A systematic review on mutation markers for bladder cancer diagnosis in urine

Anouk E. Hentschel\textsuperscript{1}, Emma E. van der Toom\textsuperscript{1}, André N. Vis\textsuperscript{1}, Johannes C.F. Ket\textsuperscript{3}, Judith Bosschieter\textsuperscript{1}, Martijn W. Heymans\textsuperscript{4}, R. Jeroen A. van Moorselaar\textsuperscript{1}, Renske D.M. Steenbergen\textsuperscript{2} and Jakko A. Nieuwenhuijzen\textsuperscript{1}

\textsuperscript{1}Departments of Urology, \textsuperscript{2}Pathology, Amsterdam University Medical Centers, Cancer Center Amsterdam, \textsuperscript{3}Medical Library, Vrije Universiteit Amsterdam, and \textsuperscript{4}Amsterdam, Epidemiology & Biostatistics, Amsterdam Public Health, Amsterdam, The Netherlands

**Objectives**

To systematically summarise the available evidence on urinary bladder cancer (BC) mutation markers. Gene mutations are expected to provide novel biomarkers for urinary BC diagnosis. To date, evidence on urinary BC mutation markers has not proven sufficient to be adopted by clinical guidelines. In the present systematic review, diagnostic accuracy of urinary mutation analysis is separately assessed for primary BC diagnosis (BC detection) and for follow-up of BC patients (BC surveillance).

**Methods**

A literature search (PubMed, Embase.com and Wiley/Cochrane Library) and systematic review was performed up to 31 October 2019. As studies were too heterogeneous, no quantitative analysis could be performed.

**Results**

In total, 25 studies were summarised by qualitative analysis. For BC detection, diagnostic accuracy differed considerably for single mutation markers (sensitivity 1–85%, specificity 84–100%), and for marker panels (sensitivity 50–94%, specificity 43–97%). Similarly, for BC surveillance, diagnostic accuracy was highly variable for single mutation markers (sensitivity 0–85%, specificity 66–100%), and for marker panels (sensitivity 51–84%, specificity 66–96%).

**Conclusion**

Urinary mutation analysis showed to be a promising diagnostic tool for non-invasive BC diagnosis. Nonetheless, we observed substantial differences in diagnostic accuracy of urinary BC mutation markers among publications. To translate the data summarised in the present review to future clinical practice, heterogeneity in research design, BC population, mutation analysis technique and urinary DNA should be considered. Eventual clinical implementation of urinary BC mutation markers can only be achieved by collecting more and stronger evidence. Combining different molecular assays might overcome current shortcomings of urinary mutation analysis.

**Keywords**

biomarkers, mutation, molecular diagnostics, urinary bladder neoplasms, urine analysis, #BladderCancer, #blcsm

**Introduction**

Bladder cancer (BC) is a worldwide clinical problem, as it is present among the top 10 most commonly diagnosed cancers. A first indication of BC is often painless haematuria without other symptoms. After transurethral resection of the bladder tumour (TURBT), patients are either diagnosed with non-muscle-invasive (NMIBC) or muscle-invasive BC (MIBC). In organ-confined MIBC, radical cystectomy or chemoradiation are indicated for local tumour control, because poor prognosis necessitates a radical approach [1]. As patients with NMIBC have a more favourable prognosis, the bladder can be preserved in these patients. Treatment consists of TURBT, followed by adjuvant instillations dependent on the risk classification: low-, intermediate- or high-risk [2].

The risk of recurrence (31–78%) or progression (1–45%) remains substantial for patients with NMIBC [3]. Therefore,
follow-up visits are required at regular intervals during a minimum period of 1 year for low-risk and 5 years for intermediate-risk patients, while high-risk patients need lifelong follow-up [2]. Cystoscopy is the ‘gold standard’ for primary BC diagnosis (BC detection) and for follow-up of BC patients (BC surveillance) [2]. Following cystoscopy, patients can experience irritative urinary symptoms and, although rare, even severe complications (e.g. urosepsis). Besides the fact that cystoscopy is an invasive procedure, it is also expensive and time-consuming [4]. Urinary analysis is considered a non-invasive and affordable alternative for cystoscopy. Urinary cytology has a sensitivity of 48%, but is only part of clinical practice in high-grade (HG) disease, as here sensitivity increases to 84%, while in low-grade (LG) disease sensitivity decreases to 16% [2,5]. In order to replace cystoscopy, urinary analysis should perform well in all grades and stages of disease. The past decade, several potential urinary biomarkers for BC diagnosis have been proposed at DNA, RNA and protein level, such as DNA methylation, microRNA and Survivin protein, respectively [6]. Many studies reported on this topic, but evidence on urinary biomarkers has not proven sufficient to be adopted by clinical guidelines [2,7].

In addition to DNA methylation, which we previously assessed in a systematic review, other urine-based biomarkers at the DNA level include DNA point mutations, as well as copy number and microsatellite changes. In the present review, we focus on BC mutation markers because of the high mutational load in BC and the extensive number of published reports on DNA mutation analysis in urine for BC diagnosis [8]. DNA point mutations linked to development of BC (e.g. in fibroblast growth factor receptor 3 [FGFR3], phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit α [PIK3CA] and telomerase reverse transcriptase [TERT] promoter) can be present at different hotspots; e.g. FGFR3 hotspot mutations are particularly found at specific positions in exon 7, 10 or 15, and PIK3CA hotspot mutations are mainly detected in exon 9 or 20 [9,10]. Some mutation markers are frequently detected in NMIBC (e.g. FGFR3 hotspot mutations), while others are more often found in MIBC (e.g. tumour protein p53 [TP53] hotspot mutations) [8]. Studies have not only evaluated single mutation markers (e.g. FGFR3 only), but have also investigated panels of two or more mutation markers and have even performed genome-wide screening for mutation markers [6].

To our knowledge, this is the first systematic review that specifically focusses on mutation markers for urine-based BC diagnosis. Diagnostic accuracy of urinary mutation analysis is separately assessed for BC detection and for BC surveillance. We present a detailed insight into the available literature, a critical review of the existing evidence and an overview of the most promising urinary mutation marker(s) for future practice. Furthermore, we explore heterogeneity between studies and make suggestions for future research.

**Methods**

**Search strategy**

A systematic review of the literature was conducted according to the Cochrane Methods Group for Systematic Review of Screening and Diagnostic Tests [11,12]. PubMed, Embase.com and Wiley/Cochrane Library were searched from inception up to 31 October 2019, for relevant publications (by J.C.F.K. and A.E.H.) as represented in the Appendix S1. The search included indexed terms and free-text words for ‘bladder cancer’, and ‘urine’, and ‘DNA’ or ‘mutation marker’, and ‘sensitivity’ or ‘specificity’. Two reviewers independently (A.E.H. and E.E.T.) checked the references of the selected full-text articles for relevant records. Duplicate publications were removed. If a research group published twice on the same population, the latest publication was included.

**Study selection**

The online tool Rayyan Qatar Computing Research Institute (QCRI) (https://rayyan.qcri.org) was used to manage the selected records from the bibliographic databases [13]. Publications were independently screened on title and abstract by two reviewers (A.E.H. and E.E.T.). If title and abstract were inconclusive, full-texts were screened. Original articles on DNA mutation markers for urine-based BC diagnosis were eligible for inclusion. Authors could use any kind of technique for urinary mutation analysis, but outcomes of urinary mutation analysis had to be compared with the current ‘gold standard’ for BC diagnosis (cystoscopy/histology). Studies had to be written in English and had to include a minimum of 10 patients with BC. Animal studies and studies without primary data (e.g. reviews, commentaries) were excluded. Studies that focussed on BC caused by occupational exposure or Bilharzia were excluded as well. These in- and exclusion criteria are largely in accordance with an earlier systematic review conducted by our research group on methylation markers for BC diagnosis in urine [14]. Disagreements between the reviewers were discussed in a consensus meeting with an expert (J.A.N.).

**Data extraction and quality assessment**

If available online, the full-text articles were judged independently by the two reviewers (A.E.H. and E.E.T.). Sensitivities, specificities, negative and positive predictive values were retrieved from the published data on patients with BC and controls. Data on single mutation markers and marker panels were collected.
Risk of bias (RoB) assessment

Reviewers A.E.H. and E.E.T. independently assessed the RoB of the included articles by using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 tool [15]. Reproducibility of the QUADAS-2 tool was piloted in two studies. Disagreements were again discussed in a consensus meeting together with an expert (J.A.N.).

Data analysis

Reviewers A.E.H. and E.E.T. independently abstracted data of the included articles. Diagnostic accuracy of urinary mutation analysis was assessed for BC detection and for BC surveillance. Sensitivity was defined as the percentage of true-positive results in the patients with BC. Specificity was defined as the percentage of true-negative results in the controls. Sensitivity was determined at patient level (1 urine sample/patient), whereas specificity could be determined at patient level or at urine sample level (≥1 urine samples/patient). Tumour-informed sensitivity was defined as the percentage of true-positive results in the bladder tumour tissues with mutations. Tumour-informed specificity was defined as the percentage of true-negative results in the bladder tumour tissues without mutations.

For single-mutation markers, sensitivity and specificity were visualised in forest plots if ≥10 studies reported on the same mutation marker (irrespective of the number of patients included). Sensitivity and specificity were described from five or more studies (irrespective of the number of patients included). Tumour-informed sensitivity and specificity were described when ≥100 tumours were included in the study.

For marker panels, sensitivity and specificity, and tumour-informed sensitivity and specificity were presented in tables. The most promising marker panels with a sensitivity and specificity of ≥80% were described.

Review methods were established in a protocol prior to the conduct of the review and no significant deviations from the protocol were made.

As studies were too heterogeneous, we could not perform a meta-analysis of the data. We therefore decided to summarise the data by performing a qualitative analysis.

Review Manager Version 5.3 (Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014) was used for the construction of graphs.

Results

Quantity of evidence identified

We selected 25 studies from the literature, as shown in the Preferred Reporting Items for Systematic and Meta-Analysis (PRISMA) flow diagram (Fig. 1) [16–40]. An overview of the studies included is given in Table 1 [16–40]. Urinary mutation analysis was used for BC detection in 15 studies [19–22,24–29,31,32,34,39,40], for BC surveillance in three studies [18,30,35], and for BC detection and surveillance in seven studies [16,17,23,33,36–38]. Single mutation markers were assessed in 13 studies [18,19,21–27,30,33,36,39] and marker panels in 12 studies [16,17,20,28,29,31,32,34,35,37,38,40]. For BC detection, 27 single-mutation markers were investigated (Table S1). For BC surveillance, 20 single-mutation markers were evaluated (Table S2). The number of mutation markers included in the panels ranged from two to 20. From the publications on multiple mutation markers, data were retrieved on the marker panel and on the single-mutation markers (if data were provided). Tumour-informed sensitivity and specificity were reported in 10 studies [17,19,25–29,33,35,37], including three studies that reported on tumour-informed analysis only [19,27,28].

RoB of included studies

In 20/25 studies, high RoB was scored for patient selection. In most studies, high RoB was introduced by non-consecutive collection of urine samples or by case-control designs (Fig. 2). For eight of the 25 studies, high RoB was scored for index test (urine) test, because the threshold of urinary mutation analysis was not specified, or because it was not stated whether reviewers of urinary mutation results were blinded to the gold standard results. In five of the 25 studies, high applicability concerns were scored for patient selection, as studies solely reported on the diagnostic accuracy of tumour-informed analysis, or as studies selected patients according to their tumour mutation status/their urinary cytology status. Lastly, two of the 25 studies scored high applicability concerns for the index test, as positivity of urinary mutation analysis was also considered true-positive if it preceded positivity of the gold standard by several months. Judgements on bias and applicability were all scored low for three of the 25 studies [20,24,30].

BC detection

Single-mutation markers

Among 18 studies, data could be retrieved on 27 single-mutation markers for urine-based BC detection, with sensitivities from 1% to 85%, and specificities from 84% to 100% (Table S1). Three studies investigated several single mutation markers, but only found negative outcomes for one of their single-mutation markers in both patients with BC and controls: NRAS proto-oncogene, GTPase (NRAS), lysine demethylase 6A (KDM6A) and Erb-B2 receptor tyrosine kinase 3 (ERBB3), respectively [32,34,37]. Sensitivity and specificity of FGFR3 was reported in 10 studies. The results
are summarised in a forest plot and a receiver operating characteristic (ROC) plot (Fig. 3A,B). Allory et al. [16], Beukers et al. [17] and Kandimalla et al. [23] solely reported on FGFR3 sensitivity for urinary BC detection and were therefore not included. All three studies showed comparable sensitivities: 36–39%. Diagnostic accuracy of TERT was reported in 10 studies, and results are shown in Fig. 4A,B. The study by Beukers et al. [17] was not included, because they only assessed TERT sensitivity (73%), but no specificity. The total of 11 studies on TERT all focussed on mutations in the promoter region of the gene. Sensitivity and specificity of HRas proto-oncogene, GTPase (HRAS) was reported in six studies and of PIK3CA in five studies (Table S1). HRAS sensitivity ranged from 1% to 44% and specificity was 100% in all studies. PIK3CA sensitivity varied from 13% to 19% and specificity from 96% to 100%.

**Single-mutation markers: tumour-informed analysis**

Across six studies, data could be collected on six single-mutation markers for tumour-informed urinary BC detection, with sensitivities from 34% to 100% and specificities from 38% to 100% (Table S1). Noel et al. [28] and Serizawa et al. [29] investigated the diagnostic accuracy of single-mutation markers in ≥100 tumours. For FGFR3, Noel et al. [28] found a sensitivity of 43% (95% CI 27–61%) at a specificity of 98% (95% CI 92–100%), whereas Serizawa et al. [29] reported a sensitivity of 63% (95% CI 48–77%) at a specificity of 98% (95% CI 90–100%). For TP53, Noel et al. [28] reported a sensitivity of 34% (95% CI 22–48%) and a specificity of 87% (95% CI 74–95%) compared to Serizawa et al. [29] with a sensitivity of 67% (95% CI 41–87%) at a specificity of 99% (95% CI 93–100%). Serizawa et al. [29] also investigated PIK3CA and found a sensitivity of 53% (95% CI 29–76%) at a specificity of 94% (95% CI 86–98%).

**Marker panels**

A total of 11 studies reported diagnostic accuracy of eight different marker panels for urinary BC detection (Table 2 [16,17,20,28,29,31,32,34,37,38,40]). Sensitivity ranged from 50% to 94%, whereas specificity ranged from 43% to 97%. Sensitivity and specificity were most promising (≥80%) for urinary marker panels of Dahmcke et al. [20], Dudley et al. [37] and Rodriguez Pena et al. [38]. Dahmcke et al. [20] included two mutation markers (FGFR3 and TERT), whereas
| Study ID | First author (year) | BC patients/controls, n | Primary/recurrent, n | Tumour-informed analysis* | Level analysis | Mutation marker(s) | Controls | NMIBC/MIBC, % | Urinary DNA| Mutation analysis technique | Ref. |
|---------|---------------------|-------------------------|---------------------|-------------------------|----------------|------------------|----------|----------------|---------|-----------------------------|------|
| 1.      | Allory (2014)       | 278/124                 | M (135/143)         | No                      | Both           | FGFR3, TERT     | S        | 90/8*          | Cellular| Sanger Seq, SNaPshot        | [16] |
| 2.      | Avogbe (2019)       | Test: 94/93 Validation: 50/50 | M (95/48) | No | Patient | TERT | M | 81/19 | Cellular/ Cell-free | SNaPshot | [36] |
| 3.      | Beukers (2017)      | 977 BC cases (305 samples of primary BC cases and multiple negative control samples during FU) | M (977/578) | Both | Both | FGFR3, TERT | S | 100/0 (132 MIBC cases were excluded) | Cellular | | [17] |
| 4.      | Couffignal (2015)   | 74 (multiple negative control samples during FU) | R | No | Urine sample | FGFR3 | S | 100/0 | Cellular | allele-specific PCR | [18] |
| 5.      | Curtigliano (2001)  | 10'14 (validated in 10 non-BC cases) | P | Yes | Urine sample | TP53 | BC cases with WT TP53 | 100/0 (T1 only) | Cellular | DGGE | [19] |
| 6.      | Dahmcke (2016)      | 99/376                  | P | No | Patient | FGFR3, TERT | S | 85/13* | Cellular | Droplet digital PCR, hydrolysis probe-based assays | [20] |
| 7.      | Descotes (2017)     | 348/167                 | P | No | Patient | TERT | M | 82/18 | Cellular | nested PCR, Sanger Seq | NGS | [21] |
| 8.      | Dudley (2019)       | 91/94                   | M (54/37) | Both | Patient | AKT1, ARID1A, BRAF, CDKN1A, CDKN2A, EP300, ERBB2, ERBB3, FXXW7, FGFR3, KDM6A, KRAS, MED12, PIK3CA, PLEKHS1, RB1, STAG2, TERT, TP53, TSC1 | M | 85/15 | Cellular | NGS | [37] |
| 9.      | Fitzgerald (1995)    | 100/20                  | P | No | Patient | HRAS | H | U | Cellular | SSCP | [22] |
| 10.     | Kandimalla (2013)   | Test: 140/70 Validation: 95/130 | M (39/196) | No | Patient | FGFR3 | M | 93/6* | Cellular | SNaPshot | [23] |
| 11.     | Karnes (2012)       | Test: 48/240 Validation: 58/690 | P | No | Patient | FGFR3 | S | 91/9 | Genomic | PCR clamping | [24] |
| 12.     | Millholland (2012)  | 43/24                   | P | Both | Patient | FGFR3 | S | 84/16 | Genomic | NGS | [25] |
| 13.     | Miyake (2007)       | 13/20                   | P | Both | Patient | FGFR3 | H | 100/0 (MIBC were considered as controls and were therefore excluded) | Cellular | PCR clamping, direct Seq | [26] |
| 14.     | Miyake (2010)       | 24/21 (not validated in non-BC cases) | P | Yes | Patient | FGFR3 | BC cases with WT FGFR3 | 100/0 | Cellular | PCR clamping, direct Seq | [27] |
| Study ID | First author (year) | BC patients/controls, n | Primary/recurrent, n | Tumour-informed analysis* | Level analysis | Mutation marker(s) | Controls | NMIBC/MIBC, % | Urinary DNA† | Mutation analysis technique | Ref. |
|----------|---------------------|------------------------|---------------------|-------------------------|----------------|-------------------|----------|---------------|-------------|-----------------------------|-----|
| 15. Noel (2015) | 76/27 (validated in 5 non-BC cases) | P | Yes | Patient | FGFR3, TP53 | BC cases with WT FGFR3 or TP53 | S | 75/25 | Cellular | SNaphot, functional assay | [28] |
| 16. Rodriguez Pena (2019) | 260/186 | M (25/235) | No | Urine sample | CKN2A, ERBB2, FGFR3, HRAS, KRAS, MET, MLL, PIK3CA, TERT, TP53, VHL | S | 75/10† | Cellular | NGS, SafeSeqS | [38] |
| 17. Serizawa (2011) | 118/33 | P | Both | Patient | FGFR3, HRAS, KRAS, NRAS, PIK3CA, TERT | H | 83/17 | Cellular | DGGE, direct Seq | [29] |
| 18. Shore (2012) | 63/670 | R | No | Patient | FGFR3 | S | 98/0‡ | Genomic | PCR damping | NGS | [30] |
| 19. Stasik (2019) | 53/36 | P | No | Patient | FGFR3 | S | 81/19 | Genomic | Cell-free | SNaphot | [31] |
| 20. van Kessel (2016) | 74/80 | P | No | Patient | FGFR3, HRAS, KRAS, NRAS, PIK3CA, TERT | S | 68/32 | Cellular | Snaphot | [32] |
| 21. van Kessel (2017) | 97/103 | P | No | Patient | FGFR3, HRAS, TERT | S | 82/8§ | Cellular | SNaphot | [33] |
| 22. van Kessel (2017) | 51/15 | M (U) | Both | Patient | FGFR3 | S | 80/19† | Cellular | SSCP, Seq | [34] |
| 23. van Kessel (2017) | 122/109 | P (2/122 were recurrent) | No | Patient | FGFR3, HRAS, PIK3CA, TERT, TP53 | M | 66/32† | Cellular | NGS | [35] |
| 24. Ward (2016) | 95/67 | P | No | Patient | ARID1A, CDKN2A, CREBBP, ERBB2, ERBB3, FGFR1, FGFR3, HRAS, KMT2D, PIK3CA, TAC2, TP53, TSC1 | S | 100/0 | Cellular | NGS | [36] |
| 25. Zuiverloon (2013) | 136 BC cases (multiple negative control samples during FU) | R | Both | Urine sample | FGFR3, HRAS, KRAS, NRAS, PIK3CA | S | 100/0 | Cellular, cell-free | SNaphot | [37] |

ARID1A, AT-rich interaction domain 1A; DGGE, denaturing gradient gel electrophoresis; FU, follow-up; H, healthy controls; M, mixed controls; P, primary; PLEKHS1, leckstrin homology domain-containing family S member 1; R, recurrent; RB1, retinoblastoma; Ref., reference; RXRA, Retinoid X receptor alpha; S, symptomatic patients or patients under surveillance; SSNaphot, single-strand conformation polymorphism; STAG2, stromal antigen 2; TSC1, TSC complex subunit 1; U, unknown; VHL, Von Hippel-Lindau tumour suppressor; WT, wild-type. *Tumour-informed analysis: tumour-informed sensitivity/specificity represent the % true-positive urines in mutated tumours/the % true-negative urines in non-mutated tumours. †T stage is not known for all BC cases. ‡Cellular, cell-free and genomic refers to DNA isolated from urine pellet, urine supernatant and full void urine, respectively.
### Fig. 2 Overview of RoB and applicability concerns according to QUADAS-2.

|                      | Risk of Bias | Applicability Concerns |
|----------------------|--------------|------------------------|
|                      | Patient Selection | Index Test | Reference Standard | Flow and Timing | Patient Selection | Index Test | Reference Standard |
| Allory Y, 2014       | +            | +          | +                  | +              | +            | +          | +                  |
| Avogbe PH, 2019      | +            | +          | +                  | +              | +            | +          | +                  |
| Beukers W, 2017      | +            | +          | +                  | +              | +            | +          | +                  |
| Couffignal C, 2015   | +            | +          | +                  | +              | +            | +          | +                  |
| Curigliano G, 2001   | +            | +          | +                  | +              | +            | +          | +                  |
| Dahmcke CM, 2016     | +            | +          | +                  | +              | +            | +          | +                  |
| Descotes F, 2017     | +            | +          | +                  | +              | +            | +          | +                  |
| Dudley JC, 2019      | +            | +          | +                  | +              | +            | +          | +                  |
| Fitzgerald JM, 1995  | +            | +          | +                  | +              | +            | +          | +                  |
| Kandimalla R, 2013   | +            | +          | +                  | +              | +            | +          | +                  |
| Karnes RJ, 2012      | +            | +          | +                  | +              | +            | +          | +                  |
| Milholland JM, 2012  | +            | +          | +                  | +              | +            | +          | +                  |
| Miyake M, 2007       | +            | +          | +                  | +              | +            | +          | +                  |
| Miyake M, 2010       | +            | +          | +                  | +              | +            | +          | +                  |
| Noel N, 2015         | +            | +          | +                  | +              | +            | +          | +                  |
| Rodriguez Pena MDC, 2019 | +    | +          | +                  | +              | +            | +          | +                  |
| Serizawa RR, 2011    | +            | +          | +                  | +              | +            | +          | +                  |
| Shore ND, 2012       | +            | +          | +                  | +              | +            | +          | +                  |
| Stasik S, 2019       | +            | +          | +                  | +              | +            | +          | +                  |
| van Kessel KEM, 2016 | +            | +          | +                  | +              | +            | +          | +                  |
| van Keessel KEM, 2017| +            | +          | +                  | +              | +            | +          | +                  |
| van Rhijn BWG, 2003  | +            | +          | +                  | +              | +            | +          | +                  |
| Ward DG, 2016        | +            | +          | +                  | +              | +            | +          | +                  |
| Zhu F, 2019          | +            | +          | +                  | +              | +            | +          | +                  |
| Zuiverloon TCM, 2013 | +            | +          | +                  | +              | +            | +          | +                  |

Key:
- **High**
- **Unclear**
- **Low**
Fig. 3 Forest plot (A) and ROC plot (B) of the estimated diagnostic accuracy of FGFR3 for urine-based BC detection. The number of true-positive (TP), false-positive (FP), false-negative (FN) and true-negative (TN) results are provided.

A

| Study                      | TP  | FP  | FN  | TN  | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|----------------------------|-----|-----|-----|-----|----------------------|----------------------|----------------------|----------------------|
| Dahmcke CM, 2016           | 41  | 8   | 58  | 368 | 0.41 [0.32, 0.52]    | 0.98 [0.96, 0.99]    |                      |                      |
| Dudley JC, 2019            | 16  | 0   | 38  | 34  | 0.30 [0.18, 0.44]    | 1.00 [0.90, 1.00]    |                      |                      |
| Karnes RJ, 2012            | 5   | 2   | 43  | 238 | 0.10 [0.03, 0.23]    | 0.99 [0.97, 1.00]    |                      |                      |
| Milholland JM, 2012        | 24  | 0   | 19  | 24  | 0.56 [0.40, 0.71]    | 1.00 [0.86, 1.00]    |                      |                      |
| Miyake M, 2007             | 11  | 0   | 2   | 20  | 0.85 [0.55, 0.98]    | 1.00 [0.83, 1.00]    |                      |                      |
| Serizawa RR, 2011          | 32  | 0   | 81  | 33  | 0.28 [0.20, 0.38]    | 1.00 [0.89, 1.00]    |                      |                      |
| van Kessel KEM, 2016       | 21  | 1   | 48  | 76  | 0.30 [0.20, 0.43]    | 0.99 [0.93, 1.00]    |                      |                      |
| van Kessel KEM, 2017       | 33  | 1   | 62  | 98  | 0.35 [0.25, 0.45]    | 0.99 [0.95, 1.00]    |                      |                      |
| Ward DG, 2016              | 37  | 1   | 85  | 108 | 0.30 [0.22, 0.39]    | 0.99 [0.95, 1.00]    |                      |                      |
| Zhu F, 2019                | 26  | 7   | 69  | 60  | 0.27 [0.19, 0.37]    | 0.90 [0.80, 0.96]    |                      |                      |

B

**Legend**

1 van Kessel KEM, 2017
2 Ward DG, 2016
3 Serizawa RR, 2011
4 van Kessel KEM, 2016
5 Milholland JM, 2012
6 Miyake M, 2007
7 Dudley JC, 2019
8 Karnes RJ, 2012
9 Zhu F, 2019
10 Dahmcke CM, 2016

© 2020 The Authors
BJU International published by John Wiley & Sons Ltd on behalf of BJU International
**Fig. 4** Forest plot (A) and ROC plot (B) of the estimated diagnostic accuracy of TERT for urine-based BC detection. The number of true-positive (TP), false-positive (FP), false-negative (FN) and true-negative (TN) results are provided.

### A

| Study                          | TP | FP | FN | TN | Sensitivity (95% CI) | Specificity (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) |
|-------------------------------|----|----|----|----|----------------------|----------------------|----------------------|----------------------|
| Allory Y, 2014                | 73 | 4  | 45 | 35 | 0.62 [0.52, 0.71]    | 0.90 [0.76, 0.97]    |                      |                      |
| Avogbe PH, 2019               | 38 | 5  | 7  | 88 | 0.84 [0.71, 0.94]    | 0.95 [0.88, 0.98]    |                      |                      |
| Dahmcke CM, 2016              | 81 | 62 | 18 | 314| 0.82 [0.73, 0.89]   | 0.84 [0.79, 0.87]   |                      |                      |
| Descotes F, 2017              | 280| 17 | 68 | 150| 0.80 [0.76, 0.84]   | 0.90 [0.84, 0.94]   |                      |                      |
| Dudley JC, 2019               | 30 | 1  | 24 | 33 | 0.56 [0.41, 0.69]   | 0.97 [0.85, 1.00]   |                      |                      |
| Rodriguez Pena MDC, 2019      | 15 | 9  | 10 | 80 | 0.60 [0.39, 0.79]   | 0.90 [0.82, 0.95]   |                      |                      |
| Stasik S, 2019                | 40 | 1  | 12 | 30 | 0.77 [0.63, 0.87]   | 0.97 [0.83, 1.00]   |                      |                      |
| van Kessel KEM, 2016          | 42 | 5  | 26 | 69 | 0.62 [0.49, 0.73]   | 0.93 [0.85, 0.98]   |                      |                      |
| van Kessel KEM, 2017          | 70 | 3  | 26 | 96 | 0.73 [0.63, 0.81]   | 0.97 [0.91, 0.99]   |                      |                      |
| Ward DG, 2016                 | 67 | 2  | 55 | 107| 0.55 [0.46, 0.64]   | 0.98 [0.94, 1.00]   |                      |                      |

### B

**Legend**

1 Avgbe PH, 2019
2 Allory Y, 2014
3 Descotes F, 2017
4 Dahmcke CM, 2016
5 Rodriguez Pena MDC, 2019
6 Dudley JC, 2019
7 van Kessel KEM, 2016
8 Stasik S, 2019
9 Ward DG, 2016
10 van Kessel KEM, 2017

© 2020 The Authors
BJU International published by John Wiley & Sons Ltd on behalf of BJU International
Dudley et al. [37] and Rodriguez Pena et al. [38] included 20 and 10 mutation markers, respectively. The combination FGFR3/TERT was reported in three studies with comparable sensitivities in Allory et al. [16] (70%) and Beukers et al. [17] (79%), and a higher diagnostic accuracy in Dahmcke et al. [20] (sensitivity 89% and specificity 82%). Marker panel FGFR3/HRAS/TERT was studied by the same research group (van Kessel et al. [31,32]) in 2016 and 2017, with similar diagnostic accuracy in both cohorts: sensitivity 72% vs 77% and specificity 93% vs 97%, respectively.

### Marker panels: tumour-informed analysis

Three studies described diagnostic accuracy of three different marker panels for tumour-informed urinary BC detection. Sensitivity varied from 46% to 93% and specificity varied from 81% to 96% [28,29,37]. Diagnostic accuracy was most promising for Dudley et al. [37], with a sensitivity of 93% and a specificity of 96%.

### BC surveillance

### Single-mutation markers

Among nine studies, data could be retrieved on 20 single-mutation markers for urinary BC surveillance, with sensitivities from 0% to 85% and specificities from 66% to 100% (Table S2). Dudley et al. [37] only found negative results in both patients with BC and controls for six of 20 mutation markers: serine/threonine-protein kinase 1 (AKT1), B-Raf proto-oncogene, serine/threonine kinase (BRAF), cyclin-dependent kinase inhibitor 2A (CDKN2A), E1A binding protein P300 (EP300), F-Box and WD repeat domain containing 7 (FBXW7) and mediator complex subunit 12 (MED12) [37]. FGFR3 and TERT were investigated in more than five studies. Across seven studies, FGFR3 sensitivity differed from 3% to 73% and specificity from 66% to 100%. In five studies, TERT sensitivity was 43% to 83% and specificity was 68% to 100%.

### Single-mutation markers: tumour-informed analysis

In two studies, data could be collected on two single-mutation markers for tumour-informed urinary BC surveillance in ≥100 tumours [17,35]. For FGFR3, Beukers et al. [17] found a sensitivity of 44% (95% CI 35–52%) at a specificity of 81% (95% CI 75–87%) and Zuiverloon et al. [35] reached a sensitivity of 66% (95% CI 60–72%) at a specificity of 50% (95% CI 40–60%). For TERT, Beukers et al. [17] found a sensitivity of 71% (95% CI 61–80%) and a specificity of 56% (95% CI 46–66%).
Four studies investigated diagnostic accuracy of three different marker panels for urinary BC surveillance (Table 3 [16,17,35,37,38]). Sensitivity ranged from 51% to 84% and specificity ranged from 66% to 96%. Sensitivity and specificity were most promising (≥80%) for Dudley et al. [37] who described a sensitivity of 84% at a specificity of 96% for their urinary marker panel.

### Marker panels: tumour-informed analysis

Three studies evaluated diagnostic accuracy of three different marker panels for tumour-informed urinary BC surveillance. Sensitivity varied from 67% to 91% and specificity varied from 56% to 100%. Dudley et al. [37] described the most promising urinary marker panel with a sensitivity of 91% and a specificity of 100%.

### BC detection vs BC surveillance

We evaluated seven studies that included both patients with primary and recurrent BC, of which six studies provided separate data for both groups. Allory et al. [16], Beukers et al. [17] and Kandimalla et al. [23] reported higher FGFR3 sensitivities for BC detection than for BC surveillance: 36% vs 19%, 36% vs 23% and 39% vs 10%, respectively (Tables S1 and S2). Allory et al. [16] and Beukers et al. [17] also detected higher TERT sensitivities for BC detection than for BC surveillance: 62% vs 42% and 73% vs 49%, respectively. However, Avogbe et al. [36] and Rodriguez Pena et al. [38] found comparable TERT sensitivities for BC detection and surveillance: 84% vs 85% and 60% vs 65%, respectively. Dudley et al. [37] found that their urinary marker panel had a similar sensitivity in the BC detection and surveillance setting (83% vs 84%), whereas Rodriguez Pena et al. [38] reported a higher sensitivity of their urinary marker panel for BC detection than for BC surveillance (88% vs 54%).

### Discussion

Urinary mutation analysis seems to provide a promising diagnostic tool for non-invasive BC diagnosis. Systematic review of available literature revealed acceptable diagnostic potential for single-mutation markers and for marker panels. Yet, diagnostic accuracy of urinary BC-mutation markers differed considerably among publications. This was particularly evident for single-mutation markers, not only showing varying diagnostic performance between markers, but even so for the same marker (e.g. FGFR3, Fig. 3). For marker panels, differences in diagnostic performance were less evident, but still substantial. The varying results can partly be explained by heterogeneity in study characteristics, e.g. differences in the sample size, the (non-)consecutive/prospective collection of samples, the proportion of patients with NMIBC/MIBC, the comparison to (non-)matched controls, and the type of mutation analysis technique. Present data do not yet allow for clinical decision making, and

### Table 3 Marker panels of urinary mutation markers for bladder cancer surveillance.

| Marker panels                  | First author (year) | Ref. | BC patients/controls, n | Sensitivity, % | Specificity, % | Tumour-informed sensitivity, % | Tumour-informed specificity, % |
|--------------------------------|---------------------|------|------------------------|----------------|----------------|-------------------|-----------------------------|
| AKT1/ARID1A/BRCA1/CDKN1A/CDKN2A/EF300/ERBB2/ERBB3/FBXW7/FGFR3/KDM6A/KRAS/MED12/PK3CA/PLEKHS1/RB1/STAG2/TER/TP53/TNCI | Dudley (2019) | [37] | 91/94                  | 83.8           | 96.3           | 91                | 100                         |
| CDKN2A/ERBB2/FGFR3/HRAS/KRAS/MLL/PIK3CA/TP53/VHL | Rodriguez Pena (2019) | [38] | 260/186                | 53.6           | 88.7           | –                 | –                           |
| FGFR3/TER | Allory (2014) | [16] | 278/124                | 50.5           | 70.6           | –                 | –                           |
| FGFR3/TER | Beukers (2017) | [17] | 977/multiple negative control samples during FU | 54.3           | 66.1           | 66.7              | 55.6                        |
| FGFR3/PIK3CA/RAS* | Zuiverloon (2013) | [35] | 136/multiple negative control samples during FU | –              | –              | 71.4              | 62.5                        |

FU, follow-up; Ref., reference. *RAS = HRAS/KRAS/NRAS.
diagnostic use of urinary BC-mutation markers can only be achieved by gathering more and stronger evidence.

In most studies, diagnostic accuracy of urinary mutation analysis was based on the presence or absence of BC. However, some studies calculated diagnostic accuracy of urinary mutation analysis based on the presence or absence of concordant mutations in the tumour (so-called tumour-informed analysis). We believe that tumour-informed analysis is less likely to be implemented for urine-based BC diagnosis, as this can only be applied in patients with histologically confirmed BC in which the mutational status has been determined. Also, tumour-informed specificities were generally lower. Tumour mutation analysis represents only a small part of the tumour and this may result in more ‘false-positive’ urines [41]. Therefore, we will discuss the most promising (defined as sensitivity and specificity ≥80%) single-mutation markers and marker panels for urinary mutation analysis based on the presence or absence of BC.

For BC detection, TERT reached a sensitivity and specificity of ≥80% across three studies [20,21,36]. Avogbe et al. [36] reported the highest values with a TERT sensitivity of 84% at a specificity of 95% in the test set (45 patients with BC/93 controls). However, TERT sensitivity decreased to 68% in the validation set, while specificity remained high at 98% (50 patients with BC/50 controls) [36]. The test set was prospectively collected in France, while the validation set was retrospectively collected in Portugal. Avogbe et al. [36] suggest that differences in countries, sampling procedures or exposures may have caused varying diagnostic accuracy between sets. In addition, the validation set had unfavourable tumour characteristics with 24% LG/76% HG tumours and 64% NMIBC/36% MIBC patients compared to the test set with 40% LG/60% HG tumours and 91% NMIBC/9% MIBC patients. Also, the validation set included young healthy controls (median age 46 years), while the test set included older controls with other urological diseases or with benign colonoscopy (median age 70 years). The other two studies did not report on a validation series [20,21]. The percentage of NMIBC/MIBC patients was comparable between the three studies, but only Dahmcke et al. [20] included matched haematuria controls [20]. For BC detection, FGFR3 reached a diagnostic accuracy of ≥80% in one study: 85% sensitivity and 100% specificity [26]. However, this was a small series of 13 patients with NMIBC and 20 chronic cystitis controls. Given that six patients with MIBC without FGFR3 mutations were also considered as controls, we excluded these patients from our analyses as they do not represent ‘true’ controls. For BC detection, FGFR3/TERT was the only promising marker panel, with a sensitivity of 89% and a specificity of 82% [20]. This study was of high quality, based on the large sample size (99 patients with BC/376 matched controls), which was consecutively collected, a clinically relevant representation of patients with BC (85% NMIBC/13% MIBC), and low concerns regarding bias and applicability.

For BC surveillance, Avogbe et al. [36] reported promising results for TERT, with a sensitivity of 85% at a specificity of 95% in the test set (47 patients with BC/93 controls), but these results were not evaluated in a validation set [36]. The population of patients with BC consisted of 42% LG/58% HG and 90% NMIBC/10% MIBC, which properly reflects clinical practice of patients with BC under follow-up for recurrence. However, patients with BC were compared to non-matched controls (with other urological diseases or with benign colonoscopy), as of which specificity may be overestimated. No other studies found promising results for BC surveillance using urinary mutation marker(s).

In general, recurrent tumours are discovered earlier than primary tumours. Accordingly, recurrent tumours are smaller and less tumour DNA is expected in urine samples, which may result in lower sensitivity of urinary mutation analysis [42]. Across studies that described primary and recurrent tumours within the same publication, sensitivity was either similar for BC detection and BC surveillance, or sensitivity decreased in the BC surveillance setting. High sensitivity is important for BC detection, because patients with a negative test result will not be monitored afterwards and BC should therefore be ruled out with high certainty. For BC surveillance, requirements of test sensitivity depend on the follow-up strategy that is applied. A follow-up strategy in which cystoscopy is completely replaced by urinary mutation analysis would require high sensitivity. However, a follow-up strategy in which cystoscopy is alternated with urinary mutation analysis would allow for a somewhat lower sensitivity, because false-negative results would be encountered at cystoscopy after an acceptable time-interval. High specificity is important in both diagnostic settings, as false-positive results would lead to unnecessary cystoscopies.

Dudley et al. [37] and Rodriguez Pena et al. [38] also reported promising results of their urinary BC marker panels, consisting of 20 and 10 mutation markers, respectively. However, they followed patients with repeat diagnostic procedures to verify earlier urinary mutation analysis results, while other studies compared urinary mutation analysis results to cystoscopy/histology at one point in time. It is known that positivity of urinary mutation analysis regularly precedes positivity of standard diagnostic procedures, the so-called ‘anticipatory effect’ [16,17,35]. In their BC surveillance series, Dudley et al. [37] and Rodriguez Pena et al. [38] indeed found that positivity of their urine test preceded clinical diagnosis by an average of several months. The ‘anticipatory effect’ is also illustrated by Beukers et al. [17], showing that patients with a primary FGFR3-positive tumour and a ‘false-positive’ follow-up urine were more likely to experience a recurrence after 2 years than patients with a...
primary FGFR3-positive tumour and a negative follow-up urine: 73% vs 41% (P = 0.005) [17]. In clinical practice, such ‘false-positive’ urines could induce two possible responses. Firstly, urologists could be more attentive during cystoscopy, resulting in more positive cystoscopies. Secondly, urologists could perform cystoscopies at shorter time-intervals, which for some patients would lead to earlier diagnosis, but for other patients would lead to over-diagnosis. Future studies should pay attention to the clinical effects from these ‘false-positive’ results.

To translate the data summarised in the present review to future clinical application, heterogeneity in research design, BC population, mutation analysis technique, and urinary DNA should be considered. Firstly, research design should reflect clinical practice by prospective and consecutive collection of patient samples and comparison to matched controls. Diagnostic accuracy may be overestimated by non-consecutive sample collection and use of non-matched controls, in particular healthy individuals [22,26,29]. For example, Descotes et al. [21] found promising results for TERT (sensitivity 80%, specificity 90%) in a series of 348 patients with BC and 167 controls. Their controls varied from healthy individuals to patients with other cancers, whereas controls with haematuria were excluded, which may have introduced bias. Secondly, the BC population should reflect clinical practice. At initial presentation, ~75% is diagnosed with NMIBC and 25% is diagnosed with MIBC [2]. During follow-up, the percentage of NMIBC is higher. As the percentage of patients with NMIBC/MIBC differed considerably across studies (Table 1), results may not correspond to ‘true’ diagnostic accuracy. For BC detection, Miyake et al. [26] included 13 (100%) patients with NMIBC and found promising results for FGFR3 (sensitivity 85%, specificity 100%), while Dahmcke et al. [20] included 84 (85%) patients with NMIBC and 13 (13%) patients with MIBC and found less promising results for FGFR3 (sensitivity 41%, specificity 98%) [20,26]. Dahmcke et al. [20] showed that FGFR3 sensitivity decreased with tumour stage in their series (Ta 59%, T1 30% and ≥T2 31%), supporting the notion that FGFR3 sensitivity is strongly correlated to tumour stage [20,43]. Thirdly, as we did not apply any restrictions regarding mutation analysis technique, diagnostic accuracy will also depend on the type of mutation analysis technique to be eventually used in clinical practice. Millholland et al. [25] demonstrated that within the same series of 43 patients with BC, FGFR3 sensitivity improved from 12% with quantitative PCR to 56% with ultra-deep next-generation sequencing (NGS). Specificity was 100% with the latter technique, but was not assessed by quantitative PCR. Their PCR vs NGS results emphasise that the type of mutation analysis technique can have large impact on diagnostic accuracy. Advantages of PCR are its low costs, its relatively short processing time, and the extensive experience with PCR in routine diagnostic laboratories. The advantages of NGS, on the other hand, include the comprehensive analysis of multiple mutations and the potential to increase sensitivity by increasing sequencing coverage, which however comes at higher costs [44,45]. The method of choice in clinical practice should be based on assay characteristics (e.g. single-mutation marker vs extensive marker panel), clinical feasibility (e.g. time and costs) and diagnostic accuracy. The latter will also rely on the type of urinary DNA analysed [44,46,47]. Most studies used cellular DNA from urine pellet [16–23,26–29,31–40], of which two recent studies also assessed cell-free DNA from urine supernatant (Table 1) [36,39]. Avogbe et al. [36] found that diagnostic accuracy of TERT was similar for cellular DNA and cell-free DNA analyses, but diagnostic accuracy was highest when both analyses were combined in primary (sensitivity 87%, specificity 95%) and recurrent (sensitivity 88%, specificity 95%) disease, which is consistent with previous work [36,44]. Stasik et al. [39] concluded that TERT sensitivity was higher for cellular DNA (77%) than for cell-free DNA (63%) at comparable specificities (97% vs 100%, respectively) [39]. In the present review, we only included cellular DNA results from the latter two studies to reduce heterogeneity between studies and because cellular DNA sensitivity (somewhat) outperformed cell-free DNA sensitivity. Three studies used DNA isolated from full void urine, also referred to as genomic DNA [24,25,30]. To date, researchers primarily gained experience with mutated cellular DNA for urine-based BC diagnosis. Focus now seems to have shifted to mutated cell-free DNA, which is released upon apoptosis and necrosis of bladder tumour cells resulting in potential enrichment of tumour DNA and an improved sensitivity [44,46,47]. Analogous to our recent study on urinary methylation analysis [48], future studies should elaborate on which type of urinary DNA is to be preferred for mutation analysis in clinical practice: cellular DNA, cell-free DNA, a combination of these two, or genomic DNA as obtained from full void urine.

Three studies were of highest quality [20,24,30]. Findings by Dahmcke et al. [20] were discussed previously. The other studies were performed by the same research group and focussed on FGFR3 mutations. For BC detection, Karnes et al. [24] included patients with primary BC (test 48, validation 58) with 91% NMIBC/9% MIBC, and compared these to matched controls (test 240, validation 690). They found low sensitivity (test 10%, validation 35%), but high specificity (test 99%, validation 100%). For BC surveillance, Shore et al. [30] included 63 patients with recurrent NMIBC and 670 patients with previous NMIBC under follow-up for recurrence, and reported 30% sensitivity and 96% specificity in the validation set. They expanded their FGFR3 assay with methylation and protein markers, and found that sensitivity improved for BC detection and surveillance (88% and 91%, respectively), but this was at the cost of specificity (56% and 35%, respectively).
An optimal urine test for BC diagnosis should perform adequately across all grades and stages of disease. A number of studies determined sensitivities of their urine test per BC grade and/or stage [17,20–23,25,26,29,34–39], some of which also calculated $P$ values between sensitivities per BC grade and stage [20,21,36,37]. In general, $TERT$ sensitivity remained comparable across BC grade and stage [20,21,36]. This is in accordance with a landscape study by Hurst et al. [43], in which $TERT$ is found to be frequently present in patients with BC irrespective of grade and stage. On the other hand, Descotes et al. [21] found that $TERT$ sensitivity was significantly higher for HG tumours than for LG tumours ($P = 0.015$), while $TERT$ sensitivity did not differ across BC stage ($P = 0.498$). Dahmcke et al. [20] found that FGFR3 sensitivity was significantly higher for Ta tumours than for other tumour stages ($P = 0.002$) [20]. This is consistent with earlier studies, which also showed that FGFR3 mutations most frequently occur in Ta tumours [49]. Dudley et al. [37] reported that their marker panel, consisting of 20 mutation markers, had a higher sensitivity for HG tumours than for LG tumours ($P = 0.022$) and a higher sensitivity for ≥T2/Tis tumours than for Ta tumours, although the latter was not statistically significant ($P = 0.054$) [37].

This systematic review has some limitations that need to be considered. Firstly, we could not perform a quantitative analysis of the data, because studies were too heterogeneous for a meta-analysis. As mentioned earlier concerns were raised about heterogeneity in study characteristics. Secondly, we did not collect data on the predictive and/or prognostic potential of urinary mutation analysis (although a number of studies did report on this) [16,18,21,27,37,40]. Thirdly, we did not review the combined performance of urinary mutation analysis and other tests. Yet, papers on combined analysis all reported higher sensitivities after expanding their mutation analysis and other tests. Mutation and methylation markers have already been subject to a large number of publications. An upcoming field of interest involves copy number and microsatellite changes. All together a combination of these four in a combined assay might hold the best promise for reliable urinary BC diagnosis [14,47,50]. Certainly when ongoing technological developments are considered, combined analysis may prove a sensitive method for urinary BC diagnosis in the coming years. Fourthly, we did not consider BC mutation markers in other liquid biopsies (e.g. blood). Nonetheless, recent sequencing data of blood samples from 586 patients with BC revealed a large number of potential gene mutations for BC diagnosis [51]. However, two studies which simultaneously analysed blood and urine samples of patients with BC concluded that the urine samples contained higher amounts of (mutated) tumour DNA [44,52]. This analytical advantage underlines the potential benefit of urine over blood, let alone the fact that urine is a ‘true’ non-invasive liquid biopsy.

Taken together, we believe that the existing evidence on mutation markers for BC diagnosis in urine does not allow for clinical implementation at present. Combining different molecular markers, such as mutation and methylation markers, may even provide a better option for non-invasive BC diagnosis, and future studies should therefore focus on identifying adequate marker panels. Large, prospective studies with consecutive sampling methods and matched control groups are required to expedite future clinical application.

**Funding**

This work was supported by the Weijerhorst Foundation.

**Conflict of interest**

R.D.M. Steenbergen has a minority share in Self-screen BV, a spin-off company of Amsterdam UMC, location VUMc. J. Bosschieter, R.D.M. Steenbergen and J.A. Nieuwenhuijzen are inventors on patent(s) related to the work.

**References**

1. Leow JJ, Bedke J, Chami K et al. SIU-JCUD consultation on bladder cancer: treatment of muscle-invasive bladder cancer. *World J Urol* 2019; 37: 61–83
2. Bladder Cancer: Diagnosis and Management of Bladder Cancer: ©NICE (2015). Bladder cancer: diagnosis and management of bladder cancer. *BJU Int* 2017; 120: 755–65
3. Sylvester RJ, van der Meijden AP, Oosterlinck W et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. *Eur Urol* 2006; 49: 466–5
4. Swatek RS, Hollenbeck BK, Holmang S et al. The economics of bladder cancer: costs and considerations of caring for this disease. *Eur Urol* 2014; 66: 253–62
5. Yafi FA, Brimo F, Steinberg J, Aprikian AG, Tanguay S, Kassouf W. Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer. *Urol Oncol* 2015; 33: 66.e25–66.e31
6. Tan WS, Tan WP, Tan MY et al. Novel urinary biomarkers for the detection of bladder cancer: a systematic review. *Cancer Treat Rev* 2018; 69: 39–52
7. 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: 639–57
8. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat Rev Cancer* 2015; 15: 25–41
9. Zuiverloon TC, Van Der Aa MN, Van Der Kwast TH et al. Fibroblast growth factor receptor 3 mutation analysis on voided urine for surveillance of patients with low-grade non-muscle – invasive bladder cancer. *Clin Cancer Res* 2010; 16: 3011–8
10. López-Knowles E, Hernandez S, Malats N et al. PIK3CA mutations are an early genetic alteration associated with FGFR3 mutations in superficial papillary bladder tumors. *Cancer Res* 2006; 66: 7401–4
Beukers W, Allory Y, Whiting PF, Miyake M, Bosschieter J, Lutz C, Segerink Li et al. The diagnostic accuracy of methylation markers in urine for the detection of bladder cancer: a systematic review. *Epigenomics* 2018; 10: 673–87

Whiting PF, Rutjes AW, Westwood ME et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011; 155: 529–36

Allory Y, Beukers W, Sagarera A et al. Telomerase reverse transcriptase promoter mutations in bladder cancer: high frequency across stages, detection in urine, and lack of association with outcome. *Eur Urol* 2014; 65: 360–6

Beukers W, van der Keur KA, Kandimalla R et al. FGFR3, TERT and OTX1 as a urinary biomarker combination for surveillance of patients with bladder cancer in a large prospective multicenter study. *J Urol* 2017; 197: 1410–8

Couffignal C, Desgrandchamps F, Mongiat-Arthus P et al. Diagnostic and prognostic performance of urinary FGFR3 mutation analysis in bladder cancer surveillance: a prospective multicenter study. *Urology* 2015; 86: 1185–90

Curigliano G, Ferretti G, Flamini G et al. Diagnosis of T1 bladder transitional cell carcinoma by denaturing gradient gel electrophoresis urinalysis. *Anticancer Res* 2001; 21: 3015–20

Dahmcke CM, Steven KE, Larsen JK et al. A prospective blinded evaluation of urine-DNA testing for detection of urothelial bladder carcinoma in patients with gross hematuria. *Eur Urol* 2016; 70: 916–9

Descotes F, Kara N, Deccaussin-Petrucci M et al. Non-invasive prediction of recurrence in bladder cancer by detecting somatic TERT promoter mutations in urine. *Br J Cancer* 2017; 117: 583–7

Fitzgerald JM, Ramchurren N, Rieger K et al. Identification of H-RAS mutations in urine sediments complements cytology in the detection of bladder tumors. *J Natl Cancer Inst* 1995; 87: 129–33

Kandimalla R, Masius R, Beukers W et al. A 3-plex methylation assay combined with the FGFR3 mutation assay sensitively detects recurrent bladder cancer in voided urine. *Clin Cancer Res* 2013; 19: 4760–9

Karnes RJ, Fernandez CA, Shuber AP. A noninvasive multianalyte urine-based diagnostic assay for urothelial cancer of the bladder in the evaluation of hematuria. *Mayo Clinic Proc* 2012; 87: 835–42

Millholand JM, Li S, Fernandez CA, Shuber AP. Detection of low frequency FGFR3 mutations in the urine of bladder cancer patients using next-generation deep sequencing. *Open Access J Urol* 2012; 4: 33–40

Miyake M, Sugano K, Kawashima K et al. Sensitive detection of FGFR3 mutations in bladder cancer and urine sediments by peptide nucleic acid-mediated real-time PCR clamping. *Biochem Biophys Res Commun* 2007; 362: 865–71

Miyake M, Sugano K, Sugino H et al. Fibroblast growth factor receptor 3 mutation in voided urine is a useful diagnostic marker and significant indicator of tumor recurrence in non-muscle invasive bladder cancer. *Cancer Sci* 2010; 101: 250–8

Noel N, Couteau J, Maillet G et al. TP53 and FGFR3 gene mutation assessment in urine: Pilot study for bladder cancer diagnosis. *Anticancer Res* 2015; 35: 4915–22

Serizawa RR, Rafikiar U, Steven K et al. Integrated genetic and epigenetic analysis of bladder cancer reveals an additive diagnostic value of FGFR3 mutations and hypermethylation events. *Int J Cancer* 2011; 129: 78–87

Shore ND, Fernandez CA, Shuber AP. Noninvasive multianalyte diagnostic assay for monitoring bladder cancer recurrence. *Open Access J Urol* 2012; 4: 49–56

van Kessel KE, Beukers W, Lurkin I et al. Validation of a DNA methylation-mutation urine assay to select patients with hematuria for cystoscopy. *J Urol* 2017; 197: 590–5

van Kessel KE, Van Neste L, Lurkin I, Zwarthoff EC, Van Criekinge W. Evaluation of an epigenetic profile for the detection of bladder cancer in patients with hematuria. *J Urol* 2016; 195: 601–7

van Rhijs BW, Lurkin I, Chopin DK et al. Combined microsatellite and FGFR3 mutation analysis enables a highly sensitive detection of urothelial cell carcinoma in voided urine. *Clin Cancer Res* 2003; 9: 257–63

Ward DG, Baxter L, Gordon NS et al. Multiplex PCR and Next generation sequencing for the non-invasive detection of bladder cancer. *PLOS One* 2016; 11: e0149756

Zuiverloon TC, Beukers W, van der Keur KA et al. Combinations of urinary biomarkers for surveillance of patients with incident nonmuscle invasive bladder cancer: the European FP7 UROMOL project. *J Urol* 2013; 189: 1945–51

Avogbe PH, Manel A, Vian E et al. Urinary TERT promoter mutations as non-invasive biomarkers for the comprehensive detection of urothelial cancer. *EBioMedicine* 2019; 44: 431–8

Dudley JC, Schroers-Martin J, Lazzarelli DV et al. Detection and surveillance of bladder cancer using urine tumor DNA. *Cancer Discov* 2019; 9: 500–9

Rodriguez Pena MD, Springer SU, Taheri D et al. Performance of novel non-invasive urine assay UroSEEK in cohorts of equivocal urine cytology. *Virchows Arch* 2020; 476: 423–9

Stask S, Salomo K, Heberling U et al. Evaluation of TERT promoter mutations in urinary cell-free DNA and sediment DNA for detection of bladder cancer. *Clin Biochem* 2019; 64: 60–3

Zhu F, Zhang Y, Shi L et al. Gene mutation detection of urinary sediment cells for NMIBC early diagnose and prediction of NMIBC relapse after surgery. *Medicine (Baltimore)* 2019; 98: e16451

da Costa JB, Gibb EA, Nykopp TK et al. Molecular tumor heterogeneity in muscle invasive bladder cancer: biomarkers, subtypes, and implications for therapy. *Urol Oncol* 2018 [Online ahead of print]. https://doi.org/10.1016/j.urolonc.2018.11.015

Boman H, Hedelin H, Holmgren S. Four bladder tumor markers have a disappointingly low sensitivity for small size and low grade recurrence. *J Urol* 2002; 167: 80–3

Hurst CD, Knowles MA. Mutational landscape of non-muscle-invasive bladder cancer. *Urol Oncol* 2018 [Online ahead of print]. https://doi.org/10.1016/j.urolonc.2018.10.015

Patel KM, van der Vos KE, Smith CG et al. Association of plasma and urinary mutant DNA with clinical outcomes in muscle invasive bladder cancer. *Sci Rep* 2017; 7: 5554

Ward DG, Bryan RT. Massively parallel sequencing of urinary DNA—the dawn of non-invasive bladder cancer detection and surveillance? *Transl Cancer Res* 2019; 8(Suppl 2): S204–7

Szavas T, Kovalszky I, Bedi K et al. Deletion analysis of tumor and urinary DNA to detect bladder cancer: urine supernatant versus urine sediment. *Oncol Rep* 2007; 18: 405–9

Togneri FS, Ward DG, Foster JM et al. Genomic complexity of urothelial bladder cancer revealed in urinary cDNA. *Eur J Hum Genet* 2016; 24: 1167–74

Hentschel AE, Nieuwenhuijzen JA, Bosschieter J et al. Comparative analysis of urine fractions for optimal bladder cancer detection using DNA methylation markers. *Cancers (Basel)* 2020; 12: 859

Billerey C, Chopin D, Aubriot-Lorton MH et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. *Am J Pathol* 2001; 158: 1955–9
van Tilborg AA, Kompier LC, Lurkin I et al. Selection of microsatellite markers for bladder cancer diagnosis without the need for corresponding blood. *PLoS One* 2012; 7: e43345

Carlo MI, Ravichandran V, Srinavasan P et al. Cancer susceptibility mutations in patients with urothelial malignancies. *J Clin Oncol* 2020; 38: 406–14

Birkenkamp-Demtroder K, Nordentoft I, Christensen E et al. Genomic alterations in liquid biopsies from patients with bladder cancer. *Eur Urol* 2016; 70: 75–82

Correspondence: Jakko A. Nieuwenhuijzen, Department of Urology, Amsterdam University Medical Centers, Vrije Universiteit Amsterdam, Post box 7057, 1007 MB Amsterdam, The Netherlands.

e-mail: j.nieuwenhuijzen@amsterdamumc.nl

Abbreviations: AKT1, serine/threonine-protein kinase 1; BC, bladder cancer; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CDKN2A, cyclin-dependent kinase inhibitor 2A; EP300, E1A binding protein P300; ERBB3, Erb-B2 receptor tyrosine kinase 3; FBXW7, F-box and WD repeat domain containing 7; FGFR3, fibroblast growth factor receptor 3; HG, high grade; HRAS, HRas proto-oncogene, GTPase; KDM6A, lysine demethylase 6A; LG, low grade; MED12, mediator complex subunit 12; (N)MIBC, (non-)muscle-invasive BC; NGS, next-generation sequencing; NRAS, NRAS proto-oncogene, GTPase; PIK3CA, phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit α; PRISMA, Preferred Reporting Items for Systematic and Meta-Analysis; QUADAS, Quality Assessment of Diagnostic Accuracy Studies; RoB, risk of bias; ROC, receiver operating characteristic; TERT, telomerase reverse transcriptase; TP53, tumour protein p53; TURBT, transurethral resection of the bladder tumour.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Overview of single-mutation markers for urine-based BC detection.

**Table S2.** Overview of single-mutation markers for urine-based BC surveillance.

**Appendix S1.** Search strategy.