivocal/red lesion							
Cxbladder negative	5	0	0	0	0	0	0
Cxbladder positive ^a	9	2 (22)	0	0	1	1	3
Negative cystoscopy							
Cxbladder negative	32	0	0	0	0	0	1
Cxbladder positive ^a	49	0	0	0	0	0	0
Total	118	19 (16)	10	4	3	1	4

Cis = flat noninvasive carcinoma in situ; HG = high grade; LG = low grade; Ta = noninvasive tumor; UC = urothelial carcinoma.

^a A positive Cxbladder-Triage or Cxbladder-Monitor test result was the absence of a rule-out result and a recommendation for the physician to conduct further follow-up tests.

Rule-out tests with high sensitivity combined with a high NPV provide better clinical resolution for ruling out patients who have a low probability of disease [19,20] than tests with only a high NPV. These findings support previous published results, which have shown that all Cxbladder tests significantly outperformed urine cytology for identifying patients without UC [14–16,23].

This study provides more evidence for using Cxbladder to evaluate hematuria in the diagnostic pathway for bladder cancer. First, because of the high NPV and the low false-negative rate of Cxbladder, a voided urine sample can rule out patients who do not have UC, sparing the need for cystoscopy or other imaging modalities with the associated costs and discomfort. Indeed, a published study showed that using CxbT in the clinical pathway for UC reduced the need for cystoscopy by 44% and CT scan by 20% in real-world patients [22]. Second, this study demonstrates that no additional diagnostic resolution is provided by using cytology in investigation of hematuria because its low sensitivity and significant number of atypical results require adjudication. Use of experts and the Paris system of cytology classification may reduce the number of atypical cytology results [23], but would not overcome the consistently reported high rate of false-negative results with urine cytology.

Cytology misses >50% of UC-positive patients, including a substantial proportion with high-grade and/or muscle-invasive tumors [6]. In this study, cytology missed 63% of the tumors overall and 55% in those with hematuria, but did not identify any UC cases that were not previously identified by Cxbladder. The false-positive rate with cytology was 7.6%, with a positive predictive value of 67%, comparable with that reported by Tan et al. [6] (Supplementary Table 12).

Approximately 20% of atypical cytology samples are found to be malignant using other diagnostic methods [24,25]. In this study, Cxbladder was able to correctly adjudicate all the UC-positive samples among the 153 atypical cytology results and rule out UC diagnosis in 47 samples (31%), thereby sparing patients from invasive workup. Among patients with atypical cytology, 14 also had an equivocal cystoscopy and two were diagnosed with UC (one high grade \geq T1 and one Cis). Both tested positive for Cxbladder. These results provide a strong argument for using Cxbladder instead of cytology, thereby avoiding atypical cytology results and consequently minimizing the number of cystoscopies performed during hematuria assessment. These data do not address the value of centralized cytology, with its expert reading. However, centralized cytology reading is not feasible in most practice settings.

Other published studies have investigated UroVysion fluorescence in situ hybridization (FISH) as a reflex test to cytology on urine samples collected at the time of cystoscopy, and found it useful for identifying cancers in patients with negative or equivocal cystoscopy results [26,27]. However, a significant number of patients had false-negative UroVysion FISH results, indicating that a negative UroVysion FISH result does not preclude a UC diagnosis, although the test did not miss any high-grade cancers

[26,27]. By contrast, in this study, none of the patients with atypical cytology and a negative Cxbladder result had UC. Furthermore, Cxbladder identified two patients with equivocal cystoscopy who were confirmed as having UC. ImmunoCyt has been used as a reflex test to adjudicate atypical cytology with overall sensitivity and specificity of 87% and 21%, respectively, when examining patients with and without a prior diagnosis of UC [28]. Albeit in a different study cohort and within the modality of a rule-out test, the Cxbladder results presented here compare favorably.

When CxbT and CxbD are used concurrently in the evaluation of hematuria, CxbT optimizes the rule out of patients (73%) with a low risk of having UC, and further clinical resolution is obtained when CxbD is used on samples that test positive for CxbT. This finding supports previous data that concurrent use of both Cxbladder test modalities provided additional clinical utility relative to using CxbT alone in the diagnostic process [21]. CxbD is also used in the diagnostic pathway to detect cancer with high specificity and positive predictive value [22].

These data indicate that Cxbladder is an effective reflex test in patients with an atypical result on cytology, with CxbT used to evaluate hematuria and CxbM to monitor for recurrence.

Limitations of our study include the fact that cytology interpretation was not centralized, rather a deliberate choice to better reflect real-world clinical conditions. In addition, although the proportion of atypical cytologies was within the representative range, there were a limited number of atypical cytologies with equivocal cystoscopies.

5. Conclusions

The Cxbladder test significantly outperformed urine cytology for identifying patients with UC, and Cxbladder correctly adjudicated all atypical cytology and atypical cytology with equivocal cystoscopy. Cxbladder and cystoscopy were concordant for all positive cases previously categorized as atypical by local cytology, including all positive UC cases that were atypical for cytology and equivocal for cystoscopy. Significant utility is gained from the inclusion of Cxbladder in the evaluation of patients for UC in both hematuria and monitoring settings, with 35% of patients avoiding cystoscopies. Cxbladder, either as a reflex to cytology or as a replacement for cytology, would remove the diagnostic dilemma associated with atypical cytology results and equivocal cystoscopy.

Author contributions: Badrinath Konety had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Konety, Shore, Kader, Porten, Daneshmand, Lough, Lotan.

Acquisition of data: Konety, Shore, Kader, Porten, Daneshmand, Lough, Lotan.

Analysis and interpretation of data: Konety, Shore, Kader, Porten, Daneshmand, Lough, Lotan.

Drafting of the manuscript: Konety, Shore, Kader, Porten, Daneshmand, Lough, Lotan.

Critical revision of the manuscript for important intellectual content: Konety, Shore, Kader, Porten, Daneshmand, Lough, Lotan.

Statistical analysis: Konety, Shore, Kader, Porten, Daneshmand, Lough, Lotan.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.eururo.2019.04.035>.

References

- [1] Davis R, Jones JS, Barocas DA, et al. Diagnosis, evaluation and follow-up of asymptomatic microhematuria (AMH) in adults: AUA guideline. *J Urol* 2012;188(6 Suppl.):2473–81.
- [2] Sanli O, Dobruch J, Knowles MA, et al. Bladder cancer. *Nat Rev Dis Primers* 2017;3:17022.
- [3] Chang SS, Boorjian SA, Chou R, et al. Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. *J Urol* 2016;196:1021–9.
- [4] Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S, Kassouf W. Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. *Urol Oncol* 2015;33:66.e25–3.
- [5] Caraway NP, Katz RL. A review on the current state of urine cytology emphasizing the role of fluorescence in situ hybridization as an adjunct to diagnosis. *Cancer Cytopathol* 2010;118:175–83.
- [6] Tan WS, Sarpong R, Khetrpal P, et al. Does urinary cytology have a role in haematuria investigations? *BJU Int* 2019;123:74–81.
- [7] Nabi G, Greene DR, O'Donnell M. How important is urinary cytology in the diagnosis of urological malignancies? *Eur Urol* 2003;43:632–6.
- [8] Strittmatter F, Buchner A, Karl A, et al. Individual learning curve reduces the clinical value of urinary cytology. *Clin Genitourin Cancer* 2011;9:22–6.
- [9] Karakiewicz PI, Benayoun S, Zippe C, et al. Institutional variability in the accuracy of urinary cytology for predicting recurrence of transitional cell carcinoma of the bladder. *BJU Int* 2006;97:997–1001.
- [10] Paez A, Coba JM, Murillo N, et al. Reliability of the routine cytological diagnosis in bladder cancer. *Eur Urol* 1999;35:228–32.
- [11] Raitanen MP, Aine R, Rintala E, et al. Differences between local and review urinary cytology in diagnosis of bladder cancer. An interobserver multicenter analysis. *Eur Urol* 2002;41:284–9.
- [12] VandenBussche CJ, Allison DB, Gupta M, Ali SZ, Rosenthal DL. A 20-year and 46,000-specimen journey to Paris reveals the influence of reporting systems and passive peer feedback on pathologist practice patterns. *Cancer Cytopathol* 2018;126:381–9.
- [13] de Bekker-Grob EW, van der Aa MN, Zwarthoff EC, et al. Non-muscle-invasive bladder cancer surveillance for which cystoscopy is partly replaced by microsatellite analysis of urine: a cost-effective alternative? *BJU Int* 2009;104:41–7.
- [14] Kavalieris L, O'Sullivan P, Frampton C, et al. Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. *J Urol* 2017;197:1419–26.
- [15] Kavalieris L, O'Sullivan PJ, Suttie JM, et al. 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* 2015;15:23.
- [16] O'Sullivan P, Sharples K, Dalphin M, et al. A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. *J Urol* 2012;188:741–7.
- [17] Altman DG, Bland JM. Diagnostic tests 2: predictive values. *BMJ* 1994;309:102.
- [18] Altman DG, Bland JM. Diagnostic tests. 1: sensitivity and specificity. *BMJ* 1994;308:1552.
- [19] Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. *Acta Paediatr* 2007;96:338–41.
- [20] Umberger RA, Hatfield LA, Speck PM. Understanding Negative Predictive Value of Diagnostic Tests Used in Clinical Practice. *Dimens Crit Care Nurs* 2017;36:22–9.
- [21] Darling D, Luxmanan C, O'Sullivan P, Lough T, Suttie J. Clinical utility of Cxbladder for the diagnosis of urothelial carcinoma. *Adv Ther* 2017;34:1087–96.
- [22] Rosenthal DL, Wojcik EM, Kurtycz DFI. The Paris system for reporting urine cytology. Switzerland: Springer International Publishing; 2016.
- [23] Lotan Y, O'Sullivan P, Raman JD, et al. Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. *Urol Oncol* 2017;35:531.e15–e.
- [24] Deshpande V, McKee GT. Analysis of atypical urine cytology in a tertiary care center. *Cancer* 2005;105:468–75.
- [25] Muus Ubago J, Mehta V, Wojcik EM, Barkan GA. Evaluation of atypical urine cytology progression to malignancy. *Cancer Cytopathol* 2013;121:387–91.
- [26] Lotan Y, Bensalah K, Ruddell T, Shariat SF, Sagalowsky AI, Ashfaq R. Prospective evaluation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. *J Urol* 2008;179:2164–9.
- [27] Schlomer BJ, Ho R, Sagalowsky A, Ashfaq R, Lotan Y. Prospective validation of the clinical usefulness of reflex fluorescence in situ hybridization assay in patients with atypical cytology for the detection of urothelial carcinoma of the bladder. *J Urol* 2010;183:62–7.
- [28] Odisho AY, Berry AB, Ahmad AE, Cooperberg MR, Carroll PR, Konety BR. Reflex ImmunoCyt testing for the diagnosis of bladder cancer in patients with atypical urine cytology. *Eur Urol* 2013;63:936–40.