


Diagnostic Predictive Value of the Bladder EpiCheck Test in the Follow-Up of Patients With Non-Muscle-Invasive Bladder Cancer

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BACKGROUND: The objective of this study was to evaluate the diagnostic accuracy of the Bladder EpiCheck test in the follow-up of patients with non-muscle-invasive bladder cancer (NMIBC) and to compare it with the accuracy of urinary cytology, cystoscopy, and/or histology. **METHODS:** In total, 243 patients were enrolled in the current study. Patients were evaluated by voided urine cytology, by the Bladder EpiCheck test, and by white-light cystoscopy. **RESULTS:** Overall sensitivity was 33.3% for cytology, 62.3% for Bladder EpiCheck, and 66.7% for the 2 tests combined. The sensitivity of cytology increased from 7.7% in low-grade (LG) tumors to 66.6% in high-grade (HG) tumors; whereas, for the Bladder EpiCheck test, the sensitivity was 46.1% in LG tumors and 83.3% in HG tumors. Combined cytology and Bladder EpiCheck testing yielded an overall sensitivity of 56.4% for LG tumors and 90% for HG tumors. Overall specificity was 98.6% for cytology, 86.3% for Bladder EpiCheck, and 85.6% for the 2 tests combined. The positive predictive value was 92% for cytology and 68.2% for Bladder EpiCheck. For the 2 tests combined, it was 68.6%. The negative predictive value was similar for the 2 tests: 75.8% for cytology, 82.9% for Bladder EpiCheck, and 84.5% for the 2 tests combined. **CONCLUSIONS:** The sensitivity of the Bladder EpiCheck test was significantly higher than that of cytology. The test performed very well in terms of specificity but could not reach the high value of cytology. The positive predictive value was higher for Bladder EpiCheck, whereas the negative predictive value was approximately the same for both tests. **Cancer Cytopathol 2019;0:1-5.** © 2019 American Cancer Society.

KEY WORDS: bladder cancer; cytology; follow-up; non-muscle-invasive; urinary marker.

INTRODUCTION

Bladder cancer (BC) is the 5th leading cancer in Europe¹ and is one of the cancers with the highest lifetime cost because of the high recurrence rate and the need for ongoing, invasive monitoring.² Cystoscopy is the most efficient method currently available for the diagnosis of primary or recurrent urothelial carcinoma (UC) of the bladder, but it is invasive and causes significant discomfort to the patient. Furthermore, flat tumors or carcinoma in situ may be difficult to detect.³ Urinary cytology is noninvasive and very effective in diagnosing high-grade (HG) lesions, but it has a sensitivity of 11% to 17% in low-grade LG tumors, which are the most common types.^{4,5}

The limitations of cytology and cystoscopy for both primary diagnosis and monitoring of patients after UC led to the ongoing research into urinary markers for the early detection of BC over decades. These tests

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are more sensitive than cytology in LG tumors, but their specificity is low, and cystoscopy is still always essential. Moreover, in HG tumors, their sensitivity is lower than that of voided urinary cytology, so that the latter, likewise, is not rendered unnecessary.⁶ Therefore, most of the developed tests have not been implemented in clinical practice.⁷

Recently, new markers have been tested, including methylation-based markers. DNA methylation in UC seems to be related to the progression of primary non-muscle-invasive BC (NMIBC) to muscle-invasive BC.⁸

The Bladder EpiCheck test (Nucleix Ltd) is a newly developed urinary marker based on DNA methylation changes associated with BC in a panel of 15 genomic biomarkers. It is intended for use as a non-invasive method of monitoring for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with BC.

The assay generates a numerical EpiScore (range, 0-100) reflecting the overall methylation level in the urine sample and does so whether or not the score is consistent with the presence or absence of UC. The test is positive when the EpiScore is ≥ 60 . The objective of this first independent study was to evaluate the diagnostic accuracy of the Bladder EpiCheck test in the follow-up of patients with NMIBC and to compare it with the accuracy of urinary cytology, cystoscopy, and/or histology.

MATERIALS AND METHODS

After approval of the local institutional ethics committee (37-2018) and after informed consent, 243 patients undergoing follow-up for NMIBC in our outpatient department were enrolled in the current study. The mean age of the patients (197 of 243 men and 46 of 243 women) was 72.5 years (age range, 37-94 years).

Patients were followed by voided urine cytology, by the Bladder EpiCheck test, and by white-light cystoscopy, according to the current European Association of Urology guidelines.⁹ We reserved photodynamic cystoscopy for patients with positive cytology and no visible bladder tumor; in our series, none of our patients underwent photodynamic cystoscopy in an outpatient setting.

Any cystoscopically suspicious lesion was biopsied or removed transurethrally, and specimens were evaluated according to the 2017 tumor, node, metastasis (TNM) classification of urinary BC and graded

according to both the 1973 and the 2004 World Health Organization classifications.^{10,11} We defined a patient as negative when white-light cystoscopy, cytology, and histology were negative.

Of the voided urine of every patient, 20 to 30 mL was added to 15 mL Cytolyt fixation liquid (Hologic Inc) in a Falcon tube for urinary cytology and sent along with a minimum of 12 mL of fresh voided urine for the Bladder EpiCheck test to the laboratory.

Cytology

The Falcon tubes were centrifuged for 10 minutes at 2000 revolutions per minute. The resulting cell pellets were resuspended in ThinPrep vials that contained PreservCyt solution and were processed using the TP 5000 System (Hologic Inc).

Cytological evaluation was performed using the Papanicolaou staining procedure and the Paris System for Reporting Urinary Cytology¹² to classify cytological specimens accordingly into negative for HGUC, atypical urothelial cells, suspicious for HGUC, HGUC, LG intraepithelial neoplasia, and not diagnostic. For statistical analysis, negative for HGUC and atypical urothelial cells were grouped as negative, whereas suspicious for HGUC, HGUC, and LG intraepithelial neoplasia were grouped as positive.

Bladder EpiCheck Test

For the Bladder EpiCheck test (Nucleix Ltd), a cell pellet of every urine sample was created. The urine sample was centrifuged twice at $\times 1000g$ for 10 minutes at room temperature. DNA was extracted from the cell pellet using the Bladder EpiCheck DNA extraction kit. The extracted DNA was digested using a methylation-sensitive restriction enzyme, which cleaves DNA at its recognition sequence if it is unmethylated. The quantitative real-time polymerase chain reaction (qRT-PCR) amplification was performed using the Rotorgene Q instrument. The samples were prepared for the PCR assay using the Bladder EpiCheck test kit, and the results were analyzed using the Bladder EpiCheck software. For samples that pass the internal control validation, the software calculates an EpiScore (a number between 0 and 100) representing the overall methylation level of the sample on the panel of biomarkers. An EpiScore ≥ 60 indicates a positive result, and a score < 60 indicates a negative result.

TABLE 1. Demographic and Clinical Characteristics of 243 Patients

Characteristic	No. of Patients (%)
Age, y	
Age: Median ± SD	74 ± 10.5
>65 y	190 (78.2)
Sex	
Men	197 (81.1)
Women	46 (18.9)
First diagnosis	
Low grade	154 (63.4)
High grade	89 (36.6)
Previous BCG	83 (34.2)
Previous MMC	11 (4.5)
Excluded because of error sign in Bladder EpiCheck	28 (11.5)

Abbreviations: BCG, Bacillus Calmette–Guerin; MMC, mitomycin C.

TABLE 2. Overall Sensitivity, Specificity, Positive Predictive Value, and Negative Predictive Value of Cytology, Bladder EpiCheck, and the Combination of the 2 Tests in 215 Evaluable Patients

Variable	Cytology (95% CI), %	EpiCheck (95% CI), %	EpiCheck and Cytology (95% CI), %
Sensitivity	33.3 (22.4-45.7)	62.3 (32.6-59.7)	66.7 (54.3-77.5)
Specificity	98.6 (95.1-99.8)	86.3 (79.6-91.4)	85.6 (78.8-90.9)
PPV	92.0 (73.6-97.9)	68.2 (57.9-77.1)	68.6 (58.8-77.1)
NPV	75.8 (72.6-78.7)	78.6 (72.5-83.9)	84.5 (79.4-84.7)

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Statistical Analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cytology, Bladder EpiCheck, and their combination were calculated and compared with those of cystoscopy/histology. Statistical analysis was performed using the Pearson chi-square test. A *P* value <.05 was considered significant. The area under receiver operating characteristic curve was calculated and tested for significance using the *z* test.

RESULTS

Among 243 patients enrolled in the study, at the time of first diagnosis, 154 tumors were classified as LG, 99 (40.7%) were classified as pathologic Ta grade 1 (pTaG1), 52 (21.4%) were classified as pTaG2, 3 (1.2%) were classified as pT1G2, 89 were classified as HG, 14 (5.7%) were classified as pTaG3, 26 (10.7%) were classified as pT1G3, and 49 (20.2%) were classified as carcinoma in situ. Eighty-three patients (34.2%) were treated with an intravesical therapy of Bacillus Calmette–Guerin, and 11 (4.5%) were treated with mitomycin. Twenty-eight patients (11.5%) had to be excluded because of an error

TABLE 3. Sensitivity of the Tests According to Grading in 69 Tumors, N = 69

Tumor Grade	Sensitivity (95% CI), %		
	Cytology	EpiCheck	Cytology and EpiCheck
Low grade, n = 39	7.7 (1.6-20.9)	46.1 (30.1-62.8)	48.7 (32.4-65.2)
High grade, n = 30	66.7 (47.2-82.7)	83.3 (65.3-94.4)	90 (73.5-97.9)

sign in the Bladder EpiCheck test. Of the 215 remaining patients, 69 (32.1%) had an NMIBC recurrence, 39 (56.5%) had LG NMIBC, and 30 (43.5%) had HG NMIBC. Demographic and clinical characteristics of the patients are provided in Table 1.

Overall sensitivity was 33.3% for cytology, 62.3% for Bladder EpiCheck, and 66.7% for the 2 tests combined. The sensitivity of cytology increased from 7.7% in LG tumors to 66.6% in HG tumors; whereas, for the Bladder EpiCheck test, the sensitivity was 46.1% in LG tumors and 83.3% in HG tumors. Combined cytology and Bladder EpiCheck yielded an overall sensitivity of 56.4% for LG tumors and 90% for HG tumors.

Overall specificity was 98.6% for cytology, 86.3% for Bladder EpiCheck, and 85.6% for the 2 tests combined. The PPV for cytology was 92% and, for Bladder EpiCheck, it was 68.2%. For the 2 tests combined, the PPV was 68.6%. The NPV was very similar for the 2 tests: 75.8% for cytology, 82.9% for Bladder EpiCheck, and 84.5% for the 2 tests combined. Detailed data are provided in Tables 2 and 3.

The final statistical analysis between the tests was highly significant (*P* < .0001) using the Pearson chi-square test. The diagnostic efficacy of Bladder EpiCheck was good, with an area under the curve of 0.785 (95% CI, 0.724-0.838) (Fig. 1).

DISCUSSION

BC is the 10th most commonly diagnosed cancer worldwide.¹³ Approximately 75% of patients present with a superficial tumor that is confined to mucosa or submucosa and has low aggressive potential. These patients usually need life-long follow-up with repeated cystoscopies, which are invasive, expensive, and cause discomfort as well as possible complications. In recent years, several urine markers have been developed to be used during surveillance, but none could replace cystoscopy in the diagnostic evaluation.

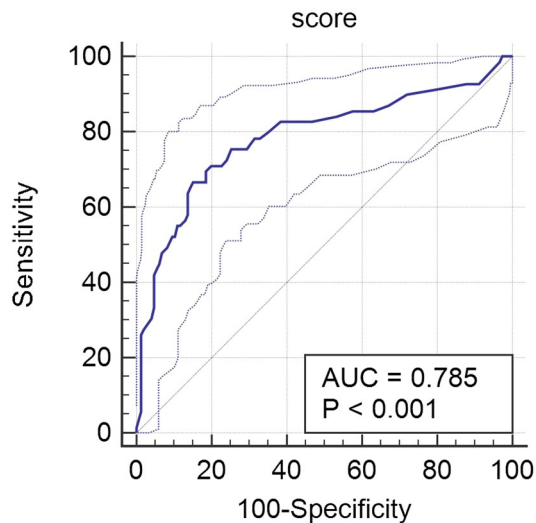


Figure 1. The area under the curve (AUC) is illustrated for all patients irrespective of tumor grade.

Cytology is the most widely used urine marker and, together with cystoscopy, it is very useful in HG tumors; but its sensitivity in LG tumors is very low. Furthermore, the cytological interpretation depends on the experience of the cytopathologist.³

A urine marker with a high sensitivity and specificity could be an additional tool to improve the sensitivity of cytology. Recently, new markers were developed based on qRT-PCR.

The Bladder EpiCheck test is a newly developed urinary marker based on DNA methylation changes associated with BC in a panel of 15 genomic biomarkers. The validation study showed a sensitivity of 90%, a specificity of 83%, and an NPV of 97% in 222 patients with NMIBC who were under surveillance.¹⁴

There are 2 recent studies. The study by Witjes et al¹⁵ included 440 patients and showed a drop-out rate of 8.4%, an overall sensitivity of 68.2%, and a specificity of 88%; and the other study, by D'Andrea et al,¹⁶ also included 440 patients and showed an overall sensitivity of 67.3% and a specificity of 88%.

In our study, 243 patients undergoing follow-up for NMIBC were analyzed with the Bladder EpiCheck test. The drop-out rate was 11.5% because of the learning curve in our laboratory. It appears that most failures were for preanalytic reasons because most were caused by insufficient DNA amounts. In fact, the failure rate dropped from 11.5% to 9% as we gained experience with the test.

The overall sensitivity for the remaining 215 patients was 62.3% for Bladder EpiCheck and 33.3% for cytology.

Combining the 2 tests, the overall sensitivity rose to 66.7%; and, evaluating only the HG tumors, Bladder EpiCheck correctly identified 83.3% and cytology correctly identified 66.6%. Compared with the validation study,¹⁴ the sensitivity in our study is significantly lower (62.3% vs 90%). Because the LG/HG distribution is approximately the same in our study, the only explanation for this difference is the selection bias, which typically occurs in a validation study.¹⁷ The sensitivity reported by Witjes et al¹⁵ and D'Andrea et al,¹⁶ in contrast, is not significantly different from that in our study (68.2% and 67.3% vs 62.3%, respectively).

The specificity reported by Wasserstrom et al¹⁴ was 83%, and that in the studies by Witjes et al¹⁵ and D'Andrea et al¹⁶ was 88%, whereas the specificity in our study was 86.3%. However, the test could not reach the excellent specificity of cytology (98%) but nonetheless performed in an excellent way.

The high NPV reported by previous studies¹⁴⁻¹⁶ could not be reached in our study (97%, 95.1%, and 94.4% vs 82.9%, respectively). The PPV in our study was superior to that reported by Witjes et al¹⁵ (68.2% vs 48.8%) but was not reported by Wassermann et al¹⁶ and thus cannot yet be discussed.

The PPV in our study was significantly higher for cytology (92%) than for Bladder EpiCheck (68.2%), whereas the NPV was very similar for the 2 tests: 75.8% for cytology and 82.9% for Bladder EpiCheck. The PPV and NPV for cytology were not reported in the previous studies.

Comparing the Bladder EpiCheck test with other bladder tumor markers, it is superior to the Bladder Xpert Monitor,¹⁸ nuclear matrix protein 22 (NMP 22) testing, the bladder tumor antigen (BTA) test,¹⁹ or fluorescence in situ hybridization,²⁰ whereas cytology is more specific¹⁸ (see also Grossman et al²¹). In terms of sensitivity, specificity, and NPV, it performs very well with an area under the curve of 0.785.

There are 2 major concerns in contrast to other markers: first, Bladder EpiCheck is an easy-to-evaluate but not an easy-to-do test because a dedicated technician is needed to perform qRT-PCR, and an accordingly equipped laboratory is required. Second, it is not cheap, which would be an important requirement for a marker.

However, Bladder EpiCheck is a highly specific test (86.3%). It can be introduced into the daily routine in molecular pathology laboratories with dedicated technicians. Combining both cytology and Bladder EpiCheck

in the follow-up of patients achieves higher sensitivity, especially in HG tumors (90%). The use of both tests in combination may have the potential to reduce the number of cystoscopies during follow-up for patients with low-risk BC.

Conclusion

In the current study, we report our first experience with the new methylation-based Bladder EpiCheck test. Its sensitivity was significantly higher than that of cytology. The test performed very well in terms of specificity but could not reach the high value of cytology. The PPV was higher for Bladder EpiCheck, whereas the NPV performed approximately the same for both cytology and Bladder EpiCheck.

This new methylation-based test is promising as a urinary marker but needs further optimization in terms of performance and costs. It may be used in combination with cytology to reduce invasiveness in the follow-up of NMIBC, decreasing discomfort for the patients and related costs.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Emanuela Trenti: Acquisition of data and writing—initial draft. **Carolina D’Elia:** Acquisition of data and statistical analysis. **Christine Mian:** Conception and design, acquisition of data, analysis and interpretation of data, and writing—initial draft. **Christine Schwienbacher:** Acquisition of data and analysis and interpretation of data. **Esther Hanspeter:** Acquisition of data and critical revision of the article for important intellectual content. **Alexander Pycha:** Acquisition of data and writing—initial draft. **Mona Kafka:** Critical revision of the article for important intellectual content. **Stephan Degener:** Acquisition of data. **Hansjörg Danuser:** Critical revision of the article for important intellectual content and supervision. **Stephan Roth:** Critical revision of the article for important intellectual content and supervision. **Armin Pycha:** Conception and design, critical revision of the article for important intellectual content, and supervision.

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