Methylation study of the Paris system for reporting urinary (TPS) categories

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ABSTRACT

Aims Bladder EpiCheck is one of several urinary tests studied to identify bladder tumours and analyses 15 methylation biomarkers determining bladder cancer presence on the basis of methylation profile.

Methods 374 patients diagnosed with high-grade non-muscle invasive bladder cancer were treated and followed for 1 year with voided urine cytology and white-light cystoscopy and biopsies according to European Association of Urology Guidelines. 268 cases were diagnosed with high-grade papillary carcinoma, while 106 cases were carcinoma in situ. Bladder EpiCheck test was performed together with cytology in all cases.

Results Comparing cytological categories of negative for high-grade urothelial carcinoma (NHGUC) and atypical urothelial cells (AUCs), we found that an EpiScore <60 correlates with NHGUC (p=0.0003, Fisher's exact test), while comparing AUC and suspicious for high-grade urothelial carcinoma (SHGUC) or SHGUC and high-grade urothelial carcinoma (HGUC) categories, an EpiScore ≥60 correlates with SHGUC and HGUC, respectively (p=0.0031 and p=0.0027, Fisher's exact test). In each TPS category, we found that sensitivity, specificity, Positive Predicitve Value (PPV) and Negative Predictive Value (NPV) of the Bladder EpiCheck test in HGUC category were higher than those observed in SHGUC group (sensitivity=98%, specificity=100%, NPV=85.7%, PPV=100% vs sensitivity=86.6%, specificity=52.3%, NPV=84.6%, PPV=56.5%).

Conclusions Analysing methylation study results, we demonstrated that different TPS cytological categories also carry a distinct molecular signature. Moreover, our results confirm that cytological categories SHGUC and HGUC are different entities also from a molecular point of view and should continue to represent distinct groups in TPS.

INTRODUCTION

In the last few years, numerous urinary tests have been studied to detect bladder tumours, including DNA methylation markers. DNA methylation alters gene expression without changing the underlying DNA sequence, typically hypermethylating tumour suppressor genes and hypomethylating oncogenes.[1]

Bladder EpiCheck test analyses 15 methylation biomarkers and determines bladder cancer presence on the basis of methylation profile.

In late 2015 the International Academy of Cytology and the American Society of Cytopathology published the guidelines of reporting urine cytology, known as The Paris System for Reporting Urinary Cytology (TPS).[2] Urine cytology has low sensitivity and specificity in identifying low-grade urothelial carcinoma (LGUC) and non-urothelial neoplasia, and better sensitivity and specificity in detecting high-grade carcinoma. On this basis, TPS was conceived to detect high-grade urothelial carcinoma (HGUC), identifying different categories for reporting urinary cytology: (1) negative for high-grade urothelial carcinoma (NHGUC); (2) atypical urothelial cells (AUCs); (3) suspicious for high-grade urothelial carcinoma (SHGUC); (4) HGUC; (5) low-grade urothelial neoplasia; and (6) unsatisfactory/non-diagnostic.[2]

In this study, we analysed Bladder EpiCheck scores in different TPS categories during the follow-up of patients diagnosed with non-muscle invasive bladder cancer (NMIBC). Our aim was to assess the validity of this classification not only from a cytological, but also from a molecular point of view.

MATERIALS AND METHODS

The present study represents a retrospective analysis of 374 patients (235 men and 139 women) diagnosed with NMIBC from January 2018 to November 2019. All patients were treated and followed for 1 year at our department. Mean age was 70.5 years (range 45–93 years).

A total of 268 patients had a histological diagnosis of high-grade papillary urothelial carcinoma (180 G3T1 and 88 G2T1), while 106 patients received a diagnosis of carcinoma in situ (CIS).

The treatment consisted of an intravesical instillation of BCG in 305 patients, while 69 patients were treated with mitomycin-C.

During the follow-up patients were evaluated by voided urine cytology and white-light cystoscopy, according to European Association of Urology Guidelines.[3]

One sample of voided urine was collected for each patient for cytological examination and the remaining material was stored for Bladder EpiCheck test.

Cases with cytological diagnosis of NHGUC were followed over time repeating urinary cytology either with voided specimens or with bladder washing at follow-up cystoscopy.

Moreover, patients with a diagnosis of AUC, SHGUC or HGUC underwent cystoscopy within 3 months after urinary cytology and a biopsy was performed during the procedure if a lesion could be identified.

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In cystoscopically negative cases, multiple random bladder biopsies were performed during cystoscopy to identify CIS.[4]

For cytological diagnoses, slides were reviewed by two experienced uropathologists (FP and MM) and in doubtful cases a third uropathologist (LML) was consulted to reach group consensus.[5]

Cytology

Samples were centrifugated for 10 min at 2000 rpm and resulting pellets were resuspended in Thin Prep PreservCyt solution and processed using TP 5000 System (Hologic Inc).

Cytological evaluation was performed using Papanicolau staining procedure and the diagnosis was formulated according to TPS classifying cytological specimens in NHGUC, AUCs, SHGUC, positive for HGUC and unsatisfactory/non-diagnostic.[2]

Bladder EpiCheck test

For Bladder EpiCheck test (Nucleix Ltd), urine sample was centrifugated twice at 1000 g for 10 min at room temperature. DNA extracted using Bladder EpiCheck DNA extraction kit was digested using a methylation-sensitive restriction enzyme, which cleaves DNA at its recongnition sequence if it is unmethylathed. Samples were prepared for PCR assay using Bladder EpiCheck test kit and results were analysed using Bladder EpiCheck software.

Bladder EpiCheck is based on a panel of 15 proprietary DNA methylation biomarkers and on an algorithm developed and validated on patients with NMIBC undergoing monitoring.

For samples passing internal control validation, an EpiScore (a number between 0 and 100) was calculated and in accordance with manufacturer's indications, an EpiScore ≥60 indicated a positive result (high risk for HGUC), while a score <60 indicated a negative result (low risk for HGUC). Moreover, in accordance with manufacturer's indications, an EpiScore ≥90 indicated HGUC.[6 7]

Statistical analysis

Statistical analysis was performed using MedCalc V.10.2.0.0 (StataCorp LP, Texas, USA) and the GraphPad-Prism V.5 software (Graph Pad Software, San Diego, California, USA). Differences between categorical variables were determined using χ^2 test or Fisher's exact test as appropriate.

A p value<0.05 was accepted for statistical significance.

RESULTS

One hundred and sixty out of 374 (43%) cases were classified as NHGUC, 66/374 (18%) cases as AUC, 36/374 (10%) as SHGUC and in 112/374 (29%) a diagnosis of HGUC was made.

In the NHGUC group, we found an EpiScore <60 in 143 (89%)/160 patients and ≥60 in 17 (11%)/160 patients; 66 patients with a diagnosis of AUC showed an EpiScore <60 in 45 (68%) cases while in 21 (32%) cases EpiScore was ≥60; 36 patients with a diagnosis of SHGUC showed an EpiScore <60 in 13 (36%) cases and an EpiScore ≥60 in 23 (64%) cases. Patients with a diagnosis of HGUC showed an EpiScore ≥60 in 98 (87.5%) cases, while the remaining 14 (22.5%) cases had an EpiScore <60.

Considering Bladder EpiCheck results, we found that EpiCheck score increases in TPS categories from NHGUC to HGUC and comparing cytological categories of NHGUC and AUC, we found that an EpiScore <60 correlates with NHGUC (p=0.0003, OR 3.925, 95% CI 1.907 to 8.081, Fisher's exact test), while comparing AUC and SHGUC or SHGUC and HGUC categories, an EpiScore ≥60 correlates with SHGUC and HGUC, respectively (p=0.0031, OR 3.791, 95% CI 1.612 to 8.915 and p=0.0027, OR 3.957, 95% CI 1.639 to 9.550, Fisher's exact test), suggesting differences between TPS categories not only from a cytological point of view, but also from a molecular one (table 1).

Table 1 Epicheck score in different TPS categories

	NHGUC	AUC	
EpiScore <60	143	45	p=0.0003
EpiScore ≥60	17	21	
	AUC	SHGUC	
EpiScore <60	45	13	p=0.0031
EpiScore ≥60	21	23	
	SHGUC	HGUC	
EpiScore <60	13	14	p=0.0027
EpiScore ≥60	23	98	
	SHGUC	HGUC	
EpiScore 60–89	22	52	p=0.0001
EpiScore ≥90	1	46	

AUC, atypical urothelial cell; HGUC, high-grade urothelial carcinoma; NHGUC, negative for high-grade urothelial carcinoma; SHGUC, suspicious for high-grade urothelial carcinoma.

Moreover, analysing patients with HGUC and SHGUC, we found that an EpiScore ≥90 was found in 46 (41%) cases of HGUC and in only 1 (3%) case of SHGUC and that this EpiScore, indicating HGUC, significantly correlates with HGUC (p<0.0001, OR 19.4, 95% CI 2.522 to 150.2, Fisher's exact test).

Correlating EpiScore test results with follow-up in each TPS category, we found that in the NHGUC group all cases with an EpiScore <60 were NHGUC during follow-up, while in patients with an EpiScore ≥60 only 1 case was positive for HGUC after cystoscopy and histology and the remaining 16 cases were negative (sensitivity=100%, specificity=89.9%, Negative Predictive Value (NPV)=100%, Positive Predicitve Value (PPV)=5,5%). In the AUC category, an EpiScore ≥60 was found in 21 cases (9 positive and 12 NHGUC, respectively), while in 45 patients with an Episcore <60 only two cases were positive for HGUC after cystoscopy and histology (sensitivity=81.8%, specificity=52.3%, NPV=95.5%, PPV=42.8%). In the SHGUC group, 13 out of 23 patients with an EpiScore ≥60 were positive for HGUC, while only 2 out of 13 patients with an EpiScore <60 received a diagnosis of HGUC (sensitivity=86.6%, specificity=52.3%, NPV=84.6%, PPV=56.5%). In the HGUC group, all patients with an EpiScore ≥60 were positive for HGUC, while only 2 out of 14 patients with an EpiScore <60 received a diagnosis of recurrent neoplasia (sensitivity=98%, specificity=100%, NPV=85.7%, PPV=100%, table 2).

Table 2 Epicheck score in different TPS categories: Sensitivity, Specificity, PPV and NPV

	NHGUC	AUC	SHGUC	HGUC
Sensitivity	1	0.818	0.866	0.988
Specificity	0.899	0.523	0.523	1
PPV	1	0.428	0.565	1
NPV	0.05	0.846	0.846	0.857

AUC, atypical urothelial cell; HGUC, high-grade urothelial carcinoma; NHGUC, negative for high-grade urothelial carcinoma; NPV, Negative Predictive Value; PPV, Positive Predicitve Value; SHGUC, suspicious for high-grade urothelial carcinoma.

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DISCUSSION

Aberrant methylation is a common epigenetic abnormality in bladder carcinoma with an important role in tumour initiation and progression.[8]

Recently, numerous prognostic methylation markers have been investigated and it has been demonstrated that aberrant methylation of gene promoters can regulate cellular growth, survival and differentiation of neoplastic cells.[9–12]

Bladder EpiCheck test is a urinary assay based on DNA methylation changes associated with bladder carcinoma in a panel of 15 genomic biomarkers. Recently, two different studies validated this test showing an overall sensitivity of 68.2% and 67.3% and a specificity of 88%, respectively.[6 7]

Result of this assay is an EpiScore (a number between 0 and 100) and a value ≥60 indicates a positive result (high risk for HGUC), while a score <60 indicates a negative result (low risk for HGUC). Moreover, an EpiScore ≥90 indicates HGUC.[6 7]

In 2015, The International Academy of Cytology and the American Society of Cytopathology published the guidelines for reporting urine cytology, known as TPS, with the aim to identify HGUC, well knowing poor sensitivity and specificity of urinary cytology in detecting LGUC.[2]

TPS identified four criteria corresponding to different cytological features of urothelial cells: (1) non-superficial and non-degenerated urothelial cells with an increased nuclear/cytoplasmic (N/C) ratio; (2) nuclear hyperchromasia; (3) irregular nuclear membranes; and (4) irregular coarse or clumped chromatin. The presence of all these cytological features in at least 5 to 10 urothelial cells was necessary to make a diagnosis of HGUC, while the presence of the first two criteria and at least one between the third and the fourth identified SHGUC. Moreover, an increased N/C ratio plus one of the remaining three criteria were needed to make a diagnosis of AUCs. All other cases were diagnosed as NHGUC.

In our study, we analysed EpiCheck scores for each TPS category and we found that EpiScore increases in TPS categories from NHGUC to HGUC and comparing cytological categories of AUC and SHGUC or SHGUC and HGUC categories, an EpiScore ≥60 correlates with SHGUC and HGUC, respectively (p=0.0031, OR 3.791, 95% CI 1.612 to 8.915 and p=0.0027, OR 3.957, 95% CI 1.639 to 9.550, Fisher's exact test).

These results suggest that TPS identifies different categories not only from a cytological point of view, but also from a molecular one.

Moreover, a debate has recently arisen about the risk of malignancy of SHGUC and HGUC categories. In fact, several studies have shown a significant concordance between cytology and subsequent histology,[13 14] while other studies demonstrated a lack of concordance, arguing against the necessity of having distinct cytological categories.[15–17]

Our results showed that a Bladder EpiCheck score ≥90 identifies HGUC and this value seems to correlate with HGUC if we compare SHGUC and HGUC categories (p<0.0001, OR 19.4, 95% CI 2.522 to 150.2, Fisher's exact test).

Moreover, analysing Bladder EpiCheck results in each TPS category, we found that sensitivity, specificity, PPV and NPV in HGUC category were higher than those observed in SHGUC group (sensitivity=98%, specificity=100%, NPV=85.7%, PPV=100% and sensitivity=86.6%, specificity=52.3%, NPV=84.6%, PPV=56.5%, respectively).

These data support the hypothesis that, considering methylation levels in a panel of 15 genomic biomarkers, SHGUC and HGUC are different categories not only from a cytological, but also from molecular point of view, and that should remain separate as indicated by TPS.

Our study has some limitations. Primarily, we examined voided urine from patients with NMIBC who were treated with mitomycin-C or BCG-immunotherapy during their follow-up: much evidence shows that these therapies may cause morphological changes, increasing the number of AUCs in urinary samples and, consequently, the number of AUC or SHGUC diagnoses.[18–22] This could represent an important bias and, therefore, all cases were reviewed by at least two experienced uropathologists to reduce hypothetical 'false' AUC or SHGUC diagnoses.[5]

Moreover, it has been described that mitomycin-C can induce DNA methylation changes, altering epigenetic mechanisms that control proteic expression in neoplastic cells: we therefore analysed a methylation gene pattern that could have been altered by therapy.[23–25]

CONCLUSIONS

Analysis of 15 informative DNA methylation biomarkers performed by Bladder EpiCheck test seems to support guidelines of reporting urine cytology, known as TPS, published in 2015 by The International Academy of Cytology and the American Society of Cytopathology. TPS identifies different cytological categories apparently having a different molecular profile and our results confirm that SHGUC and HGUC TPS categories should remain distinct from a molecular point of view.

Multicentre studies with a larger number of cases are needed to confirm our findings.

Take home message

- Bladder EpiCheck test seems to support guidelines of The Paris System for Reporting Urinary Cytology (TPS).
- TPS identifies cytological categories apparently having a different molecular methylation profile.
- Suspicious for high-grade urothelial carcinoma and high-grade urothelial carcinoma TPS categories should remain distinct from a molecular point of view.

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