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Performance of the Bladder EpiCheck™ Methylation Test for Patients Under Surveillance for Non–muscle-invasive Bladder Cancer: Results of a Multicenter, Prospective, Blinded Clinical Trial

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Abstract

Background: The highly frequent strategy of surveillance for non–muscle-invasive bladder cancer (NMIBC) involves cystoscopy and cytology. Urine assays currently available have not shown performance sufficient to replace the current gold standard for follow-up, which would require a very high negative predictive value (NPV), especially for high-grade tumors. Bladder EpiCheck (BE) is a novel urine assay that uses 15 proprietary DNA methylation biomarkers to assess the presence of bladder cancer.

Objective: To assess the performance of BE for NMIBC recurrence.

Design, setting, and participants: This was a blinded, single-arm, prospective multicenter study. The inclusion criteria were age ≥ 22 yr, urothelial carcinoma (UC) being monitored cystoscopically at 3-mo intervals, all UC resected within 12 mo, able to produce 10 ml of urine, and able to consent.

Outcome measurements and statistical analysis: The BE test characteristics were calculated and compared to cytology and cystoscopy results confirmed by pathology.

Results and limitations: Out of 440 patients recruited, 353 were eligible for the performance analysis. Overall sensitivity, specificity, NPV, and positive predictive value were 68.2%, 88.0%, 95.1%, and 44.8%, respectively. Excluding low-grade (LG) Ta recurrences, the sensitivity was 91.7% and NPV was 99.3%. The area under receiver operating characteristic (ROC) curves with and without LG Ta lesions was 0.82 and 0.94, respectively.

Conclusions: In follow-up of NMIBC patients, the BE test showed an overall high NPV of 95.1%, and 99.3% when excluding LG Ta recurrences. With high specificity of 88.0%, the test could be incorporated in NMIBC follow-up since high-grade recurrences would be instantly detected with high confidence. Thus, the current burden of repeat cystoscopies and cytology tests could be reduced.

Patient summary: The Bladder EpiCheck urine test has a clinically relevant and high negative predictive value. Its use in clinical routine could reduce the number of follow-up cystoscopies, and thus associated patient and financial burdens.

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1. Introduction

For decades, urinary tests have been studied for the detection of bladder tumors. However, most of these tests have not been implemented in clinical practice because of cost issues, practical aspects, or insufficient sensitivity or specificity as compared to the gold standard [1]. Consequently, in patients for whom a bladder tumor is suspected, cystoscopy or ultrasound imaging is usually performed rather than relying on a urinary test. In follow-up, however, the situation may be different. Missing an asymptomatic low-risk recurrence that can be subsequently picked up by the next cystoscopy appears to be safe and costs-effective, decreases the patient burden, and meets their requirements [2,3]. This would require a test with a high negative predictive value (NPV) at least for high-risk tumors.

In the last couple of years, new markers have been developed and tested, including DNA methylation markers, amongst others. DNA methylation alters gene expression without changing the underlying DNA sequence. Typically, there is hypermethylation of tumor suppressor genes and hypomethylation of oncogenes [4]. DNA methylation in bladder cancer has been described, and validated to be related to progression of primary pTaG1/2 bladder tumors to muscle-invasive disease [5]. Methylation was even reported as being useful in hematuria patients for reducing diagnostic cystoscopies [6].

The Bladder EpiCheck test analyzes 15 methylation biomarkers and determines whether this pattern is consistent with bladder cancer presence or absence. The validation study [7] showed 90% sensitivity, 83% specificity, and NPV of 97% among 222 NMIBC patients undergoing surveillance.

The primary objective of the current study was to determine the sensitivity and specificity of Bladder EpiCheck for patients undergoing surveillance for recurrent bladder cancer. The sensitivity and specificity of the test were compared to a prespecified reference standard of cystoscopy, cytology, and histology.

2. Patients and methods

This was a multicenter, prospective, blinded, single-arm, single-visit cohort study. The Bladder EpiCheck result was not intended to be used in patient management, and investigators were blinded to the test results. Urine for testing was collected before standard-of-care cystoscopy at the outpatient urology clinic. If cystoscopy or cytology was suspicious for recurrence, histology was performed, histology samples were obtained via either direct biopsy or a planned transurethral resection of bladder tumor (TURBT). In defining the reference standard (cystoscopy, cytology, and histology), pathology was used as the key determinant; if pathology was available and positive, then the sample was deemed "positive". If the cytology was positive but pathology was negative, the result was considered inconclusive by the reference standard and was excluded from the final analysis. If a patient had positive cystoscopy but the pathology was absent, the reference standard was considered positive if there was a clinical decision to start

oncologic treatment (ie, only patients for whom TURBT was planned but refused, and whose physicians stated there was clear disease present). Patients with positive or equivocal results for either cystoscopy or cytology who lacked confirmatory pathology and a clinical decision to treat were also classified as having an inconclusive diagnosis by the reference standard and were excluded from the final analysis.

The inclusion criteria were: (1) incident or recurrent bladder urothelial carcinoma (UC) undergoing cystoscopic surveillance at 3-mo intervals (adjuvant intravesical therapy allowed); (2) all UC resected within the previous 12 mo; (3) able to provide informed consent; and (4) age ≥ 22 yr. The exclusion criterion was planning to undergo radical cystectomy or chemotherapy-radiation for UC. All data were collected during one visit. Demographic information including risk factors, medical history, and relevant family history was collected. Results for cystoscopy and cytology were noted, and if a lesion was detected, the date and result of histological confirmation were recorded. A urine sample for the Bladder EpiCheck test was collected. Subjects were free to withdraw from participation for any reason at any time. Subjects who withdrew from the study were not replaced. The study was approved by the hospitals' ethics committees and all subjects signed informed consent before any study-related procedure (NCT02647112).

Bladder EpiCheck (Nucleix, Rehovot, Israel) is a urine test developed to monitor recurrence of bladder cancer according to 15 DNA methylation biomarkers. The test was performed on ≥ 10 ml of urine and processed within 5 d in a central laboratory (Germany or Israel). Processing includes centrifugation to separate the cell pellet, from which DNA is extracted. The extracted DNA is digested using a methylation-sensitive restriction enzyme that cleaves DNA at recognition sequences if it is unmethylated, while leaving methylated sequences intact. Digested DNA is then amplified via real-time polymerase chain reaction with locus-specific primers and probes (8 wells per sample), and the resulting data are analyzed using the Bladder EpiCheck software. The report for each patient contains a quantitative score (EpiScore) and a positive/negative interpretation. The EpiScore is a number between 0 and 100, with a higher score indicating more methylation; an EpiScore ≥ 60 is considered a positive result.

All data complied with the *Journal of the National Cancer Institute* biomarker reporting standards.

2.1. Statistical methods

The primary efficacy endpoint was a binary diagnosis (negative or positive) for both Bladder EpiCheck and the reference standard. A secondary endpoint for each subject was the EpiScore continuous measure. This study had two co-primary aims; to show that sensitivity is $\geq 65\%$ and that specificity is $\geq 65\%$ with 95% confidence. The study plan was to enroll a sample size of approximately 400 subjects. Given the prevalence reported, this sample was expected to yield approximately 22 positive cases. Assuming device sensitivity of 90% and specificity of 80%, this sample size would

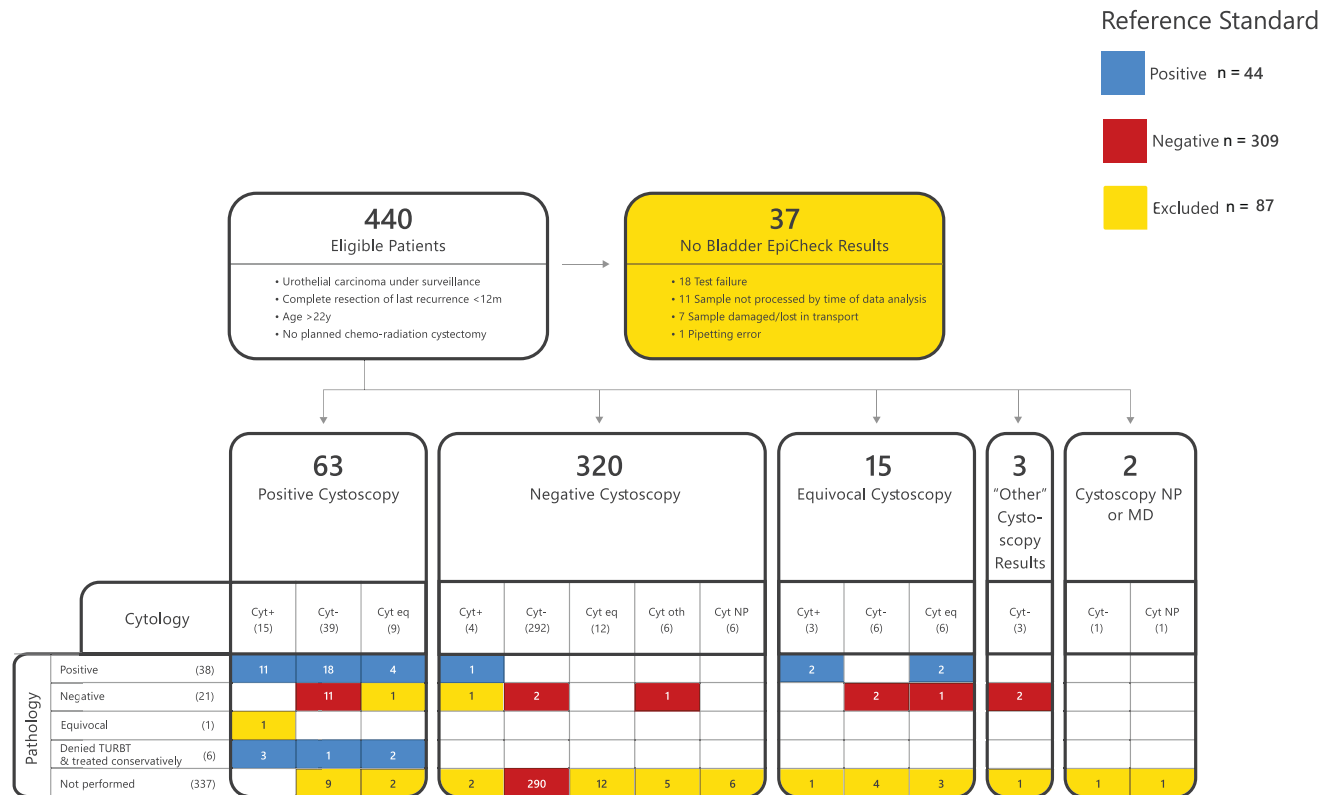


Fig. 1 – Flow chart for performance analysis. Cyt+ = positive cytology; Cyt- = negative cytology; Cyt eq = equivocal cytology; Cyt oth = other cytological result; Cyt NP = Cytology not performed; NP = not performed; MD = missing data.

provide precision of 14% in estimating sensitivity and of 5% in estimating specificity with the corresponding 95% exact binomial confidence intervals (CIs). Additional accuracy statistics were planned, including positive predictive value (PPV) and NPV, the positive and negative likelihood ratio (PLR and NLR), and a ROC curve, with the area under the ROC curve (AUC) presented as a summary measure for the EpiScore continuous variable. For all accuracy parameters, both point estimates and 95% two-sided CIs are provided. Statistical analyses were performed using SAS v9.4 (SAS Institute, Cary NC, USA).

3. Results

3.1. Data set

Data for 440 subjects attending for routine surveillance from December 2015 until January 2017 were included in study. The performance analysis included 353 subjects; 87 subjects were excluded because of inconclusive diagnosis according to the reference standard ($n = 50$), no Bladder EpiCheck results ($n = 30$), or both ($n = 7$). Figure 1 details the categorization by reference standard to positive, negative, or excluded on the basis of the cystoscopy, cytology, and pathology results, and the availability of Bladder EpiCheck results. Demographics are detailed in Table 1 and are consistent with the bladder cancer population in Europe.

3.2. Test results

Forty-six patients were defined as positive according to the reference standard: 40 based on cystoscopy/cytology and confirmatory pathology (21 LG Ta, 8 high-grade [HG] Ta, 7 HG T1, 1 T2, 3 carcinoma in situ [CIS]), and another six based on cystoscopy and/or cytology and the clinical decision of the treating urologist. Two of the 46 positives did not have Bladder EpiCheck results (1 LG Ta, 1 HG Ta), so the total number of positives included in the performance analysis was 44.

For the 403 patients with a Bladder EpiCheck result, 320 samples (81.1%) were negative and 83 (18.9%) were positive; the mean EpiScore was 31.2 (standard deviation 26.6). Bladder EpiCheck results versus the reference standard are presented in Table 2.

The overall sensitivity was 68.2% (30/44) and overall specificity was 88.0% (272/309). Table 3 lists results for the primary and secondary endpoints, including overall agreement (85.6%), NPV (95.1%), and PPV (44.8%). Table 3 also shows subgroup analyses such as the sensitivity and NPV when LG Ta recurrences are excluded (91.7% and 99.3%, respectively). The two HG tumors that were missed were HG Ta and T2. Five cases were excluded because of positive cytology and negative, equivocal, or no pathology. When including these cases in the performance analysis, the overall sensitivity (67.3%) and NPV (94.4%) remained

Table 1 – Demographic data

Parameter	Result
Median age, yr (range)	70.5 (31.7–92.2)
Age group, n (%)	
<50 yr	22 (5.0)
50–59 yr	48 (10.9)
60–69 yr	142 (32.3)
70–79 yr	140 (31.8)
≥80 yr	88 (20.0)
Gender, n (%)	
Male	341 (77.5)
Female	99 (22.5)
Caucasian race, n (%)	440 (100)
Not Hispanic or Latino ethnicity, n (%)	440 (100)
Smoking status, n (%)	
Never smoked	81 (18.4)
Former smoker	252 (57.3)
Current smoker	107 (24.3)
Occupational exposure, n (%) ^a	
No	231 (52.5)
Yes	56 (12.7)
Don't know/cannot remember	153 (34.8)
Stage and grade of last recurrence, n (%)	
PUNLMP	21 (4.8)
Ta low grade	191 (43.4)
Ta high grade	55 (12.5)
T1	117 (26.6)
T2	1 (0.2)
Carcinoma in situ	47 (10.7)
Unknown	8 (1.8)
Primary tumor, n (%)	
Yes	228 (51.8)
No	210 (47.7)
Unknown	2 (0.5)
Time from recent TURBT to urine collection, n (%)	
≤3 mo	25 (5.7)
3–6 mo	165 (37.5)
6–12 mo	197 (44.8)
>12 mo	46 (10.5)
Missing	7 (1.6)
Treated for recent recurrence, n (%) ^b	
Yes	314 (71.4)
No	126 (28.6)
Treatment type for recent recurrence, n (%)	
Bacillus Calmette–Guérin	87 (19.8)
Mitomycin C	135 (30.7)
Bacillus Calmette–Guérin + mitomycin C	74 (16.8)
Other ^c	18 (4.1)

TURBT = transurethral resection of bladder tumor.

^a Bus driver, rubber worker, motor mechanics, leather (including shoe) worker, blacksmith, machine setter, mechanics, paint industry, or hairdresser.^b Information on treatments was collected for recent recurrence only.^c Other treatments include radiation, chemotherapy (systemic or intravesical), and intravesical antibiotic treatment.

similar. When including these cases in the non-LG Ta performance analysis, the sensitivity and NPV remained excellent under worst and best case assumptions (sensitivity 86.2% and 92.6%, NPV 98.6% and 99.3%, respectively). In total, 38 patients who had positive or equivocal cystoscopy or cytology results without the confirmatory pathology were excluded from the analysis. Figure 2 shows the analysis of specificity and sensitivity (except for LG Ta recurrences) by various risk factors and by study center. Age, gender, treatment for recent recurrence (stopped or ongoing), smoking history, and occupational exposure

Table 2 – Bladder EpiCheck result versus valid reference standard

Bladder EpiCheck result	Valid reference standard			
	Negative	Positive	Excluded	Total
Negative	272	14	34	320
Positive	37	30	16	83
None	28	2	7	37
Total	337	46	57	440

Table 3 – Test characteristics

Parameter	n/N	Result, % (95% CI)
Sensitivity	30/44	68.2 (52.4–81.4)
Specificity	272/309	88.0 (83.9–91.4)
Negative predictive value	272/286	95.1 (91.9–97.3)
Positive predictive value	30/67	44.8 (32.6–57.4)
Sensitivity excluding Ta low grade	22/24	91.7 (73.0–99.0)
Negative predictive value excluding Ta low grade	272/274	99.3 (97.4–99.9)
Sensitivity by subgroup		
Ta	14/27	51.9 (32.0–71.3)
Ta low grade	8/20	40.0 (19.1–64.0)
Ta high grade	6/7	85.7 (42.1–99.6)
T1	7/7	100.0 (59.0–100.0)
Carcinoma in situ	3/3	100.0 (29.2–100.0)
Low grade	8/20	40.0 (19.1–64.0)
High grade	16/18	88.9 (65.3–98.6)
Overall agreement	302/353	85.6 (81.5–89.1)

CI = confidence interval.

had no impact on the test performance. Specificity was significantly different between two sites, probably because of the low volume at one of them ($n = 9$). The test performance was similar in the presence of hematuria (data not shown). Figure 3 shows ROC curves for the test; the AUC is 0.82 when LG Ta recurrences are included, and 0.94 when LG Ta recurrences are excluded. Sixty-seven patients had a positive test result, resulting in a PPV of 44.8%. PPV significantly increased with the EpiScore deciles (Supplementary Fig. 1).

4. Discussion

High recurrence rates of up to 52% after 5 yr mean that the prevalence of NMIBC is high, and it is estimated that more than two million patients worldwide are living with bladder cancer [8]. This implies a high patient and physician burden, and a significant economic impact. Bladder cancer costs in the European Union (EU) were recently reported to be 3% of EU cancer costs and 5% of total EU health care costs [9]. Considering the high recurrence rate of NMIBC and the subsequent frequent, costly, and long follow-up, this is an area that deserves attention.

Standard follow-up tools involve a combination of cystoscopy and cytology. However, cystoscopy does not detect all lesions and is subject to experience. Voided cytology requires trained cytopathologists and has the potential for interobserver variability. Cystoscopy has improved with the introduction of digital cameras and the use of enhanced imaging such as photodynamic

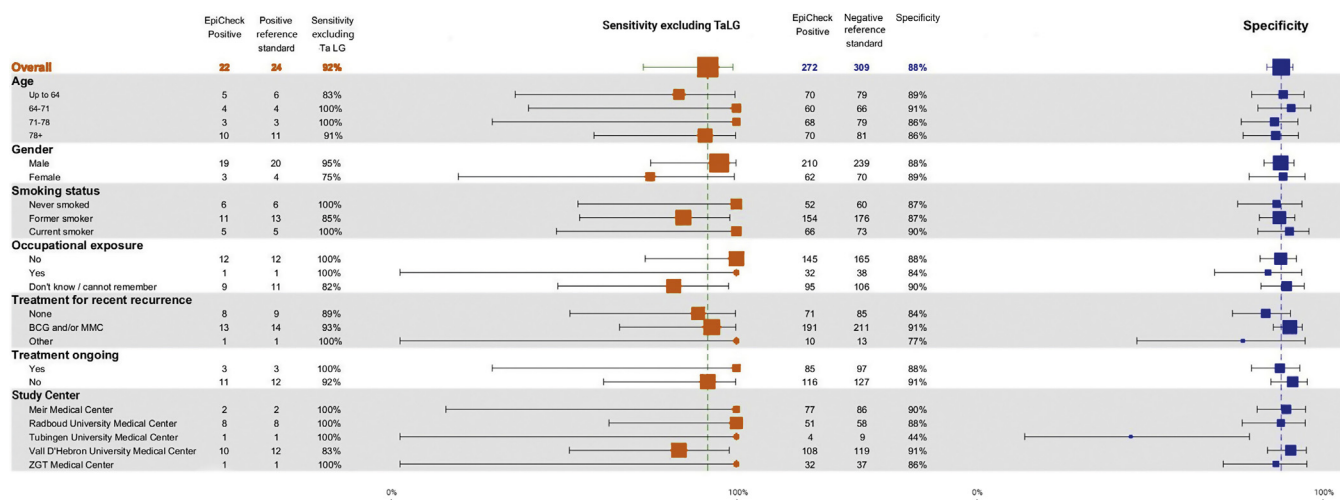


Fig. 2 – Sensitivity excluding LG Ta and Specificity by risk factors and study center. BCG = bacillus Calmette-Guérin; MMC = mitomycin C; LG = low grade.

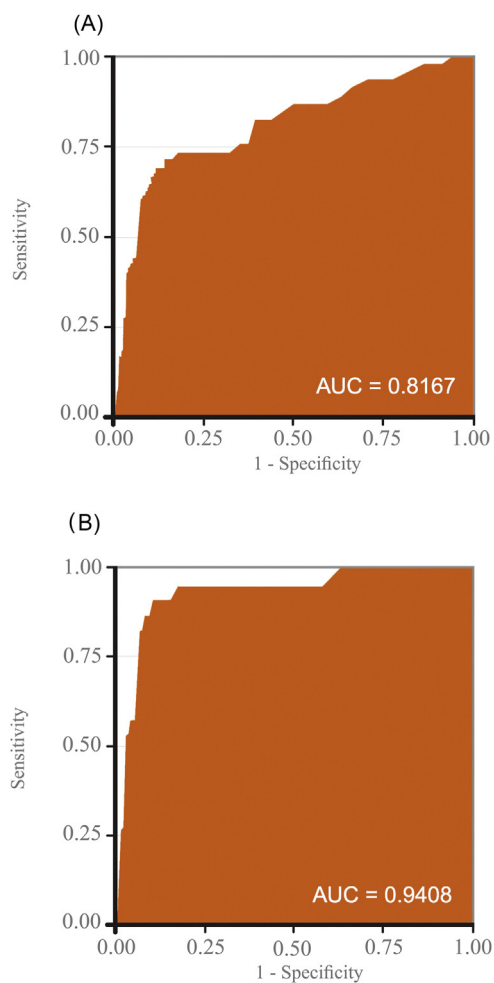


Fig. 3 – Receiver operating characteristic curve for Bladder EpiCheck. (A) overall and (B) non-Ta low grade.

diagnosis (PDD) and narrow-band imaging (NBI) [10–12]. However, PDD is predominantly carried out with rigid instruments during resection. By contrast, NBI is easy to perform during outpatient flexible cystoscopy, but improvements in tumor detection still remain to be proven [12].

Urinary tests instead of or in conjunction with urinary cytology have been studied for decades. Some of these tests have been approved by the US Food and Drug Administration (FDA). The NMP22 point-of-care test, for example, with overall sensitivity (56%) and specificity (86%) is considered insufficient to replace cystoscopy [1]. Better results were reported for the UroVysion fluorescence in situ hybridization test, which has FDA approval for bladder cancer detection and follow-up. The overall sensitivity was 74%, and even 100% in CIS [13]. A false-positive test result was thought to be predictive for future tumor recurrence, since close to 90% of patients with a false-positive test were found to have positive bladder biopsies within a year of their positive test [14]. However, these two tests are not used widely.

Results for a multigene urine biomarker were recently published [15,16]. This test achieved an internally validated sensitivity of 93%, specificity of ~40%, and an NPV of 97%. It was suggested that this test could be useful in postponing follow-up cystoscopies, but its low specificity does not provide a cost-effective solution as the majority of patients would receive a positive result leading to cystoscopy.

Bladder EpiCheck is an easy test that uses 15 DNA methylation biomarkers to assess the presence of bladder cancer. This is the second independent study of Bladder EpiCheck, and our results are consistent with those from the first independent study (validation study). Our study showed a high NPV of 95.1% for the entire cohort, with overall sensitivity of 68.2%. More importantly, the assay can exclude the presence of HG tumors with an NPV of 99.3% and detect their presence with sensitivity of 91.7%. These

results, and the high specificity of 88.0%, could allow incorporation of Bladder EpiCheck in NMIBC follow-up because a HG recurrence would be instantly detected with high likelihood. Age, gender, smoking history, occupational exposure, and recent or ongoing treatment since the last TURBT did not influence the test performance. Such consistent results make this test attractive for use in clinical decision-making across a variety of patients and situations in which existing techniques have a substantial rate of equivocal results. Application of this test could reduce the current burden of repeat cystoscopy and cytology tests, for example, by alternating between the follow-up gold standard (cystoscopy and cytology) and Bladder EpiCheck. Because most NMIBC recurrences will have the same stage and grade as the initial tumor, low-risk cases in particular (approx. 30–40% of the NMIBC cohort in follow-up [17]) could be candidates for an alternating follow-up schedule, which would be beneficial for both urologists and patients. Cost savings are also feasible, depending on the health system and whether the test price is kept in the low hundreds of euro, considering the costs for the gold standard (including cytology, cystoscopy equipment and maintenance, staff time). If LG recurrences are missed, they can be picked up in the next follow-up cystoscopy. For LG recurrences, it has been reported that an active surveillance strategy is safe and cost-effective and can prevent bladder function deterioration due to multiple resections, although this still is a concept that should be further studied [18,19]. There is a substantial unmet need to identify patients during or after recent instillation therapy whose cystoscopy is positive or equivocal, but are actually free from disease. With the Bladder EpiCheck test the chances of missing a HG recurrence are very low, and the confidence in a negative result actually being negative is very high. Therefore, adding the test to routine surveillance in this patient population could provide an opportunity to reduce unnecessary work-up and TURBTs.

As a limitation to our study, no follow-up data were collected. Thus, we were unable to correlate false-positives with later recurrences. No information was collected on treatments before the last recurrence. We did not collect data regarding active UTI, but interference tests for white blood cells showed no impact on Bladder EpiCheck performance [20]. Unlike the specificity and NPV, which have very narrow CIs owing to the large patient groups they represent, the sensitivity is based on 46 patients only and has a wider CI. More follow-up data from these patients are needed for further substantiation of these results.

5. Conclusions

In this prospective multicenter study of the Bladder EpiCheck urine test, we found clinically relevant sensitivity and NPV for patients undergoing routine cystoscopic surveillance for NMIBC. When LG Ta tumors are excluded, the test has sensitivity of 91.7% and an NPV of 99.3%, with high specificity of 88.0%. Bladder EpiCheck is easy to perform, assessing changes in 15 DNA methylation biomarkers. This test could

serve as a rule-out test and help to avoid unnecessary cystoscopic procedures in NMIBC follow-up since HG recurrence would be detected with high confidence. Alternating cystoscopy and cytology with Bladder EpiCheck could reduce the current burden of repeat cystoscopy and cytology tests, which would be beneficial for urologists, health care systems, and patients.

Author contributions: J. Alfred Witjes 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: Witjes.

Acquisition of data: Witjes, Morote, Cornel, Gakis, van Valenberg, Lozano Palacio, Sternberg, Willemsen, Hegemann, Y Paitan, I Leibovitch.

Analysis and interpretation of data: van Valenberg, Witjes.

Drafting of the manuscript: Witjes.

Critical revision of the manuscript for important intellectual content: van Valenberg, Witjes, Morote, Cornel, Sternberg, Paitan, Leibovitch.

Statistical analysis: None.

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Supervision: Witjes.

Other: None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.euo.2018.06.011](https://doi.org/10.1016/j.euo.2018.06.011).

References

- [1] Tilki D, Burger M, Dalbagni G, et al. Urine markers for detection and surveillance of non-muscle-invasive bladder cancer. *Eur Urol* 2011;60:484–92.
- [2] Vriesema JL, Poucki MH, Kiemeny LA, Witjes JA. Patient opinion of urinary tests versus flexible urethrocytoscopy in follow-up examination for superficial bladder cancer: a utility analysis. *Urology* 2000;56:793–7.
- [3] Yossepowitch O, Herr HW, Donat SM. Use of urinary biomarkers for bladder cancer surveillance: patient perspectives. *J Urol* 2007;177:1277–82.

- [4] Gonzalo S. Epigenetic alterations in aging. *J Appl Physiol* 2010;109:586–97.
- [5] Beukers W, Kandimalla R, Masius RG, et al. Stratification based on methylation of TBX2 and TBX3 into three molecular grades predicts progression in patients with pTa-bladder cancer. *Mod Pathol* 2015;28:515–22.
- [6] van Kessel KEM, Beukers W, Lurkin I, et al. Validation of a DNA methylation-mutation urine assay to select patients with hematuria for cystoscopy. *J Urol* 2017;197:590–5.
- [7] Wasserstrom A, Frumkin D, Dotan Z, et al. Molecular urine cytology-Bladder EpiCheck is a novel molecular diagnostic tool for monitoring of bladder cancer patients. *J Urol* 2016;195(Suppl):e140.
- [8] Ploeg M, Aben KK, Kiemeny LA. The present and future burden of urinary bladder cancer in the world. *World J Urol* 2009;27:289–93.
- [9] Leal J, Luengo-Fernandez R, Sullivan R, Witjes JA. Economic burden of bladder cancer across the European Union. *Eur Urol* 2016;69:438–47.
- [10] Burger M, Grossman HB, Droller M, et al. Photodynamic diagnosis of non-muscle-invasive bladder cancer with hexaminolevulinate cystoscopy: a meta-analysis of detection and recurrence based on raw data. *Eur Urol* 2013;64:846–54.
- [11] Mogensen K, Glenthøj A, Toft BG, Scheike T, Hermann GG. Outpatient photodynamic-guided diagnosis of carcinoma in situ with flexible cystoscopy: an alternative to conventional inpatient photodynamic-guided bladder biopsies in the operating theatre? *Scand J Urol* 2017;51:376–80.
- [12] Naito S, Algaba F, Babjuk M, et al. The Clinical Research Office of the Endourological Society (CROES) multicentre randomised trial of narrow band imaging-assisted transurethral resection of bladder tumour (TURBT) versus conventional white light imaging-assisted TURBT in primary non-muscle-invasive bladder cancer patients: trial protocol and 1-year results. *Eur Urol* 2016;70:506–15.
- [13] Hajdinjak T. UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. *Urol Oncol* 2008;26:646–51.
- [14] Gofrit ON, Zorn KC, Silvestre J, et al. The predictive value of multi-targeted fluorescent in-situ hybridization in patients with history of bladder cancer. *Urol Oncol* 2008;26:246–9.
- [15] Kavalieris L, O'Sullivan P, Frampton C. Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. *J Urol* 2017;197:1419–26.
- [16] 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–e22.
- [17] Miñana B, Cózar JM, Palou J, et al. Bladder cancer in Spain 2011: population based study. *J Urol* 2014;191:323–8.
- [18] Hernandez V, Llorente C, de la Penã E, et al. Long-term oncological outcomes of an active surveillance program in recurrent low grade Ta bladder cancer. *Urol Oncol* 2016;34:165.e19–23.
- [19] Hurlle R, Lazzeri M, Vanni E, et al. Active surveillance for low risk nonmuscle invasive bladder cancer: a confirmatory and resource consumption study from the BIAS project. *J Urol* 2018;199:401–6.
- [20] Bladder EpiCheck Instructions for Use with ABI 7500 Fast Dx ver 1.10.2; Bladder EpiCheck Instructions for Use with RotorGene Q, ver 1.0.