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ORIGINAL ARTICLE

DNA methylation analysis in urinary samples: A useful method to predict the risk of neoplastic recurrence in patients with urothelial carcinoma of the bladder in the high-risk group

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Abstract

Background: Recently, it was reported that the Bladder EpiCheck test is likely to represent a valid tool in the diagnostic process of patients who have suspected bladder carcinoma, with some controversial management decisions because of the technical limitations of cytology.

Methods: Two hundred ninety patients with a diagnosis of nonmuscle-invasive bladder carcinoma who were admitted at the authors' department from March 2019 to December 2019 were treated and followed for 1 year. During follow-up, all patients were evaluated by voided urine cytology, white-light cystoscopy (according to European Association of Urology guidelines), and the Bladder EpiCheck test.

Results: The cytologic diagnoses of high-grade urothelial carcinoma (HGUC) and suspicious for HGUC were histologically confirmed in 5 of 20 patients (25%) who had quantitative Bladder EpiCheck scores (EpiScores) from 60 to 69, in 23 of 36 patients (64%) who had EpiScores from 70 to 79, and in 42 of 56 patients (75%) and 57 of 63 patients (90%) who had EpiScores between 80 and 89 and EpiScores >90, respectively. Of 48 patients who had a cytologic diagnosis of HGUC or suspicious for HGUC with EpiScores ≥60 and negative histology, 20 (42%) had a recurrence of HGUC, which was cytologically and histologically confirmed, at 6–12 months during follow-up.

Conclusions: To the best of the authors' knowledge, this is the first study in which patients at high risk for HGUC were stratified using the Bladder EpiCheck EpiScore. The results validate this methylation analysis tool as a useful method for predicting

recurrent HGUC during the follow-up of patients with nonmuscle-invasive bladder carcinoma.

KEYWORDS
bladder carcinoma, high-grade urothelial carcinoma, methylation analysis, urinary cytology

INTRODUCTION

The management of nonmuscle-invasive bladder carcinoma (NMIBC) after transurethral resection of a bladder tumor consists of surveillance, including intravesical therapy and cytology with cystoscopy. The latter represent the most efficient methods currently available for the diagnosis of recurrent urothelial carcinoma during follow-up.

The Bladder EpiCheck test analyzes 15 methylation biomarkers and determines the presence bladder cancer on the basis of the methylation profile. The Bladder EpiCheck test (Nucleix Ltd.) is a newly developed urinary diagnostic test based on DNA methylation changes in a panel of genomic biomarkers that has been proven as a high-performing diagnostic tool in patients with NMIBC and potentially can reduce the number of unnecessary investigations.[1,2]

Recently, it was reported that the Bladder EpiCheck test may represent a valid tool in the diagnostic process of patients who have suspected urothelial neoplasia of the upper urinary tract and in cases that involve a difficult clinical decision because of the technical limitations of upper urinary tract biopsy and cytology. In this regard, the methylation test is likely to assist in the clinical management of these patients.[3] Therefore, use of the Bladder EpiCheck test in combination with cytology seems to reduce the invasiveness of testing during follow-up in patients with NMIBC, with potential benefits for urologists, health care systems, and, as the end point, patients.[4]

The report generated by the Bladder EpiCheck test contains a quantitative score (the EpiScore) ranging between 0 and 100, in which scores ≥60 indicate a positive result (high risk for high-grade urothelial carcinoma [HGUC]), whereas scores <60 indicate a negative result (low risk for HGUC). The objective of the current study was to stratify individuals with a high risk for HGUC, as indicated by the Bladder EpiCheck test, during the follow-up of patients with NMIBC, correlating EpiScores with the incidence of HGUC recurrences confirmed by clinical evidence or histologic biopsies.

MATERIALS AND METHODS

Two hundred ninety patients with a diagnosis of NMIBC who were admitted at our department from March 2019 to December 2019 were treated and followed for 1 year. There were 205 men and 85 women, and the mean patient age was 72.5 years (age range, 47–89 years). From a histologic point of view, the tumors were classified as high-grade papillary carcinoma (grade 3, T1 tumors) in 143 patients; moderately high-grade papillary carcinoma (grade 2, T1 tumors) in 105 patients; and carcinoma in situ in 42 patients. In addition, according to European Association of Urology guidelines,[5] all tumors were considered clinically high-grade neoplasia and thus were included in the overall results. The treatment was intravesical therapy with bacille Calmette–Guérin in 216 patients, whereas 74 patients received treatment with mitomycin.

During follow-up, all patients were evaluated by voided urine cytology and white-light cystoscopy according to European Association of Urology guidelines.[5] In these patients, regular bladder washing specimens were collected for cytology, and the remaining material was stored for the Bladder EpiCheck test. Cytologic evaluation was performed using the Papanicolaou staining procedure,[6] and the diagnoses were formulated according to The Paris System for Reporting Urinary Cytology.[7]

For the Bladder EpiCheck test, the urine sample was centrifugated twice at 1000*g* for 10 minutes at room temperature. DNA was extracted using the Bladder EpiCheck DNA-extraction kit and was digested using a methylation-sensitive restriction enzyme, which cleaves DNA at its recognition sequence if it is unmethylated. The samples were prepared for the polymerase chain reaction assay using the Bladder EpiCheck test kit, and the results were analyzed using the Bladder EpiCheck software. For the samples that passed the internal control validation, an EpiScore (a number between 0 and 100) was calculated, with an EpiScore ≥60 indicating a positive result and an EpiScore <60 indicating a negative result.[1]

Among these patients, 175 received a cytologic diagnosis of HGUC or suspicious for HGUC (SHGUC). Moreover, in patients who had negative cystoscopy results, multiple random bladder biopsies were performed, whereas a single biopsy was performed in those who had cystoscopically evident lesions.

The bladder biopsies were evaluated according to the 2017 tumor, node, metastasis (TNM) classification and graded using both the 1973 and the 2004 World Health Organization classifications.[8,9]

In 115 patients, a cytologic diagnosis of negative for HGUC (NHGUC) or atypical urothelial cells was made, and this group was followed for 1 year; we evaluated the upper urinary tract and urethra and repeated urinary cytology (voided specimens or bladder washings at follow-up cystoscopy) every 3 months for 1 year. To date, all patients in this group remain cystoscopically and cytologically negative.[5]

For cytologic and histologic diagnoses, slides were reviewed by two experienced uropathologists (F.P. and M.M.). In doubtful cases, a third uropathologist (L.M.L.) was consulted to reach group consensus.[10,11]

For all patients studied, no upper urothelial tract neoplastic lesion was found during follow-up. Moreover, in all patients who had negative bladder biopsies and urinary cytology and a Bladder EpiCheck test that was positive for HGUC, ureteroscopy and selective ureteral catheterization were performed to rule out a neoplasm of the upper urinary tract.

Statistical analysis was performed using Sigma Plot 13.0 statistical software (Systat Software Inc.) and GraphPad Prism 5 software (Graph Pad Software). Differences between categorical variables were determined using the χ^2 test or the Fisher exact test, as appropriate.

A p value < .05 was accepted for statistical significance. The area under receiver operating characteristic curve (AUC) was calculated and tested for significance using the z test.[12]

RESULTS

In the group that had positive cytology for HGUC or SHGUC, the bladder biopsies confirmed a recurrence of HGUC in 127 of 175 patients (72.6%), whereas 48 patients (37.4%) were histologically negative for HGUC, and a diagnosis of urothelial dysplasia with inflammation and reactive cytologic changes was made. The Bladder EpiCheck test showed an EpiScore ≥60 in all of these patients: in 20 of 175 patients (11.4%), the EpiScore was between 60 and 69; in 36 of 175 patients (20.6%), the EpiScore was between 70 and 79; and, in 56 of 175 patients (32%) and 63 of 175 patients (36%), the EpiScores were 80–89 and >90, respectively.

The cytologic diagnoses of HGUC and SHGUC were histologically confirmed in 5 of 20 patients (25%) who had EpiScores from 60 to 69, in 23 of 36 patients (64%) who had EpiScores from 70 to 79, and in 42 of 56 patients (75%) and 57 of 63 patients (90%) who had EpiScores of 80–89 and >90, respectively (Figure 1).

Moreover, stratifying the patients in two groups with EpiScores <80 and ≥80, we found that the histologic diagnosis of recurrent HGUC seemed to be correlated with patients who had EpiScores ≥80 (p < .0001; odds ratio, 10.45; 95% CI, 4.992–21.87), suggesting a difference between the two groups from a molecular point view that was not identified by cytologic features (Figure 2).

The diagnostic efficacy of an EpiScore >60 to diagnose HGUC was excellent, with an AUC of 0.811 (p < .001; 95% CI, 0.745–0.866; Youden index, J = 0.565; sensitivity, 83.62; specificity, 72.88; Figure 3A) and was better than the diagnostic efficacy of an EpiScore >80, which had an AUC of 0.686 (p = .0074; 95% CI, 0.595–0.768; Youden index, J = 0.316; sensitivity, 61.62; specificity, 70.00; Figure 3B).

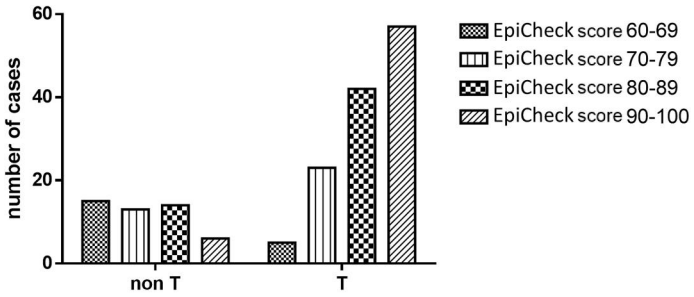
In the remaining 48 patients who had a cytologic diagnosis of HGUC or SHGUC, an EpiScore ≥60, and negative histology, 20 of 48 patients (42%) showed a recurrence of HGUC, which was cytologically and histologically confirmed, at 6–12 months during follow-up (Figure 4). This value reached a percentage of 61% when considering only patients who had EpiScores >70. In particular, we identified a recurrence of HGUC in 7 of 13 patients with EpiScores between 70 and 79 and in 7 of 14 patients with EpiScores between 80 and 89, whereas all patients with EpiScores >90 were positive for HGUC by 6 months during follow-up. No patients with EpiScores between 60 and 69 and negative histology had an HGUC recurrence identified during follow-up.

Moreover, all patients who had a cytologic diagnosis of NHGUC or atypical urothelial cells had an EpiScore <60, and none of these patients had clinical evidence of an HGUC recurrence during follow-up.

DISCUSSION

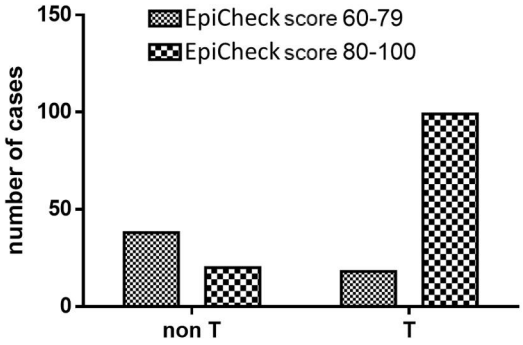
Several studies analyzing the performance of the Bladder EpiCheck test showed a sensitivity of 90% and a specificity of 83%, with a negative predictive value of 97%, in patients with NMIBC who were under surveillance.[1,2,13–15] When comparing the Bladder EpiCheck test with cytology, the former showed significantly higher sensitivity than the latter, whereas the specificity was comparable in the two assays[15]

Therefore, it has been suggested that the Bladder EpiCheck test could be introduced into daily routine practice in laboratories that



	EpiCheck score 60-69	EpiCheck score 70-79	EpiCheck score 80-89	EpiCheck score 90-100
non T	15	13	14	6
T	5	23	42	57

FIGURE 1 Correlation between the histologic recurrences of high-grade urothelial carcinoma and scores on the EpiCheck test. non T, non Tumor; T, tumor.



	EpiCheck score 60-79	EpiCheck score 80-100
non T	38	20
T	18	99

FIGURE 2 Correlation between the group of patients who had EpiScores ≥80 and a histologic diagnosis of high-grade urothelial carcinoma. non T, non Tumor; T, tumor.

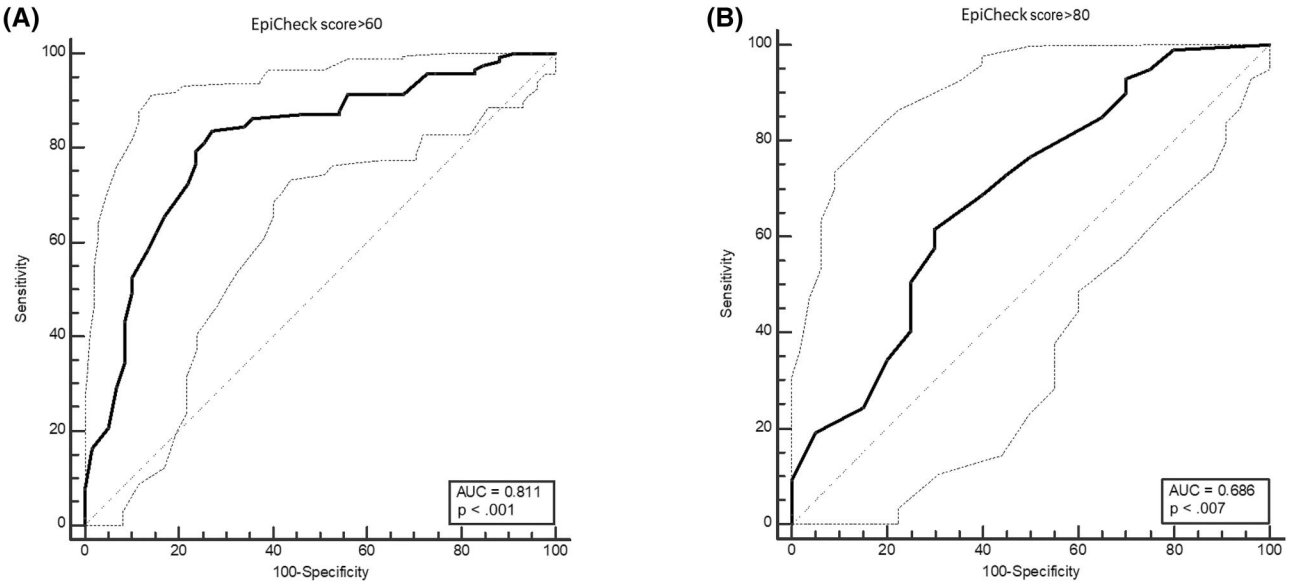


FIGURE 3 Receiver operating characteristic curves are illustrated for scores on the Bladder EpiCheck test using threshold scores of (A) ≥80 and (B) ≥60 as cutoffs for indicating a high risk of high-grade urothelial carcinoma. AUC indicates area under the receiver operating characteristic curve.

have a wide experience in molecular pathology, thus reducing the number of unnecessary investigations: this new test, used in combination with cytology, may be of great benefit to urologists, health care systems, and patients.[15,16]

The Bladder EpiCheck test analyzes 15 methylation biomarkers and determines whether the methylation pattern is consistent with the presence or absence of bladder carcinoma, indicating a high risk for high-grade carcinoma if the level of methylation (EpiScore) is ≥60.

To stratify this high risk, we analyzed a group of patients who had cytologic diagnoses of HGUC and SHGUC and EpiScores ≥60, correlating the clinical and histologic recurrence of high-grade carcinoma with EpiScore values.

In a previous study analyzing the correlation between cytology and the Bladder EpiCheck test, we demonstrated that the EpiScore value increased in The Paris System for Reporting Urinary Cytology categories from NHGUC to HGUC and that an EpiScore <60 was correlated with the cytologic diagnoses of NHGUC and atypical urothelial cells, whereas an EpiScore ≥60 seemed to correlate with the HGUC and SHGUC categories.[4]

In our group of patients who had cytology consistent with HGUC and EpiScores ≥60, the cytologic and molecular results were confirmed in 127 of 175 patients (72.6%); whereas, in 48 of 175 patients, histology was negative (37.4%), and no neoplastic lesions in the bladder or upper urothelial tract were clinically demonstrated in these patients.

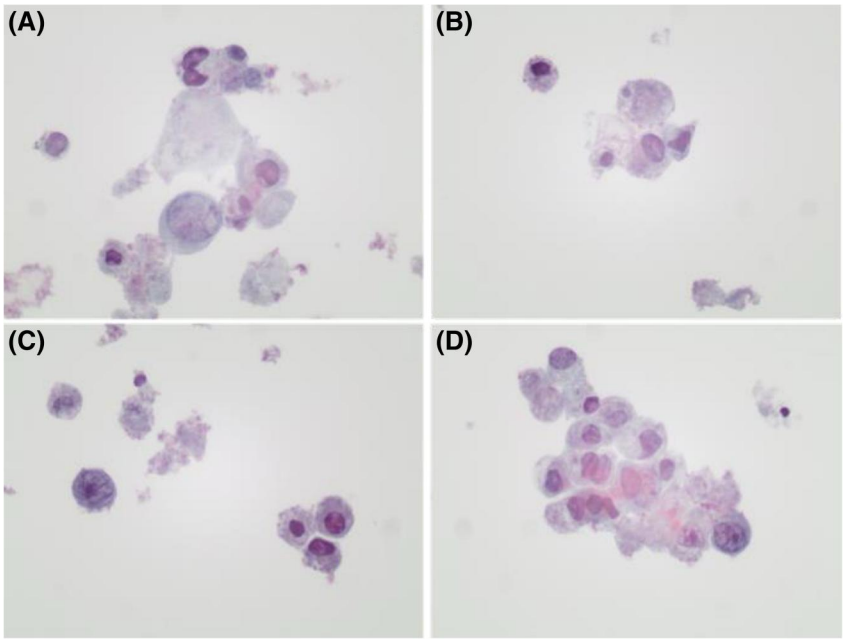


FIGURE 4 Photomicrographs of representative urinary cytology: (A) Cytology-positive for high-grade urothelial carcinoma (HGUC), Bladder EpiCheck-positive, and bladder biopsies negative. The patient remained negative during follow-up. Urothelial cells with regressive features are present. (B) Cytology-positive for HGUC, Bladder EpiCheck-positive, and bladder biopsies negative. The patient showed recurrence of HGUC after 6 months during follow-up. (C,D) Cytology-positive for HGUC, Bladder EpiCheck-positive, and bladder biopsies positive (original magnification ×40 in A–D).

Our data seem to indicate that, by analyzing the EpiScore, we could stratify patients according to their high risk for HGUC by identifying score categories with a different risk of bladder carcinoma and, in patients with NMIBC during follow-up, a different frequency of recurrence.

Patients with EpiScores between 81 and 90 had a percentage of histologic positivity for HGUC that was double that seen in patients with EpiScores <80 (64% vs. 32%), whereas patients with EpiScores >90 received a diagnosis of HGUC at a percentage four times higher than that in patients with EpiScores <70.

Moreover, when using an EpiScore threshold of ≥80, we observed a significant association between the group of patients with an EpiScore ≥80 and a histologic diagnosis of HGUC ($p < .0001$; odds ratio, 10.45; 95% CI, 4.992–21.87; Figure 2), suggesting that this EpiScore value could be used as a threshold to identify HGUC recurrences in patients with NMIBC during follow-up.

The receiver operating characteristic curve for the Bladder EpiCheck test showed that, when considering the new threshold of ≥80 as the EpiScore cutoff indicating a high risk for HGUC in these patients, the diagnostic efficacy of the Bladder EpiCheck test in terms of sensitivity and specificity was lower than that with a threshold cutoff EpiScore of ≥60 (Figure 3A,B).

Correlating histologic recurrences of high-grade carcinoma with the EpiScores, we observed that the methylation level progressively increased in concomitance with the rise in frequency of HGUC recurrences, from a value of 25% to 90%, whereas the frequency of urothelial dysplasia progressively decreased by a minimum of 10% in patients who had EpiScores >90.

This risk stratification does not seem to be influenced by intravesical therapy, because we did not observe any statistically significant correlation between patients who received treatment with either bacille Calmette–Guérin or mytomicin and their EpiScore levels.

Our data seem to support the hypothesis that methylation is a dynamic process involving urothelial dysplastic cells in a preneoplastic condition and in an early phase of bladder carcinogenesis. The increase in methylation levels could indicate progression to a neoplastic stage.

Previous studies demonstrated that considerable methylation changes are present in both preinvasive and invasive urothelial carcinoma, with a statistically significant trend toward increasing methylation levels in more aggressive tumors.[17]

Catto et al. suggested that methylation in bladder carcinoma is more typical in the invasive stage than in superficial tumor stages and that this epigenetic change occurs at an early phase of neoplastic invasion. Moreover, it has been hypothesized that the association between the presence and extent of methylation and subsequent tumor progression indicates a potential role of this process as a prognostic biomarker.[18]

Interestingly, during the follow-up of patients who had NMIBC with a cytologic diagnosis of HGUC or SHGUC, an EpiScore ≥60, and negative histology, there was a 42% rate of recurrence of HGUC over a period of time between 6 and 12 months. It is noteworthy that all

patients who had EpiScores >90 tested positive at 6 months during follow-up, whereas no patients with EpiScores between 60 and 69 had an HGUC recurrence during follow-up.

The predictive value of the methylation test could have two possible explanations. First, the Bladder EpiCheck test analyzes a greater number of urothelial cells than the number obtained in random bladder biopsies, identifying neoplastic cells present in a bladder area not studied with histologic biopsies. Second, the methylation process involves urothelial cells in a preneoplastic condition or in an early phase of urothelial carcinogenesis. The correlation between the increasing levels of methylation and the frequency of HGUC recurrence during follow-up may support this hypothesis. In addition, the predictive value of the methylation test could be very important from a clinical point of view to identify patients for whom further clinical and histologic investigations are required during follow-up.

Analyzing our results, 28 patients who had cytology and Bladder EpiCheck tests that were positive for HGUC had negative biopsies that showed urothelial dysplasia of low grade, with inflammation in >60% of cases, and this inflammatory condition could explain the cytologic features that mimic neoplastic cells. Moreover, all of these patients had EpiScores between 60 and 69, indicating that low-level methylation may represent the first step in a neoplastic process that, in some patients, could preclude a *definitive* neoplastic condition. Further studies with more patients are needed to validate this hypothesis.

A limitation of this study is the retrospective design and the relatively small number of clinical cases registered in a single center. Moreover, we examined bladder washing specimens, and further validation of the results on spontaneous urine are needed, especially considering that voided urine was used in the follow-up of urothelial neoplasms to avoid cystoscopy.

Another possible limitation is represented by the splitting the specimens for cytology and for the Bladder EpiCheck test, even if, to reduce this bias, minimum cutoff volumes of 30 ml for cytology and 10 ml for methylation analysis were observed.[1,7]

Recurrence risk stratification based on methylation levels and the ability of the methylation test to predict the risk of relapse in patients with NMIBC need further validation and investigations: nevertheless, our work could represent a starting point in addressing this topic and may pave the way for further studies on larger patient cohorts.

In conclusion, in this study, for the first time to our knowledge, we stratified the high risk for HGUC, as indicated by the Bladder EpiCheck test in patients with NMIBC during follow-up. Moreover, we validated a combined approach of cytology and the methylation test not only in terms of diagnosis of HGUC but also as a predictive method for clinical and histologic recurrences.

AUTHOR CONTRIBUTIONS

Francesco Pierconti: Planning and drafting of the article. **Esther Diana Rossi:** Planning of the article. **Tonia Cenci:** Molecular data. **Angela Carlino:** Molecular data. **Vincenzo Fiorentino:** Molecular data. **Emilio Sacco:** Clinical aspects. **Angelo Totaro:** Clinical aspects. **Giuseppe Palermo:** Clinical aspects. **Roberto Iacovelli:** Clinical aspects. **Luigi Maria Larocca:** Planning of the article. **Pier Francesco Bassi:** Clinical aspects. **Maurizio Martini:** Statistics and planning the manuscript.

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CONFLICT OF INTEREST

The authors made no disclosures.

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REFERENCES

1. Witjes JA, Morote J, Cornel EB, et al. 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. *Eur Urol Oncol*. 2018;1(4):307-313. doi:10.1016/j.euo.2018.06.011
2. D'Andrea D, Soria F, Zehetmayer S, et al. Diagnostic accuracy, clinical utility and influence on decision-making of a methylation urine biomarker test in the surveillance of non-muscle-invasive bladder cancer. *BJU Int*. 2019;123(6):959-967. doi:10.1111/bju.14673
3. Pierconti F, Martini M, Fiorentino V, et al. Upper urothelial tract high-grade carcinoma: comparison of urine cytology and DNA methylation analysis in urinary samples. *Hum Pathol*. 2021;118:42-48. doi:10.1016/j.humpath.2021.09.007
4. Pierconti F, Martini M, Cenci T, et al. Methylation study of the Paris System for Reporting Urinary (TPS) categories. *J Clin Pathol*. 2021;74(2):102-105. doi:10.1136/jclinpath-2020-206633
5. Babjuk M, Burger M, Comperat EM, et al. European Association of Urology guidelines on non-muscle-invasive bladder cancer (TaT1 and carcinoma in situ)—2019 update. *Eur Urol*. 2019;76(5):639-657. doi:10.1016/j.eururo.2019.08.016
6. Rossi ED, Mule A, Russo RM, Pierconti F, Fadda G. Application of liquid-based preparation to non-gynaecologic exfoliative cytology. *Pathologica*. 2008;100:461-465.
7. Rosenthal DL, Wojcik EM, Kurtycz DFI, eds. *The Paris System for Reporting Urinary Cytology*. Springer International Publishing AG; 2016.
8. Brierley JD, Gospodarowicz MK, Wittekind C. *TNM Classification of Malignant Tumours*. 8th ed. Wiley Blackwell; 2017.
9. Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasm of the urinary bladder. Bladder Consensus Conference Committee. *Am J Surg Pathol*. 1998;22(12):1435-1448. doi:10.1097/00000478-199812000-00001
10. Pierconti F, Rossi ED, Straccia P, et al. The risk of malignancy of atypical urothelial cells of undetermined significance in patients treated with chemohyperthermia or electromotive drug administration. *Cancer Cytopathol*. 2018;126(3):200-206. doi:10.1002/cncy.21957
11. Pierconti F, Milardi D, Martini M, et al. Pituitary-tumour-transforming-gene 1 expression in testicular cancer. *Andrologia*. 2015;47(4):427-432. doi:10.1111/and.12283

12. Bacci L, Aiello A, Ripoli C, et al. H19-dependent transcriptional regulation of β3 and β4 integrins upon estrogen and hypoxia favors metastatic potential in prostate cancer. *Int J Mol Sci*. 2019;20(16):4012. doi:10.3390/ijms20164012
13. Lotan Y, Shariat SF, Lotan Y, et al. Statistical consideration for clinical biomarker research in bladder cancer. *Urol Oncol*. 2010;28(4):389-400. doi:10.1016/j.urolonc.2010.02.011
14. Bensalah K, Montorsi F, Shariat SF. Challenges of cancer biomarker profiling. *Eur Urol*. 2007;52(6):1601-1609. doi:10.1016/j.eururo.2007.09.036
15. Trenti E, D'Elia C, Mian C, et al. Diagnostic predictive value of the Bladder EpiCheck test in the follow-up of patients with nonmuscle-invasive bladder cancer. *Cancer Cytopathol*. 2019;127(7):465-469. doi:10.1002/cncy.22152
16. Pierconti F, Martini M, Fiorentino V, et al. The combination cytology/EpiCheck test in non muscle invasive bladder carcinoma follow-up: effective tool or useless expense? *Urol Oncol*. 2021;39(2):131.e17-131.e21. doi:10.1016/j.urolonc.2020.06.018
17. Salem C, Liang G, Tsai YC, et al. Progressive increases in de novo methylation of CpG islands in bladder cancer. *Cancer Res*. 2000;60(9):2473-2476.
18. Catto JW, Azzouzi AR, Rehman I, et al. Promoter hypermethylation is associated with tumor location, stage, and subsequent progression in transitional cell carcinoma. *J Clin Oncol*. 2005;23(13):2903-2910. doi:10.1200/JCO.2005.03.163

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