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Research Report

Potential of an mRNA-Based Urine Assay (Xpert® Bladder Cancer Detection[1]) in Hematuria Patients - Results from a Cohort Study

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Abstract.
BACKGROUND AND OBJECTIVE: Assessment of patients with hematuria (aH) remains a challenge in urological practice, balancing the benefits of diagnosing a potentially underlying bladder cancer (UCa) against the risks of possibly unnecessary diagnostic interventions. This study analyzes the potential of an mRNA-based urine assay, the Xpert® Bladder Cancer Detection- CE-IVD (Xpert BC-D), in patients with hematuria.
MATERIALS AND METHODS: Overall, 368 patients with newly observed painless hematuria and no history of UCa were included in this observational study. Patients received urological workup, including urethrocystoscopy (WLC), upper tract imaging, urine cytology and Xpert BC-D. Patients with positive WLC were recommended to undergo tumor resection (TUR-B).
RESULTS: After excluding non-assessable cases, 324 patients were considered for analysis (188 males, 136 females; median age: 61 years). Eight of twenty-eight patients with a positive TUR-B had Ta low grade (LG) tumors; the others were diagnosed with high grade (HG) lesions (Ta: 4, CIS: 2, T1:11, >T1:3). The Xpert BC-D was more sensitive than urine cytology (96% vs. 61%) ($p = 0.002$). Increased risk ratios (RR) were observed for gross hematuria, gender, urine cytology, and positive Xpert BC-D (all $p < 0.05$). Age and positive Xpert BC-D remained independent predictors of UCa in multivariate analysis. Simulating a triage with WLC restricted to patients with positive Xpert BC-D could have saved 240 (74.1%) assessments at the cost of missing one pTa LG tumor.

[1]CE-IVD. *In Vitro* Diagnostic Medical Device. May not be available in all countries. Not available in the United States.

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CONCLUSIONS: The results suggest a potential role for Xpert BC-D in preselecting patients with hematuria for either further invasive diagnosis or an alternate diagnostic procedure.

Keywords: Hematuria, microhematuria, gross hematuria, bladder cancer, mRNA, urine markers

INTRODUCTION

Asymptomatic hematuria (aH) is a frequent urologic symptom and may be a sign of serious underlying disease of the urinary tract, including malignancy. For asymptomatic microhematuria (aMh), a disease prevalence of up to 24% in general population has been reported [1]. However, particularly in patients with aMh, the prevalence of urothelial cancer (UCa) is infrequent [2].

Several guidelines on aMh have been developed to permit stratification of patients for or against further assessment based on individual risks [1, 3, 4]. However, these recommendations 1) differ widely, 2) are mostly complicated dichotomized algorithms, 3) lack prospective validation, and 4) are of questionable efficacy [4]. Consequently, acceptance in the urologic community has been reported to be poor [5, 6].

As commonly accepted standards regarding urologic assessment of aH patients are missing, current work-up typically includes urine analysis, urethrocystoscopy (WLC), upper tract imaging, and – at some institutions - urine cytology. WLC is invasive, accompanied by patient discomfort, may trigger urinary tract infection, gross hematuria, and, although infrequent, even injure bladder and/or urethra [7, 8].

Urine cytology has a good sensitivity for detecting high grade (HG) tumors, while its sensitivity for low-grade (LG) lesions, representing the most common type of UCa, is low [1, 4]. Moreover, it suffers from interrater variability and test performance strongly depends on the expertise of the cytopathologist [1, 9]. With regards to imaging, computed tomography urography (CTU) has been forwarded as a standard; however, its relevance in aMh assessment remains controversial [10].

This situation has fueled interest in the development and validation of urine biomarkers for UCa in the assessment of hematuria patients. A recently developed urine-based mRNA signature (Xpert® Bladder Cancer Detection, CE-IVD (Xpert BC-D)) measuring 5 mRNA targets frequently overexpressed in UCa patients [11] has demonstrated potential to stratify hematuria patients being at risk for subsequent UCa diagnosis [12].

While previous multicenter studies were performed at tertiary academic referral centers, it may be speculated that UCa prevalence might be higher in these cohorts and therefore, assay performance could be overestimated. This monocenter study analyzes the performance of the Xpert BC-D in a contemporary hematuria cohort in routine practice. In addition, we simulated the effects of restricting invasive diagnosis (WLC) to patients with a positive Xpert BC-D result.

MATERIALS AND METHODS

Patients with newly observed painless hematuria and no history of UCa were eligible for this non-interventional study [DRKS 00027469]. IRB approval was obtained, and all patients provided informed consent. Patients were included retrospectively after identification in the institutional patient data base and prospectively between May 1st, 2020 and December 31th, 2022.

Ah was defined as 1) acute or recent gross hematuria (Gh), 2) referral by the family doctor for repetitive aMh or 3) an incidental finding of aMh while presenting for non-related conditions. The latter patients were included after $\geq$2 independent microscopic urine examinations yielding $\geq$3 Erythrocytes/hpf.

Patients were considered for analysis if they had valid Xpert BC-D testing and a sufficient assessment including personal history including exploration of confounding or risk factors (vigorous exercise, menstruation, etc.), basic urological examination including physical examination, urine analysis, bladder and upper tract imaging by ultrasound, and, if considered necessary by the urologist in charge, advanced imaging (CT or magnetic resonance imaging (MRI)). Urine cytology was performed at the discretion of the urologist. Patients without a valid Xpert BC-D result or not undergoing WLC were excluded from analysis. Patients with a positive WLC were recommended to undergo tumor resection (TUR-B).

Urine cytology (Paris classification) and the Xpert BC-D (according to the manufacturer's instructions)

Table 1
Demographic parameters (prospectively and retrospectively included patients)

Parameter	(%)	(%)	Comment
Age (years)	Mean: 60.6	Median: 61	Range: [20, 90]
Sex	Male: 188 (58)	Female: 136 (42)	Ratio: 1.38:1
Recruitment	prosp.: 263 (81.2)	retrosp.: 61 (18.8)	
Haematuria	Micro.: 213 (65.7)	Gross: 111 (34.2)	

were performed by experienced technicians (> 4.000 cytological and > 250 Xpert BC examinations annually). The Xpert BC-D, performed in voided urine specimens, is based on RT-qPCR and measures five mRNA targets (ABL1, CRH, IGF2, UPK1B, and ANXA10) frequently overexpressed in UCa. The reaction is performed in a cartridge and results are provided within 1.5 hours [11]. For the Xpert BC-D the recommended cut-off of 0.45 based on total Linear Discriminant Analysis was used. Results were communicated to the urologist in charge. Laboratory staff was blinded against clinical information.

Performance of urine cytology and the Xpert BC-D were studied analyzing sensitivity, specificity, positive and negative predictive values (PPV, NPV). Patients referred to TUR-B that did not undergo tumor resection were not considered for analysis. Risk ratios for the Xpert BC-D and established UCa risk factors were calculated. In addition, a simulation (triage) was performed to investigate the effects if clinical work-up was based on either a positive Xpert BC-D result or a positive urine cytology.

Patients with a positive urine cytology or Xpert BC-D but no UCa diagnosis were followed within the limitations of a non-interventional study.

Statistical analysis was performed using R v 4.3.0 via RStudio 2023.06.0 Build 421.

RESULTS

Overall, 368 patients were enrolled in the study. Forty-four patients were excluded due to: invalid Xpert BC-D results ($n = 2$ (0.5%)), withdrawal of consent/rejecting adequate work-up ($n = 13$), screen failure ($n = 13$), incomplete data (no Xpert BC-D test) ($n = 12$), and other reasons ($n = 4$) (supplementary Figure 1, supplementary Table 1).

Of the remaining 324 cases, 61 patients were included retrospectively after identification from the institutional data base and 263 patients were prospectively enrolled. Demographic data for retrospective and prospective patient cohorts showed no significant differences (supplementary Table 2). Therefore, both cohorts were combined for analysis.

The final data set comprised 188 males and 136 females (median age: 61 years), 213 patients presented with aMh and another 111 cases had Gh. Of these, 3 patients with positive WLC rejecting TUR-B were excluded from analysis of performance. Patient characteristics are summarized in Table 1.

Imaging was performed by ultrasound only in 191 (73%) of patients, 57 patients (22%) were referred for an additional CT scan and 12 patients (5%) underwent MRI. Urine cytology was performed in 297 (92.5%) patients.

Twenty-eight of fifty patients (56%) undergoing TUR-B based on a positive WLC or (a positive urine cytology or suspicious imaging result (2 cases)) had a histologically confirmed UCa. Eight patients had Ta low grade (LG) tumors, the remaining 20 cases were classified as high grade (HG) lesions (Ta: 4, CIS: 2, T1:11, > T1:3). In 22 patients (44%), histopathology did not confirm the results of WLC.

Xpert BC-D showed high sensitivity (96.4%) and was positive in all HG tumors at a specificity of 80.1% (Table 2). PPV was 0.329 and NPV was 0.996. Xpert BC-D was significantly more sensitive than urine cytology (60.7%) ($p < 0.05$), while specificity of cytology was superior (80% vs. 86%, respectively, ($p = 0.032$)).

Univariate analysis showed increased risk ratios (RR) for gross hematuria (RR = 4.8; 95% CI: 2.18–10.53, $p < 0.001$), gender (RR = 2.66; 95% CI 1.09, 6.39, p 0.026), urine cytology (RR = 7.72; 95% CI: 3.60–14.50, $p < 0.001$), and a positive Xpert BC-D result (RR = 78.70; 95% CI: 10.86–570.03, $p < 0{,}001$) to predict UCa diagnosis, while smoking status and professional exposure were not predictive (Table 3). Area under the Receiver Operating Characteristic curve (AUC) for Xpert BC-D and urine cytology were 0.89 and 0.74, respectively (supplementary Figure 2). Addition of urine cytology results did not improve sensitivity of Xpert BC-D.

A multivariate analysis based on R package "ordinal" was fit via response variable, with tumor grade coded as follows: 0 as negatives, 1 as low grade and 2 as high grade. As a high grade tumor is consid-

Table 2a
Performance of urine cytology and Xpert BC-D vs. reference standard (histopathology) Paired design for differences of sensitivity and specificity

	Xpert BC-D (%)	Urine Cytology (%)	% Diff (SE)	[Diff 95% CI], p.value
Sensitivity	96.4	60.7	35.7 (± 9.1)	[–60.46, –10.97], $p = 0.002$
Specificity	80.1	86.2	5.6 (± 2.4)	[0.12, 11.03], $p = 0.032$

Table 2b
Sensitivity and Specificity from ROC analysis for Xpert BC-D and urine cytology versus UCa (see supplementary Fig. 2)

	PPV	PPV CI	NPV	NPV CI
Xpert BC-D	0.329	[0.228 – 0.431]	0.996	[0.988 – 1.00]
Cytology	0.327	[0.199 – 0.454]	0.955	[0.929 – 0.981]

ered higher risk, the ordinal logistic regression will be more sensitive than just using a binary logistic regression and allows more predictor variables to be fitted. Age, and Xpert BC-D were the only significant predictors from Ordinal Logistic Regression from multivariate analysis (Table 5).

Simulating a triage comprising 321 patients with complete data for WLC and histopathology restricting WLC to patients with a positive Xpert BC-D or positive urine cytology suggested that 239 WLCs (74.5 %) ($p < 0.001$) and 6 of 22 histopathological negative tumor resections (TUR-Bs) (n.s.) would have been abandoned. These effects must be balanced against the delayed detection of a small (1 g) Ta LG tumor. Alternatively, if triage was based on urine cytology results, a similar number of WLCs ($n = 243$) (75.7%) would have been avoided, along with 7 negative TUR-Bs, but at the cost of 11 overlooked tumors, including 3 T1 cases and 2 muscle-invasive (T2) cancers.

Follow-up information was obtained for 23 out of 41 patients (56.1%) with a positive urine cytology and/or a positive Xpert BC-D and a negative WLC. After a median follow-up of 54 weeks (range 5 – 189 weeks), no additional UCa was diagnosed so far.

DISCUSSION

Assessment of patients with hematuria has remained a challenge to urologists. The prevalence of asymptomatic microhematuria (aMh) is high, with reported frequencies up to 24% in otherwise healthy individuals [1]. This contrasts with a low prevalence of UCa in aMh patients that has been estimated to be approximately 2-3% [2]. On the other hand, gross hematuria (gH), while less frequent, represents a worrisome symptom with a prevalence of UCa between 10 – 20% [12, 13]. Thus, hematuria assessment demands a thorough balance between excessive diagnostic intervention on the one hand and concerns to overlook a serious underlying condition on the other.

While a single patient reported episode of gH is considered adequate to trigger urological assessment, aMh has remained a matter of controversy starting with the definition (dip stick vs. microscopy, adequate cut-offs, or the question of whether a single episode is sufficient or if repetitive confirmation is required) and controversy regarding an adequate work-up. Therefore, recommendations on the management of aMh patients differ considerably [1]. For this study a single patient-reported episode of gross hematuria, patient referral for aMh, or a confirmed microscopic observation of > 3 erythrocytes/high power field qualified a patient for consideration.

A recent evaluation of the discriminative capacity of current aMh guidelines disclosed a poor performance of current guidelines and fueled interest in the potential for molecular urinary markers to improve stratification of patients for or against extensive urologic work-up [4].

A previous study in 1182 patients with painless hematuria demonstrated that immunocytology was a strong predictor of subsequent UCa diagnosis [14]. In addition, a triage based on a nomogram including immunocytology results suggested a potential reduction of WLCs. Similar results have been reported by Beukers et al. investigating methylation changes in urine cells [15, 16] while another study using a commercially available point-of-care assay detecting cytokeratins 8 and 18 was rather discouraging [17].

The Xpert BC-D assay was recently validated in a prospective, multi-center study including 895

Table 3
Univariate risk factors in hematuria patients of subsequent UCa diagnosis

Parameter (details)	RR	95% CI	*p*-value (Fisher Exact)
Age (< 50 vs. > 50 years)	6.08	[0.84, 43.85]	0.038
Sex (M vs. F)	2.66	[1.09, 6.39]	0.026
Hematuria (gross- vs. microhematuria)	4.80	[2.18 – 10.53]	< 0.001
Smoking (Never vs. ex- and current)	1.10	[0.50 – 2.38]	1
Professional exposure	0.72	[0.175 – 2.98]	1
Urine cytology (positive vs. negative)	7.72	[3.60 – 14.50]	< 0.001
Xpert® BC-D (positive vs. negative)	78.70	[10.86 – 570.03]	< 0.001

subjects with newly diagnosed hematuria [12]. An overall sensitivity of 75.8%, a NPV of 97.8%, and a specificity of 84.6% were reported. The Xpert BC-D had a higher sensitivity for both HG and LG tumors and a higher NPV when compared to urine cytology and to the UroVysion assay. A specificity analysis in patients without hematuria and no previous UCa yielded specificities of 96.2% in patients with urinary incontinence or overactive bladder ($n = 79$), 90.8% in patients with BPH ($n = 76$), and 93.2% in patients with a history of prostate cancer ($n = 44$).

Comparing the results of this examination in a private practice with those from the Dutch multi-center study mostly conducted at academic centers, UCa prevalence was slightly higher than expected in our cohort, suggesting that referral of hematuria patients by family doctors for urologic assessment may introduce a selection bias (supplementary Table 3).

In both studies, high sensitivities for the Xpert BC-D and urine cytology were observed reflecting the fact that primary tumors tend to be larger than lesions diagnosed in patients undergoing meticulous follow-up [18–20]. Furthermore, in both studies sensitivity of the Xpert BC-D was superior to urine cytology (supplementary Table 3). While only one Ta LG UCa was negative with the Xpert BC-D in this study, it should be noted that urine cytology also missed several HG UCas, including 3 T1 HG lesions and two T2 cancers. The overall performance of the Xpert BC-D is further highlighted by an AUC of 0.89 (supplementary Figure 2) in this study.

Another mRNA signature, the Cxbladder was studied alone and in combination with phenotypic biomarkers and clinical characteristics in a cohort of 863 hematuria patients [21]. Inclusion of Cxb results within the Cxb Resolve (CxbR) test yielded a sensitivity of 92.4% along with a specificity 93.8% for identifying clinically relevant high impact tumors.

More recently, a retrospective study comprising 804 hematuria patients incorporating an enhanced Cxbladder test, adding DNA analysis of 6 single nucleotide polymorphisms for the FGFR3 and TERT genes to the current 5 mRNA biomarkers [22]. Pooled analysis using the enhanced CxbladderDetect system came up with a sensitivity of 97% and a specificity of 90%.

Another retrospective hematuria study comprising 838 patients used urine biomarker testing based on mutation status for FGFR3, TERT, and HRAS genes and methylation status for the OTX1, ONECUT2, and TWIST1 genes. Sensitivity of this assay was 95.5% at a specificity of 72.9 [23]. Application of biomarker results helped to further stratify high risk patients as defined by the 2020 AUA guidelines [3].

As data from surveillance studies suggest that approximately 10% of patients with a positive urine marker result and a negative WLC may subsequently develop tumor recurrence [24, 25]. we tried to address this question in our cohort following patients with either positive urine cytology or a positive Xpert BC-D. However, although "anticipatory positive" marker results cannot entirely be ruled out due to incomplete follow-up data, we rather speculate that a delayed diagnosis of UCa in hematuria patients is rare.

Regarding risk factors for subsequent UCa diagnosis, known risk factors e.g. gender, age and gH were confirmed, while smoking and professional exposure were not found to increase UCa risk (Table 3). The reasons behind this finding remain obscure and require re-evaluation in a larger cohort. A positive urine cytology and a positive Xpert BC-D were the strongest predictors in univariate analysis. Multivariate analysis confirmed age and a positive Xpert BC-D as independent predictors of subsequent UCa diagnosis. These observations are in line with previous findings using immunocytology [13, 14].

Only a minority of patients (27%) received advanced upper tract imaging (CT, MRI). As this was a non-interventional study, this figure reflects doubts on the benefits of an expensive and potentially hazardous diagnostic tool in a patient cohort with a low prevalence of upper tract UCa [10, 26]. However, it is remarkable that a positive Xpert BC-D result obviously triggered referral for advanced imag-

Table 4
Simulation (triage): reflex cystoscopy based on positive test result (Urine cytology vs. Xpert BC-D) based on 321 patients with full TUR-B/pathology data

	Marker positive	Marker negative	WLC positive* (%)	WLC negative (%)	TURBs	UCas diagnosed	Tumors potentially over-looked	WLCs potentially avoided	Nega-tive TURBs
SOC (WLC triage) ($n = 321$)*	NA	NA	**59*** (18.4%)	262 (81.6%)	50 (48 WLC directed)	28/28 (100%)	–	–	20/48 (41.7% WLC directed)**
Xpert BC-D ($n = 321$)*	82* (25.5%)	239 (74.5)	46(56.1%)	36 (43.9%)	43	27/28 (97.4%)	1 (pTa LG) (2.6%)	240 (74.1%)	16/48 (33.3%)
Urine cytology ($n = 297$)***	54* (18.2%)	243 (81.8%)	26 (48.1%)	28 (51.9%)	24	17/27 (63%)***	11 (5 HG) (39%)	246 (81.7%)	7/48 (14.6%)

*Of 324 patients, 3 excluded per protocol as TURBT was recommended but not completed. Leaving a total of 321 results for WLC and Xpert, and 297 results for urine cytology; 62 positive WLCs; 82 Xpert BC-D positive (2 positives excluded per protocol); 54 urine cytology positive (1 positive result excluded per protocol). **50 TURBTs completed; 48 WLC-directed; 2 recommended by other clinical measures (imaging and urine cytology). ***cytology data not available for 24 patients. 1 patient with tumor did not have cytology result.

ing (suppl. Table 4). More than 50% of patients with a positive Xpert BC-D received CT or MRI (RR 1.64, $p = 0.013$).

It is noteworthy that histopathology was negative in 44% of cases, suggesting a low specificity of WLC. This observation appears worrisome but is supported by previous findings by our group and other investigators [20, 27]. Although this observation may be partially explained by technical issues, it deserves further attention and measures to minimize the number of histopathological negative TUR-Bs are needed.

Several studies suggest a potential role of urine markers in the assessment of patients with hematuria [12, 14–16], however, concrete recommendations on the use of urine markers are missing. Based on previous experience [14], we simulated a triage restricting WLC to patients with either a positive Xpert BC-D or a positive urine cytology.

This procedure would generate significant savings of WLCs for both markers, however, with a chance of missing several high risk and even muscle invasive tumors if based on urine cytology. It is of interest that some negative TUR-Bs would also have been avoided. Restricting WLC to a positive Xpert BC-D result, 6 of the 22 histopathological negative TUR-Bs (27.3%) would have been abandoned. Considering these results, it appears useful to add urine markers to clinical decision making in patients with painless hematuria.

There is some concern that urine markers might miss clinically relevant high grade lesions [12]. Technical reasons, e.g. high fluid intake to enable a patient to provide a urine specimen or a low tumor size might yield a negative assay result in patients with a clinically relevant lesion. This risk could be reduced by a re-assessment including marker testing after 3–6 months. However, such recommendation requires validation by prospective trials.

This study has some limitations: 1) the combined analysis of retrospectively and prospectively collected patient sets may introduce some bias. However, comparison of demographic factors showed no significant differences. 2) It must be considered that this investigation was a non-interventional study. While Xpert BC-D and a baseline examination including WLC was required for patient eligibility, urine cytology was performed at the urologist's discretion; in consequence, only 92.5% of patients had a urine cytology result. However, comparing the tumor stages in both groups there is no indication of systematic bias for or against one of the urine tests. 3) Similar concerns apply to the use of advanced

Table 5
Multivariate Ordinal Logistic Regression (https://stats.oarc.ucla.edu/r/dae/ordinal-logistic-regression/)

Parameter	OR = exp (coef)	coefficients	Std. error	z-value	*p*-value
Age	1.073	0.070	0.027	2.57	**0.0103**
Sex (male)	0.835	–0.180	0.597	–0.30	0.7631
Negative Xpert BC-D	0.017	–4.073	1.072	–3.78	**0.0001**
Negative urine cytology	0.738	–0.305	0.560	–0.54	0.5868
Smoking (ex + current vs. never)	0.987	–0.013	0.252	–0.05	0.9590
Professional exposure	1.593	0.466	0.473	0.99	0.3243

OR = Odds Ratio. 321 rows, (287 included, 34 observations deleted due to „missingness“).

imaging (27%). However, given the low prevalence of upper tract UCa and the limited sensitivity of CT scan and MRI, the risk of missing a relevant number of cases is low. 4) the issue of potentially anticipatory positive testing was not systematically addressed as sufficient information on surveillance of patients with either positive urine cytology or a positive Xpert BC-D was only retrieved in 56% of cases. Nevertheless, a median follow-up interval of app. 1 year in this group rather argues against high numbers of patients with UCa after a negative primary assessment. 5) The multivariable models may be overfit and therefore underpowered for some variables in this study due to the small number of samples in the study.

CONCLUSIONS

The Xpert BC-D is a reliable urine assay for detection of bladder cancer also suited for a use in private practice. The simulation of a triage restricting invasive diagnosis to patients with a positive Xpert BC-D result would have resulted in high savings of WLC without compromising the detection of clinically relevant tumors. The results of this study support a potential role for Xpert BC-D in preselecting patients with hematuria for either further invasive diagnosis or an alternative follow-up schedule.

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AUTHOR CONTRIBUTIONS

Claudia Schmitz-Dräger - Concept, data retrieval, literature review, manuscript drafting
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Jack Chen - Biostatistics, manuscript drafting
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Bernd J. Schmitz-Dräger - Concept, data retrieval, literature review, patient assessment manuscript drafting
Natalya Benderska-Söder - Data retrieval, literature review, manuscript drafting
CSD contributed to the project in fulfillment of the requirements for obtaining the degree ‘Dr med.’ at the Friedrich-Alexander University, Erlangen-Nürnberg, Germany.

ETHICAL CONSIDERATIONS

The authors declare compliance with acknowledged ethical standards (Helsinki Declaration) and good clinical practice (GCP). IRB approval was obtained (University of Erlangen 328_20 B), and all patients provided informed consent.

This non-interventional study was performed in accordance with the STARD criteria (2015).

Registration: The study was registered at the German Registry for Clinical Trials (DRKS00027469)

CONFLICTS OF INTEREST

BJSD is consultant, speaker and trialist for Cepheid, Sunnyvale, CA, USA, and Nucleix, Rehovot, Israel/USA and trialist for Concile, Freiburg, Germany, Zetiq/NextAge, Tel Aviv, Israel, and Arquer Diagnostics Ltd, Sunderland, UK. PJG is consultant and trialist for Cepheid, PB, JC, EP, and MB are employees of Cepheid, Sunnyvale, CA, USA

BJSD and PJG are members of the Bladder Cancer Editorial Board

DATA AVAILABILITY

The study protocol and data obtained in this study may be available upon written request. The authors reserve the right to reject applications based on scientific, ethical or copy right reasons.

SUPPLEMENTARY MATERIAL

Supplementary data available online: https://dx.doi.org/10.3233/BLC230089.

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