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BladderPath Trial Management Group

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Highly Sensitive and Specific Detection of Bladder Cancer via Targeted Ultra-deep Sequencing of Urinary DNA

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Abstract

Background: There is an unmet need for an accurate, validated, noninvasive test for diagnosing and monitoring bladder cancer (BC). Detection of BC-associated mutations in urinary DNA via targeted deep sequencing could meet this need.
Objective: To test the ability of mutational analysis of urinary DNA to noninvasively detect BC within the context of haematuria investigations and non–muscle-invasive BC (NMIBC) surveillance.
Design, setting, and participants: Capture-based ultra-deep sequencing was performed for 443 somatic mutations in 23 genes in 591 urine cell-pellet DNAs from haematuria clinic patients and 293 from NMIBC surveillance patients. Variant calling was optimised to minimise false positives using urine samples from 162 haematuria clinic patients without BC.
Outcome measurements and statistical analysis: The sensitivity and specificity for BC diagnosis were determined.
Results and limitations: Mutational analysis of urinary DNA detected 144 of the 165 haematuria patients diagnosed with incident BC from two independent cohorts, yielding overall sensitivity of 87.3% (95% confidence interval [CI] 81.2–92.0%) at specificity of 84.8% (95% CI 79.9–89.0%). The sensitivity was 97.4% for grade 3, 86.5% for grade 2, and 70.8% for grade 1 BC. Among NMIBC surveillance patients, 25 out of 29 recurrent BCs were detected, yielding sensitivity of 86.2% (95% CI 70.8–97.7%) at specificity of 62.5% (95% CI 56.1–68.0%); a positive urine mutation test in the absence of clinically detectable disease was associated with a 2.6-fold increase in the risk of future recurrence. The low number of recurrences in the NMIBC surveillance cohort and the lower sensitivity for detecting grade 1 pTa BC are limitations.

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Conclusions: Detection of mutations in a small panel of BC-associated genes could facilitate noninvasive BC testing and expedite haematuria investigations. Following further validation, the test could also play a role in NMIBC surveillance.
Patient summary: Identification of alterations in genes that are frequently mutated in bladder cancer appears to be a promising strategy for detecting disease from urine samples and reducing reliance on examination of the bladder via a telescopic camera inserted through the urethra.

1. Introduction

Haematuria is the presenting symptom of bladder cancer (BC) for >90% of patients [1]. However, only 10–22% of patients investigated in haematuria clinics are diagnosed with a urinary tract malignancy, including BC (8.0–17.9%), upper tract urothelial cancer (0.3–1.2%), kidney cancer (1.0–1.5%), and prostate cancer (0.3–1.8%) [2–4].

Flexible cystoscopy is a key component in the investigation of haematuria [5] and a core modality for identification of local disease recurrence in BC surveillance following endoscopic treatment of non–muscle-invasive BC (NMIBC) or bladder-sparing treatment of MIBC [5,6]. However, flexible cystoscopy is invasive and costly and not without complications [7,8]; it is also operator-dependent and fallible, with estimated sensitivity of ≤85% and specificity of ≤87% [8,9]. Although studies (including our own [10]) demonstrate an improvement in sensitivity achievable with enhanced cystoscopy [8,9], the development of a noninvasive (urine) test for detection of new and recurrent disease remains a high priority for patients and health care professionals alike [11].

Given the genomic complexity of BC [12,13], urinary diagnostics based on single markers have not seen widespread clinical uptake because of poor sensitivity and/or specificity (eg, NMP22, BTA) [14]. Several mRNA panel–based urine tests have been commercialised (eg, Cxbladder Triage/Monitor and Xpert Bladder Cancer Monitor) [14,15]; however, urinary DNA arguably yields the most robust tumour-specific information for noninvasive BC detection.

Targeted massively parallel sequencing of urinary DNA allows detection of multiple biomarkers even when tumour DNA is a minor component of the total urinary DNA. Reliable detection of variant allele frequencies (VAFs) below 1–2% is challenging using standard deep-sequencing approaches [16,17]. The limit of detection can be improved by utilising unique molecular identifiers (UMIs) if there is sufficient DNA, efficient library preparation, and high sequencing depth [16,18]. The requirement for very high sequencing depth for sensitive detection of mutations and the need to minimise the risk of false-positive results imply that a focused BC-specific gene panel should be used rather than generic cancer panels or whole-exome or whole-genome sequencing.

We published the first application of a next-generation sequencing (NGS)-based BC-specific mutation panel for noninvasive BC detection in 2016 [17] and subsequently used a cohort of 956 BC tumour samples to develop a focused gene panel mutated in >96% of BCs [19]. We hypothesised that use of this gene panel, in combination with capture-based library preparation and UMIs, could generate a clinically useful urine test for BC rivalling the sensitivity and specificity of flexible cystoscopy. In the current study we used this diagnostic test in cell-pellet (cp) DNAs from 884 urine samples: 591 from patients attending haematuria clinics (including 165 BCs) and 293 from patients on NMIBC surveillance (including 29 BCs).

2. Patients and methods

2.1. Patients and samples

Urine samples from three independent patient cohorts were used to generate the groups indicated in Figure 1 (the haematuria clinic cohort comprised patients referred for urgent investigation of haematuria in accordance with contemporaneous UK guidelines). All patients gave written informed consent for urine collection, processing, and analysis. Patient demographics are shown in Table 1. The presence or absence of BC on the date of urine collection was determined via white light flexible cystoscopy.

The retrospective haematuria clinic cohort comprised BC patients from the Bladder Cancer Prognosis Programme (BCPP, ethics reference 06/MRE04/65) and patients without BC recruited from haematuria clinics at The Queen Elizabeth Hospital, Birmingham, UK (ethics reference 15/NW/0079). BCPP collected biospecimens for biomarker research at ten hospitals in the West Midlands region of the UK between 2005 and 2011: patients with cystoscopic suspicion of primary BC and no prior history of BC were recruited [20]. Pretreatment mid-stream urine samples (up to 50 ml) were collected in plain 50-ml centrifuge tubes, transported on ice, and centrifuged at 1000 × g for 10 min; the cell pellets and supernatants were then separated and frozen at −80 °C on the same day.

Before undertaking analysis of prospectively collected urine samples, prespecified sensitivity and specificity targets of ≥85% had to be achieved for the retrospective haematuria clinic cohort, representing a go/no-go decision for continuation of research funding.

The prospective haematuria clinic cohort consisted entirely of samples prospectively collected for this study from patients attending a haematuria clinic, initially from The Queen Elizabeth Hospital, Birmingham (ethics reference 15/NW/0079) and subsequently from other UK urology units participating in the BladderPath study (ethics refer-

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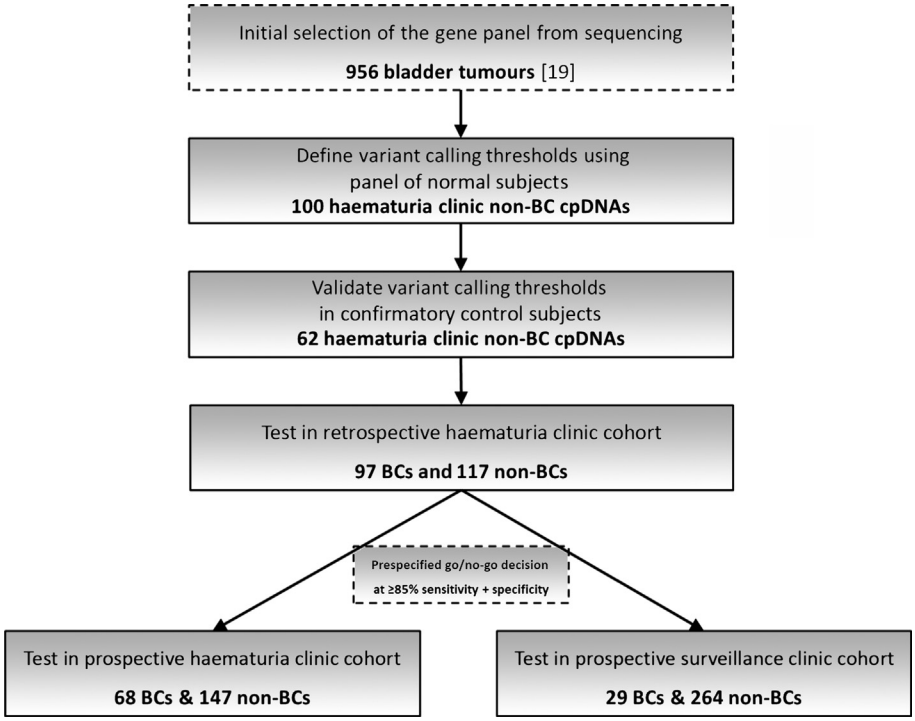

Fig. 1 – Study design. Panel development using tumour tissue DNA was previously reported [19]. Variant calling thresholds were selected using a panel of 100 non-BC cpDNAs and confirmed in a further 62 non-BC cpDNAs. The test was then applied to two haematuria clinic cohorts (retrospective and prospective) and a cohort of patients with non–muscle-invasive BC undergoing surveillance for disease recurrence after transurethral resection of bladder tumour. The haematuria clinic cohorts comprised patients referred for urgent investigation of haematuria in accordance with contemporaneous UK guidelines (https://www.baus.org.uk/_userfiles/pages/files/News/haematuria_consensus_guidelines_July_2008.pdf, https://www.baus.org.uk/_userfiles/pages/files/Publications/BAUS%20Cancer%20Guidelines%20Summary.pdf). BC = bladder cancer; cpDNA = urinary cell-pellet DNA.

Table 1 – Patient characteristics by cohort (Fig. 1)

Cohort	Disease status	No.	M/F	Median age (yr)	pT stage (*n*)					Grade (*n*)			
					Tis	Ta	T1	T2+	NR	1	2	3	NR
Panel of normal subjects	Non-BC	100	41/59	57	–	–	–	–	–	–	–	–	–
Confirmatory control subjects	Non-BC	62	37/25	59	–	–	–	–	–	–	–	–	–
Haematuria clinic (retrospective) [a]	BC	97	80/17	72.5	3	47	18	29	0	21	27	48	1
	Non-BC	117	55/62	60	–	–	–	–	–	–	–	–	–
Haematuria clinic (prospective) [a]	BC	68	52/16	72.5	0	41	16	8	3	3	26	29	10
	Non-BC	147	85/62	60	–	–	–	–	–	–	–	–	–
NMIBC surveillance (prospective)	BC	29	18/11	71	0	17	4	2	6	4	8	11	6
	Non-BC	264	175/89	74	–	–	–	–	–	–	–	–	–

BC = bladder cancer; F = female; M = male; NR = not recorded.
[a] The haematuria clinic cohorts comprised patients referred for urgent investigation of haematuria in accordance with contemporaneous UK guidelines (https://www.baus.org.uk/_userfiles/pages/files/News/haematuria_consensus_guidelines_July_2008.pdf, https://www.baus.org.uk/_userfiles/pages/files/Publications/BAUS%20Cancer%20Guidelines%20Summary.pdf).

ence 17/LO/1819) [21]. Pre-cystoscopy mid-stream urine specimens (up to 50 ml) were collected at The Queen Elizabeth Hospital in plain 50-ml centrifuge tubes on the day of clinic attendance and were either immediately manually delivered to the Human Biomaterials Resource Centre (HBRC) at the University of Birmingham at ambient temperature for processing within 2 h, or were stored overnight at 4 °C for processing the following day. For other BladderPath recruiting centres, pre-cystoscopy mid-stream urine specimens (up to 50 ml) were collected in Norgen urine collection and preservation tubes (Norgen Biotek, Thorold, ON, Canada) on the day of clinic attendance and were posted to the HBRC at the University of Birmingham at ambient temperature in UN3373 packaging. On receipt at HBRC, samples were centrifuged at 1000 × *g* for 10 min; cell pellets and supernatants were then separated and frozen at −80 °C. Patients in the non-BC group were determined to be "normal" or to have diagnoses that included calculi, benign prostatic hyperplasia, cystitis, inflammation, urinary tract infection, prostate cancer, and kidney cancer. The panel of normal subjects and the confirmatory control subjects were randomly selected from this cohort.

Samples for the prospective NMIBC surveillance cohort were prospectively collected for this study from unselected

consenting patients attending one of three dedicated NMIBC cystoscopy surveillance clinics at The Queen Elizabeth Hospital, Birmingham (ethics reference 15/NW/0079). Pre-cystoscopy mid-stream urine specimens (up to 50 ml) were collected in plain 50-ml centrifuge tubes on the day of clinic attendance and were either immediately delivered by hand to the HBRC at the University of Birmingham at ambient temperature for processing within 2 h, or were stored overnight at 4 °C for processing the following day. On receipt at HBRC, samples were centrifuged at 1000 × *g* for 10 min; cell pellets and supernatants were then separated and frozen at −80 °C immediately.

DNA was extracted from cell pellets using Quick-DNA urine kits (D3061; Zymo Research. Irvine, CA, USA) and quantitated using high-sensitivity dsDNA Qubit kits (ThermoFisher, Waltham, MA, USA). Laboratory staff were unaware of patient diagnoses.

2.2. Library preparation and sequencing

Libraries were prepared from 25 ng of urinary cpDNA (extracted from an average of 23 ml of urine) using Nonacus Cell3 Target enrichment (Supplementary material and www.nonacus.com/). DNA was enzymatically sheared, end-repaired and A-tailed, and adapters (including UMIs) were ligated to the fragments. Libraries were amplified and pooled in batches of twelve before overnight hybridisation with biotinylated probes and subsequent capture and final amplification of the NGS libraries. The probes targeted hotspots or regions of 23 genes (Supplementary Table 1). All libraries were 2 × 150 bp sequenced on a NovaSeq (Illumina, San Diego, CA, USA).

2.3. Bioinformatics

Sequencing data were demultiplexed and aligned to *hg19* using *bwa* v0.7.15-r1140. Consensus reads were built using *fgbio* v1.1.0 requiring at least three reads to produce a consensus as previously described [19] and realigned to the reference. Average raw and consensus read depths were 27 100× and 3000×, respectively (Supplementary Fig. 1). Samples with a consensus read depth of <500 at ≥10 loci were excluded (35 out of 919, 3.8%). Base calls with quality ≥30 were extracted using *bam-readcount* and used to calculate VAFs at 443 predefined genomic coordinates (a refined set of single-nucleotide variants from our study of 956 BCs [19]). An optimal variant calling strategy was developed on the basis of the maximum VAFs observed in a panel of 100 BC-negative patients with haematuria and confirmed in a further 62 BC-negative patients with haematuria.

2.4. Statistical considerations

Sample size calculations were based on 90% target sensitivity with a lower 95% confidence interval (CI) limit close to 85%; 150 BCs would yield a 95% CI of 83.8–94.1%. Descriptive statistics were prepared for sensitivity, specificity, and corresponding 95% CIs and reported separately for each of the haematuria clinic cohorts (and combined) and for the NMIBC surveillance cohort. Flexible cystoscopy findings at the time of urine sample collection represented the reference test to provide BC/non-BC status; for patients who underwent transurethral resection of bladder tumour (TURBT) or re-TURBT, the TURBT/re-TURBT findings and histopathology reports provided definitive diagnosis and the BC grade and stage. Treating clinicians were unaware of urine biomarker test results.

3. Results

3.1. Variant calling optimisation

We sequenced a panel of normal samples (100 cpDNAs from non-BC haematuria cases) and determined VAFs at 443 predefined mutation coordinates. The maximum VAF observed in any of the cpDNAs at each coordinate was <0.5% for 434 of 443 coordinates. Eight coordinates had VAF >0.5% in one to three cpDNAs, and the *TERT* promoter mutation chr5:1295228A/G had VAF >0.5% in eight cpDNAs; increasing the threshold for chr5:1295228A/G to 0.9% VAF reduced this to three cpDNAs. Consequently, a positive test was defined as detection of any one of the 443 mutations in a cpDNA sample at >0.9% VAF for chr5:129528A/G or >0.5% VAF for all other coordinates. This combination provided 89.9% specificity in the panel of normal samples and 91.2% specificity in a further 62 confirmatory control samples (cpDNAs from non-BC haematuria cases).

3.2. Sensitivity and specificity for detection of new BC cases

The variant calling strategy above was applied to cpDNAs from the retrospective haematuria clinic cohort (97 incident BC cases and 117 non-BC haematuria cases; Fig. 1). Mutations were detected in the urine of 85/97 BC cases (87.6% sensitivity) and 13/117 non-BC cases (88.9% specificity). Having achieved the prespecified sensitivity and specificity threshold of ≥85%, the test was applied blinded to the independent prospective haematuria clinic samples (68 BCs and 147 non-BCs) for validation, for which sensitivity of 86.8% at 81.0% specificity was achieved.

After combining the retrospective and prospective haematuria clinic cohorts to derive test positivity and VAFs across disease grades and stages, 144/165 BCs tested positive (sensitivity 87.3%, 95% CI 81.2–92.0%) and 223/264 non-BCs tested negative (specificity 84.8%, 95% CI 79.9–89.0%). Mutations were detected significantly more frequently (and with higher VAFs) in cpDNA from patients with all stages and grades of BC in comparison to patients without BC ($p < 0.001$; Fig. 2). The sensitivity was 97.4% (95% CI 91.4–99.7%) for grade 3 BC, 86.5% (95% CI 74.2–94.4%) for grade 2 BC, and 70.8% (95% CI 48.9–87.4%) for grade 1 BC. The sensitivity was 79.3% (95% CI 69.3–87.2) for stage pTa, 100% (95% CI 90.0–100.0%) for stage pT1, and 91.7% (95% CI 78.1–98.3) for MIBC; all three cases of solitary carcinoma in situ were detected. The median maximum VAF for incident BCs was 18.7%, versus 0.28% for non-BC cases ($p < 0.001$; Fig. 2), with a median of three mutations per BC cpDNA. The mutations most commonly detected were in *TERT*, *TP53*, *FGFR3*, *PIK3CA*, *ERCC2*, *ERBB2*, and *RHOB* (Fig. 3), mirroring previous tumour tissue data [19,22,23]. Mutation frequencies by stage and grade are detailed in Supplementary Table 1.

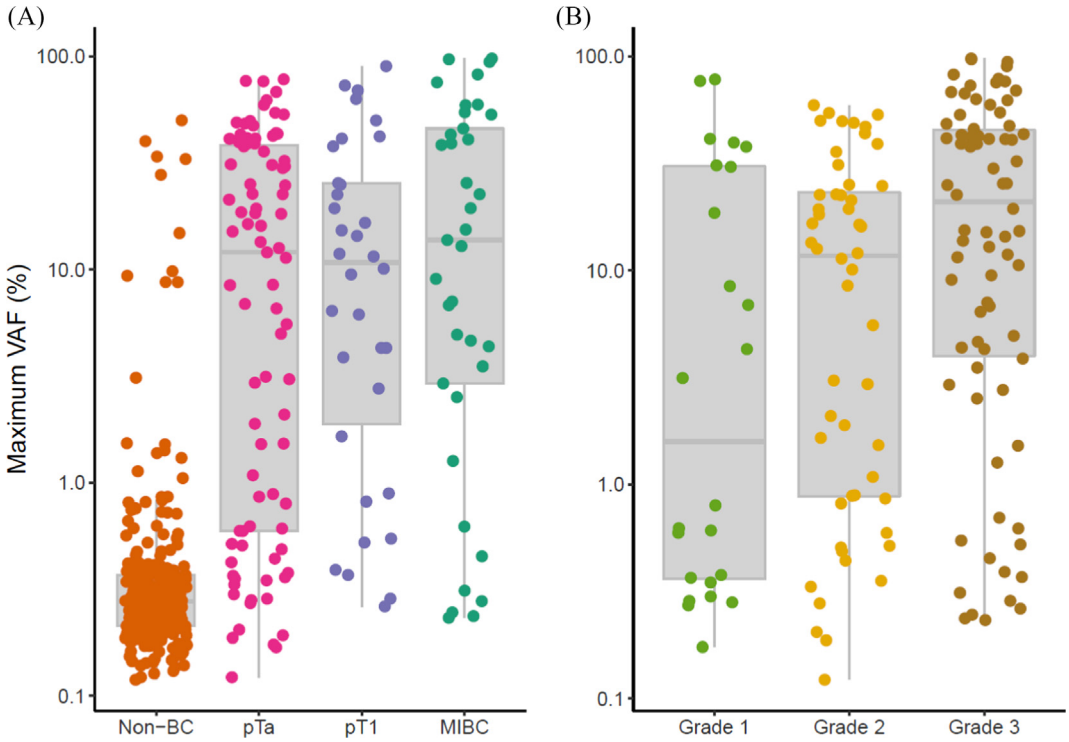

Fig. 2 – Single-nucleotide variants in urinary DNA from patients with incident BC. (A) Highest VAFs in cpDNAs from 264 non-BC (excluding non-BC cpDNAs used to develop the variant calling thresholds), 88 pTa, 34 pT1, and 37 MIBC cases. (B) Highest VAFs in BC cpDNAs by tumour grade. BC = bladder cancer; cpDNA = urinary cell-pellet DNA; MIBC = muscle-invasive BC; VAF = variant allele frequency.

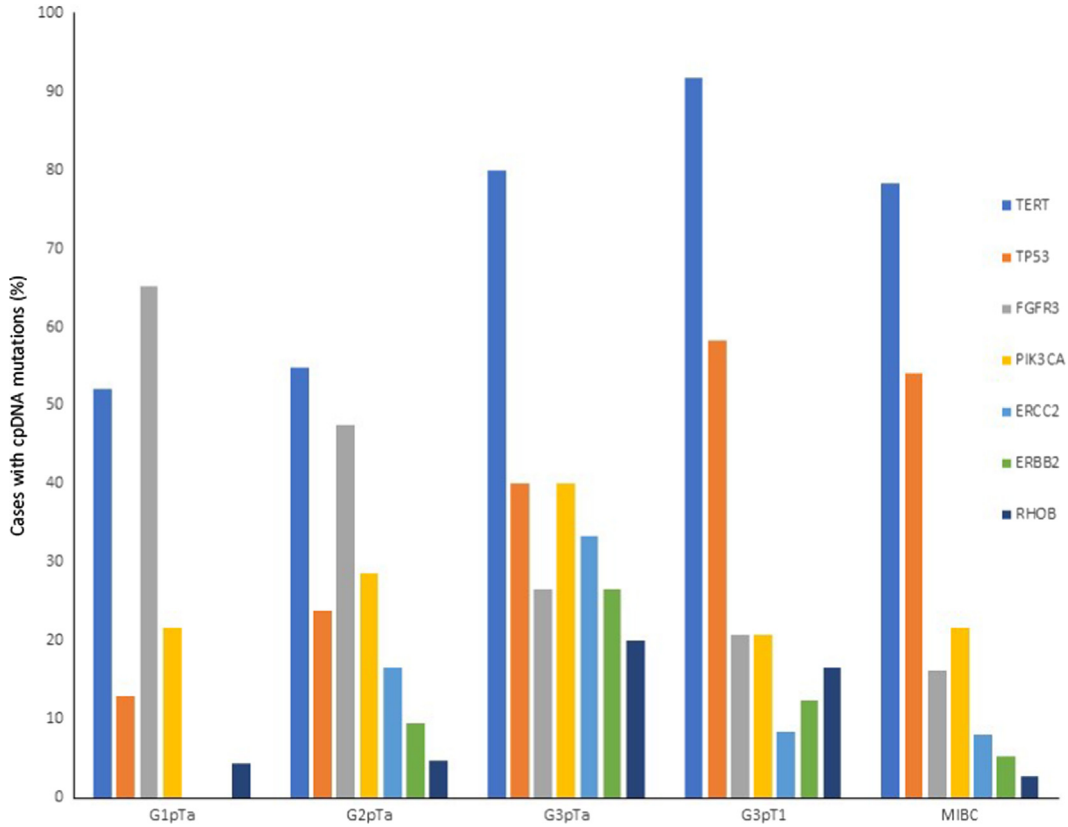

Fig. 3 – Mutation frequencies across incident bladder cancer stages and grades. Data are shown for the six genes most frequently mutated (overall) in grade 1 pTa (*n* = 23), grade 2 pTa (*n* = 42), grade 3 pTa (*n* = 15), grade 3 pT1 (*n* = 24), and grade 2 pTa (*n* = 37). cpDNA = urinary cell-pellet DNA; MIBC = muscle-invasive bladder cancer.

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3.3. *Sensitivity and specificity for detecting recurrent disease*

We analysed 293 cpDNAs from patients on NMIBC surveillance, 29 with BC detected on the day of urine collection. The median maximum VAF was significantly higher in BC recurrence cpDNAs than nonrecurrent cpDNAs (10.2% vs 0.39%; $p < 0.001$; Fig. 4A). On applying the variant calling strategy developed with haematuria clinic samples, we obtained sensitivity of 86.2% (95% CI 68.3–96.1%) at 62.5% (95% CI 56.4–68.4%) specificity in the NMIBC surveillance setting; notably, all 11 grade 3 recurrences were detected. Exploratory receiver operating characteristic analysis suggests that the ideal VAF threshold for defining a positive test result may be different for haematuria and surveillance testing and that it may be possible to achieve >70% specificity at >80% sensitivity for surveillance (Fig. 4B).

Of the 264 cpDNAs from patients cystoscopically negative for BC on the day of urine collection, 18 were from patients diagnosed with recurrence at their next cystoscopy 2–24 mo later. The pre-recurrence cpDNA VAFs were significantly higher in these 18 cpDNAs than in the 246 cpDNAs from patients without subsequent recurrence (2.1% vs 0.37%; $p < 0.05$; Fig. 4); 11 of 18 cpDNAs (61%) from patients with future recurrence tested positive, compared to 88 of 246 (36%) cpDNAs from patients without subsequent recurrence. The rate of future recurrence was 2.6-fold higher in the group with a positive test result (11.1% vs 4.2% for the group with a negative test).

4. Discussion

We previously developed a somatic mutation panel suitable for noninvasive detection of BC [19]; we have now used this panel in conjunction with error-suppressed ultra-deep sequencing to determine the sensitivity and specificity for detection of new cases of BC (haematuria clinic) and recurrences (NMIBC surveillance). Importantly, we used real-world non-BC urine samples from patients attending a haematuria clinic as "control samples" throughout. The panel comprises 443 possible mutations in 23 genes and is derived from sequencing data for 956 BCs [19]; restricting analysis to these 443 mutations (in the absence of paired germline data) decreases the risk of false-positive results from polymorphisms and sequencing errors.

In the haematuria clinic setting we obtained sensitivity of 87.3% at 84.8% specificity for detection of symptomatic incident disease, which compares favourably with existing US Food and Drug Administration–approved biomarkers such as NMP22 and BTA [14]. Following CE mark approval, the test will soon become available for clinical use as the Inform-Bladder test (Nonacus, Birmingham, UK). If confirmed in future studies (currently in set-up), we propose that this noninvasive test could be used in conjunction with imaging to reduce the number of flexible cystoscopies undertaken for the >80% of haematuria patients who ultimately do not have BC. Published data also suggest that our gene panel would diagnose up to 98% of upper tract urothelial carcinomas [24]; this warrants further investigation in future studies.

Our test may also have utility for influencing downstream disease management: we showed that 53% of patients with MIBC harboured *TP53* mutations, compared to 29% of patients with NMIBC, while 43% of patients with NMIBC harboured *FGFR3* mutations, compared to 17% of patients with MIBC. Combined with a visual Likert score for tumour appearance, mutation status may allow optimal

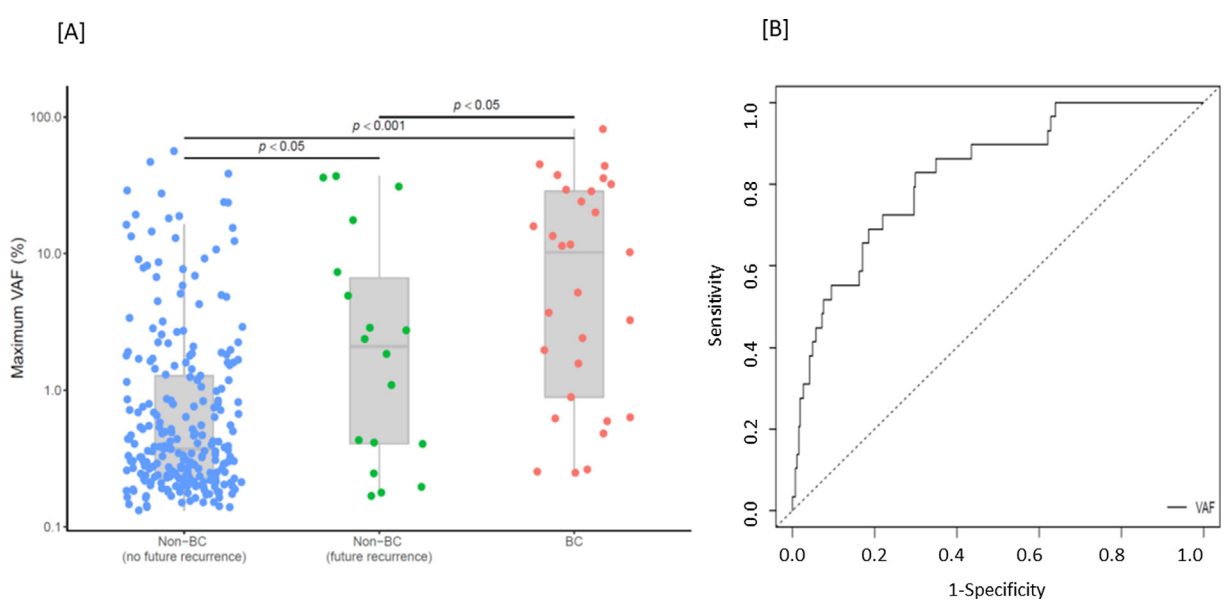

Fig. 4 – (A) VAFs for single-nucleotide variants in surveillance clinic cpDNAs and (B) ROC analysis. No BC detected on the day of urine collection or during follow-up: median VAF = 0.37% (n = 246); no BC on the day of urine collection but future recurrence: median VAF = 2.1% (n = 18); BC detected on the day of urine collection: median VAF = 10.2% (n = 29). The ROC curve was created by varying the minimum VAF required for a positive test result. The area under the curve is 0.827 (95% confidence interval 0.750–0.902). BC = bladder cancer; cpDNA = urinary cell-pellet DNA; ROC = receiver operating characteristic; VAF = variant allele frequency.

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selection of patients for multiparametric magnetic resonance imaging staging before TURBT or definitive treatment [21]. Furthermore, *RXRA*, *RHOB*, and *TERT* mutations are reportedly associated with significantly shorter time to recurrence in NMIBC [19], and *ERCC2* mutations appear to confer response to platinum-based chemotherapy in MIBC [25].

For NMIBC surveillance, we obtained sensitivity of 88.9% at 62.2% specificity. Most noninvasive biomarker tests for BC perform less well in the surveillance setting [26,27]. Lower specificity may arise if biomarkers are present in macroscopically normal epithelium, resulting from either the epigenetic/genomic changes accompanying microscopic disease [28] or accrual of somatic mutations in normal cells [29]. These phenomena also occur in symptomatic patients with incident disease but they have less impact on specificity. Nonetheless, post-treatment persistence of urinary biomarkers may indicate a higher risk of future recurrence [30], a hypothesis that is clearly supported by the data reported here. Moreover, biomarker tests of this nature may detect some tumours before they become visible on cystoscopy, such that true-positive results are interpreted as false-positives and the specificity of the test is undermined. Hence, to realise the potential of such biomarkers through clinical trials and comparative studies, the field should consider establishing consensus on an acceptable time interval from testing to subsequent clinical detection of new or recurrent disease in order to harmonise the definition of true-positive and false-positive tests. However, if validated, in future studies it may be appropriate to intensify surveillance in biomarker-positive/cystoscopy-negative patients, or even instigate pre-emptive intravesical therapy.

Limited test specificity in the NMIBC surveillance setting requires further investigation, alongside limited sensitivity (65.2%) for the detection of grade 1 pTa tumours in patients with haematuria. It should also be noted that 45 BC cases in the retrospective haematuria clinic cohort were among the 956 tumours sequenced during development of the panel [19]; since very similar sensitivities were achieved in the retrospective and prospective haematuria clinic cohorts, this does not appear to have influenced the results. Furthermore, we did not undertake a head-to-head comparison with voided urine cytology; since the diagnostic performance of urine cytology is well characterised and mediocre [31] and its role in the investigation of haematuria is being challenged internationally [31,32], we did not consider this to be a useful or relevant comparison.

Other studies have used error-suppressed ultra-deep sequencing for the non-invasive detection of BC based on mutations in urinary DNA. The best validated mutation-based approach seems to be UroSEEK [27], with 83% sensitivity at 93% specificity for detection of incident disease (n = 570) and 68% sensitivity at 80% specificity for NMIBC surveillance (n = 322). This method requires urinary DNA to be divided between three separate polymerase chain reactions (*TERT* promoter, regions of 10 cancer-associated genes, and long interspersed nucleotide element-1 retrotransposon repeats for genome-wide aneuploidy) before sequencing. Our method has advantages of simplicity and applicability to samples with low DNA yields. There has also been considerable research on DNA methylation for BC detection, with several studies reporting very high sensitivity and specificity [33]. van Kessel et al [34] developed a test that combines methylation of *ONCEUT*, *OTX1*, and *TWIST1* with mutations in *HRAS*, *FGFR3*, and the *TERT* promoter which performs well in the haematuria clinic setting (AssureMDx) [34]. DNA-based tests that use analogue techniques may have analytical limits of detection that preclude BC detection if the tumour DNA is massively diluted by non-tumour DNA. However, genomic alterations in the absence of clinically detectable disease and limited shedding of cells from small low-grade tumours appear to prevent even the most technically sensitive methods and the most comprehensive biomarker panels from attaining 100% sensitivity and specificity. Another factor inhibiting the ascertainment of true biomarker performance is comparison with the reference (but imperfect) test of flexible cystoscopy [8,9].

We used urinary cpDNA in this study. We and others have reported that urinary cell-free (cf)DNA more faithfully recapitulates the tumour genome than cpDNA [35–37]. However, urinary cfDNA yields are frequently considerably lower than cpDNA yields; it is essential for assays that have the technical ability to detect very low VAFs to also have sufficient input DNA. We thus choose to use cpDNA, as >90% of urine samples can provide the required 25 ng of DNA for input [19]. In the future, use of total urinary DNA (cfDNA + cpDNA) or methods for cfDNA extraction from large volumes of urine [38] may further improve the performance of noninvasive BC tests.

5. Conclusions

We have described the development and validation of a noninvasive test for detection of BC based on error-suppressed ultra-deep sequencing of somatic mutations in 23 genes in urinary DNA. The test has the potential to detect new cases of BC with high sensitivity and specificity and could reduce reliance on cystoscopy in the haematuria clinic setting. Further studies are required in the NMIBC surveillance setting to define and characterise the key influences on test performance.

Author contributions: Richard T. Bryan 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: Ward, Bryan.
Acquisition of data: Gordon, Wang, Patel, Piechocki, Silcock, Sale, BladderPath Trial Group, Zeegers, Cheng, James, Bryan.
Analysis and interpretation of data: Baxter, Ott, Ward, Bryan.
Drafting of the manuscript: Ward, Bryan.
Critical revision of the manuscript for important intellectual content: Zeegers, Cheng, James.
Statistical analysis: Baxter, Ott, Ward.
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Supervision: Bryan, Ward, Cheng, Zeegers, James.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euo.2022.03.005.

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