

# Diagnostic predictive value of Xpert Bladder Cancer Monitor in the follow-up of patients affected by non-muscle invasive bladder cancer

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## ABSTRACT

**Aims** Cystoscopy and urine cytology represent the gold standard for monitoring superficial bladder cancer (BC). Xpert BC Monitor is a new urinary marker based on the evaluation of five target mRNAs overexpressed in patients with bladder cancer. The aim of our study was to evaluate the diagnostic accuracy of Xpert BC Monitor in follow-up of patients with non-muscle invasive bladder cancer (NMIBC).

**Methods** 230 patients were included in this prospective study. Xpert BC Monitor cut-off was set to 0.5. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of cytology, Xpert BC Monitor and their combination were calculated and compared with cystoscopy/histology.

**Results** 52/230 patients showed a NMIBC recurrence, 45 low grade (LG) and 7 high grade (HG). Overall sensitivity was 11.5% for cytology, 46.2% for Xpert BC Monitor and 48.1% for the two tests combined. Sensitivity of cytology increased from 4.4% in LG to 57.1% in HG tumours whereas for the Xpert BC Monitor it was 40% in LG and 85.7% in HG tumours. Combined cytology and Xpert BC Monitor yielded an overall sensitivity of 42% for LG and 85.7% for HG. Overall specificity was 97.2% for cytology, 77% for Xpert BC Monitor and 75.8% for the two tests.

**Conclusions** Sensitivity for the Xpert BC Monitor Test was significantly higher than for cytology. The test performed very well in terms of specificity but could not reach the value of cytology, while PPV and NPV performed approximately the same for both tests.

## INTRODUCTION

Almost 80% of superficial bladder cancers are low grade and low stage and have a non-invasive behaviour.<sup>1</sup> Nevertheless, they are characterised by a high recurrence rate, which distinguishes them from other types of bladder cancer.

A prompt and correct diagnosis is essential to ensure the adequate therapy and follow-up for patients with superficial urothelial cancer. A big challenge for the urologist is the detection of cancer recurrence. Due to the high recurrence rates of non-muscle invasive bladder cancer (NMIBC), patients need a frequent, regular and close follow-up. This is based on a risk group stratification into low risk, intermediate risk and high risk.<sup>2</sup> However, the intermediate risk group is rather

heterogeneous and cases differ in their risk of recurrence and progression.

According to the in-use guidelines, cystoscopy and urine cytology represent the gold standard for monitoring superficial bladder cancer.<sup>3</sup> Cystoscopy is the most efficient method currently available for the detection of primary or recurrent tumours, but it is invasive and causes discomfort to the patients. Nevertheless, the sensitivity of cystoscopy is limited to the tumours that can be visualised. Tammela *et al* showed that routine follow-up cystoscopy may miss over 5% of the recurrent tumours. Even with negative cystoscopy, patients with positive urine marker status and urine cytology should be considered at risk for high-grade recurrence.<sup>4</sup>

The sensitivity of cytology in voided urine is variable; depending on the studies, sensitivity of cytology in low-grade tumours ranges from 4% by Leyh *et al*<sup>5</sup> up to 17% in a systematic review by van Rhijn *et al*.<sup>6</sup> Additionally, cytological evaluation is always prone to examiner variation, as shown by Ried *et al*, documenting a low interobserver agreement,<sup>7</sup> as well as to different atypical cells, which may complicate the definite diagnosis of bladder cancer.

Therefore, the research for an adequate urine tumour marker for the follow-up of patients with NMIBC is continuing since the ideal one has not yet been found.

As previously reported by Pichler *et al*,<sup>8</sup> 100 tumour markers described as differentially expressed in bladder cancer were selected and tested by quantitative real-time, reverse transcription PCR (RT-PCR) to identify markers that consistently show upregulation in urine specimens from subjects with bladder cancer compared with urine specimens from control subjects. After evaluation of the 10 best biomarkers (UPK1B, IGF2, CRH, ANXA10, ABL1, KRT20, AR, PIK3CA, UPK2 and MGEA5) within a training set of 444 urine specimens, the best performing five markers were further validated. The results of expression of the five markers (UPK1B, IGF2, CRH, ANXA10, ABL1) are combined in a linear model algorithm to give a positive or negative result. The new mRNA-based Xpert BC Monitor measures the level of these five target mRNAs by means of RT-PCR.

The aim of the present study was to test the diagnostic value of this new mRNA-based test in patients undergoing follow-up after NMIBC.



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**Table 1** Demographical and clinical characteristics of the patients

Age	71.32±10.6	(%)
Age >65 years	169	73.5
Gender		
Male	176	76.5
Female	54	23.5
First diagnosis		
pTaG1	116	50.4
pTaG2	32	13.9
pTaG3	14	6.1
pT1G2	3	1.3
pT1G3	11	4.8
CIS	54	23.5
Previous BCG	75	32.6
Previous MMC	7	3.3

BCG, Bacillus Calmette-Guérin; CIS, carcinoma in situ; MMC, mitomycin.

## MATERIALS AND METHODS

After approval of the local institutional ethics committee (47-2017) and following informed consent, 231 patients under follow-up for NMIBC in our outpatient department were enrolled in the present study.

Patients were routinely evaluated by voided urine cytology, by the Xpert BC Monitor (Cepheid Srl, Italy) and by white light cystoscopy, according to the current European Association of Urology (EAU) guidelines.<sup>3</sup> We reserved photodynamic cystoscopy for patients with positive cytology and no visible bladder tumour; in our series, none of our patients underwent photodynamic cystoscopy in an outpatient setting.

Any cystoscopically suspicious lesion was biopsied or removed trans-urethraly and specimens were evaluated according to the 2017 TNM classification of urinary bladder cancer and graded according to both the 1973 and the 2004 WHO grade classification.<sup>9,10</sup> We defined a patient as negative when white light cystoscopy, cytology and histology were negative.

Of the voided urine of every patient, 4.5 mL was added to the Xpert Urine Transport Reagent Kit (Cepheid), a RNA stabilising reagent, and inverted three times in order to mix it properly. Stabilised samples were stored at 4°C and analysed within 7 days after collection.

The residual urine was added to 15 mL Cytolyt fixation liquid (Hologic, Malborough, Massachusetts, USA) in a Falcon tube and sent to the laboratory along with the stabilised urine for the Xpert BC Monitor test (Cepheid).

## Cytology

The tubes were centrifuged for 10 min at 2000 rpm. The resulting cell pellets were re-suspended in ThinPrep vials containing PreservCyt solution and processed by the TP 5000 System (Hologic).

Cytological evaluation was performed using the Papanicolaou staining procedure and the Paris System for Reporting Urinary

Cytology<sup>11</sup> classifying the cytological specimens accordingly into negative for high-grade urothelial carcinoma (NHGUC), atypical urothelial cells (AUCs), suspicious for high-grade urothelial carcinoma (SHGUC), high-grade urothelial carcinoma (HGUC), low-grade intraepithelial neoplasia (LGUN) and not diagnostic.

For the statistical analysis, NHGUC and AUC were grouped as negative, and SHGUC, HGUC and LGUN as positive.

## Xpert BC Monitor

According to the manufacturer, the Xpert BC Monitor, performed on the Cepheid GeneXpert Instrument Systems (Cepheid), is a qualitative in vitro diagnostic test created to monitor the recurrence in patients previously diagnosed with bladder cancer.

This test analyses 4.5 mL of stabilised voided urine and measures the level of five target mRNAs (ABL1, CRH, IGF2, UPK1B, ANXA10) by RT-PCR. The results are interpreted by the GeneXpert Instrument System from measured fluorescent signals and embedded calculation algorithms. The Test Result, linear discriminant analysis (LDA) totals and Analyte Results are shown on the Test Report. A cut-off is set at a LDA of >0.5.

## Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of cytology, Xpert BC Monitor and their combination were calculated and compared with cystoscopy/histology. Statistical analysis was performed using the Pearson  $\chi^2$  test. A p value of less than 0.05 was considered statistically significant. Area under the curve under the receiver operating characteristic curve was calculated and tested for significance using the z-test.

## RESULTS

Of the 231 patients enrolled in the study, one patient had to be excluded because of a not diagnostic cytology due to artefacts and error sign in the Xpert BC Monitor. Mean age of the remaining 230 patients (176/230 men and 54/230 women) was 71.32 years (range 28–95).

At the time of first diagnosis, 116 cases (50.4%) were diagnosed with pTaG1 bladder cancer, 32 cases (13.9%) with pTaG2, 14 (6.1%) with pTaG3, 3 cases (1.3%) with pT1G2, 11 cases (4.8%) with pT1G3 and in 54 cases (23.5%) with carcinoma in situ (CIS). A total of 74 patients (32.2%) were treated with an intravesical therapy of BCG and 7 (3%) with mitomycin. Fifty-two out of the 230 patients (22.6%) showed a NMIBC recurrence, 45 (86.5%) low-grade NMIBC and 7 (13.5%) high-grade NMIBC. Demographical and clinical characteristics of the patients are given in table 1.

Overall sensitivity was 11.5% for cytology, 46.2% for Xpert BC Monitor and 48.1% for the two tests combined. The sensitivity of cytology increased from 4.4% in low grade (LG) to 57.1% in high grade (HG) tumours, whereas for the Xpert BC Monitor, the sensitivity was 40% in LG and 85.7% in HG

**Table 2** Performance of cytology, Xpert BC Monitor and combination of the two tests

	Cytology (95% CI)	Xpert (95% CI)	Xpert+cytology (95% CI)	Xpert cut-off 0.48 (95% CI)
Sensitivity	11.5 (2.9 to 20.2)	46.2 (32.6 to 59.7)	48.1 (34.5 to 61.7)	50 (6.4 to 63.6)
Specificity	97.2 (94.8 to 99.6)	77 (70.8 to 83.2)	75.8 (70 to 82.1)	76.4 (70.2 to 82.6)
PPV	54.5 (25.1 to 84)	36.9 (25.2 to 48.7)	36.8 (29.3 to 44.2)	38.2 (26.7 to 49.8)
NPV	79 (73.6 to 84.4)	83 (77.3 to 88.8)	83.3 (74.5 to 92.2)	84 (8.3 to 89.6)

NPV, negative predictive value; PPV, positive predictive value.

**Table 3** Performance of the tests according to grading

n=52	Cytology (95% CI)	Xpert (95% CI)	Cytology+Xpert (95% CI)
Low grade (n=45)	4.4 (0.5 to 15.1)	40 (25.7 to 55.6)	42.2 (27.6 to 57.8)
High grade (n=7)	57.1 (18.4 to 90.1)	85.7 (42.1 to 99.6)	85.7 (42.1 to 99.6)

tumours. Combined cytology and Xpert BC Monitor yielded an overall sensitivity of 42.2% for LG and 85.7% for HG tumours.

Overall specificity was 97.2% for cytology, 77% for Xpert BC Monitor and 75.8% for the two tests combined. PPV for cytology was 54.5% and for Xpert BC Monitor 36.9%. For the two tests combined, it was 36.8%. NPV was very similar for the two tests: 79% for cytology and 83% for Xpert BC Monitor and 83.3% for the two tests combined.

Detailed data are given in [tables 2 and 3](#).

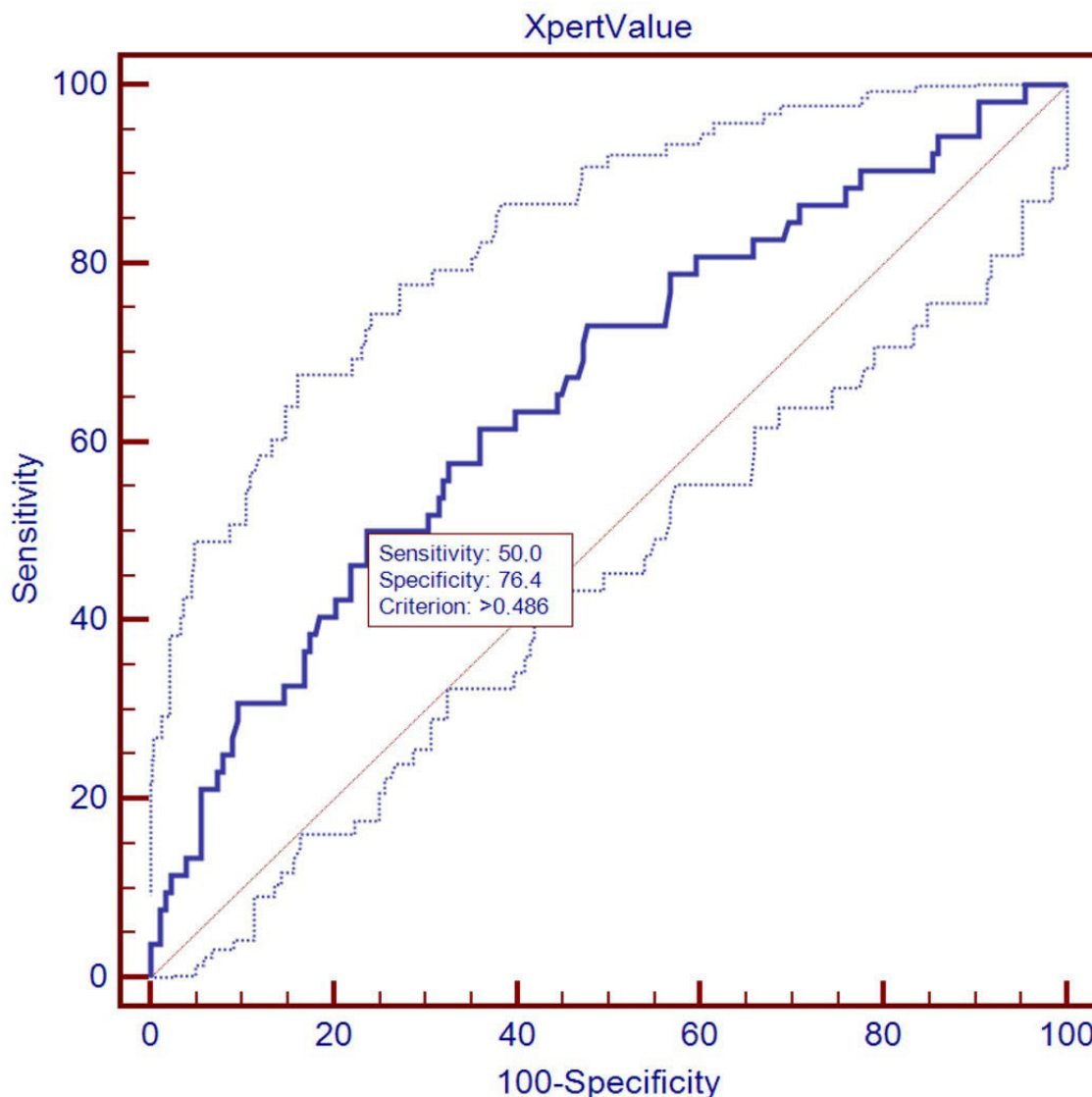
The final statistical analysis between the tests was highly significant ( $p < 0.0001$ ) with Pearson's  $\chi^2$  test. The diagnostic efficacy of Xpert BC Monitor was fair, with an area under the curve of 0.65 (95% CI 0.593 to 0.719) ([figure 1](#)). The area under the curve gave 0.48 as best cut-off. Using this cut-off, overall

sensitivity for Xpert BC Monitor was 50%, specificity 76.4%, PPV 38.2% and NPV 84%.

## DISCUSSION

Cystoscopy and urinary cytology represent the gold standard follow-up strategy for NMIBC surveillance, for both EAU and American Urological Association (AUA) Guidelines.<sup>3 12</sup> White light cystoscopy shows a poor capacity in the identification of flat tumours; urinary cytology shows a high sensitivity in high-grade tumours but a poor one in low-grade tumours with a high interobserver variability.<sup>13</sup>

Even though several urine markers have been studied to improve the diagnostics of bladder cancer and to reduce the



**Figure 1** Area under the curve of 0.65 (95% CI 0.593 to 0.719) for the Xpert Bladder Cancer Monitor.

need for cystoscopy, none has been identified that sufficiently meets these criteria. Therefore, no urinary molecular marker test has been incorporated into the treatment guidelines.

The Xpert BC Monitor, a promising diagnostic assay for bladder cancer, was introduced in October 2016. It measures the level of five target mRNAs in voided urine by RT-PCR. It works using the all-or-none law defined by the cut-off level given by the producing company.

The first study that evaluated the accuracy of Xpert BC Monitor was carried out by Pichler *et al* in a series of 140 patients. They reported an overall sensitivity of 84% for Xpert BC Monitor and 33% for bladder washing cytology.<sup>8</sup> Van Valenberg *et al* conducted a multicentre study involving 22 European centres and 255 patients, reporting lower sensitivities with 75% for Xpert BC Monitor and 29.5% for cytology, respectively.<sup>14</sup>

In our study, 230 patients under follow-up for NMIBC were analysed with this test. The overall sensitivity was 46.2% for Xpert BC Monitor and 11.5% for cytology. Combining the two tests, the overall sensitivity rose to 48.1%, and evaluating only the HG tumours, Xpert BC Monitor correctly identified 85.7% and cytology 57.1%, respectively. The significantly lower sensitivity for both tests in our study compared with Pichler *et al*<sup>8</sup> could be explained by the different analysed sample types. While we used only voided urine cytology, Pichler *et al* analysed voided urine and barbotage cytology but compared the performance of the test in terms of sensitivity, specificity, PPV and NPV only with bladder washing cytology. Voided urine of LG tumours contains no or few atypical cells compared with bladder washings.<sup>15</sup> This might have some influence on the performance of the test. In fact, of the 24 false-negative Xpert BC Monitor results in our study, 19 were cytologically negative (79.1%) and only 5 (20.9%) showed few atypical cells (AUC).

Pichler *et al*<sup>8</sup> reported a high specificity for both tests: 91% for Xpert BC Monitor versus 94% for cytology ( $p=0.41$ ). The specificity for the Xpert BC Monitor in our study was lower than for cytology with 76.9% versus 97.2%, respectively. This could be due to false-positive results in patients undergoing follow-up after bladder instillation therapy and in the case of anticipatory positives, as previously described by Mian *et al*<sup>16</sup> and Lodde *et al*<sup>17</sup> for other urinary markers. Patients under instillation therapy may continue to have a genetic instability at a time where cytology cannot detect any tumour cells and other patients may show genetic alterations due to a recurrent tumour which cannot yet be seen by cytology.<sup>16 17</sup> Specificities in Van Valenberg's study were similar to our study resulting in 80.6% for the Xpert BC Monitor, but lower for cytology (90.8% vs 97%).<sup>14</sup>

PPV was 36.9% for Xpert BC Monitor and 54.5% for cytology in contrast to 80% and 70%, respectively, as published by Pichler *et al*.<sup>8</sup> NPV for Xpert BC Monitor was similar to cytology with 83% versus 79%, but lower in comparison with the values reported by the previous two studies (93% and 93.9%).<sup>8 14</sup> The same happens when we combine the two tests (NPV 83.3%). However, important information for urologists is that there is only a small percentage of probability for recurrence left, when both tests are negative.

Our sensitivity data are almost overlapping with the performance of other bladder tumour markers, such as NMP 22, BTA,<sup>18</sup> fluorescence in situ hybridisation (FISH)<sup>19</sup> or ImmunoCyt,<sup>20</sup> whereas cytology is more specific (cytology: 97.2% in our series vs 87.3% in the series of Grossman *et al*<sup>21</sup>). A meta-analysis conducted on 2477 FISH tests showed an overall sensitivity of 72%, reaching 86% excluding the Ta tumours, and a specificity of 83%, with an area under the curve of 0.86. However, due to the cost of this procedure, its use was not indicated in all

clinical settings.<sup>19</sup> The ImmunoCyt test reported an overall sensitivity of 50%–100% and specificity ranging from 69% to 79%<sup>20</sup>; however, ImmunoCyt is currently no longer in production.

Xpert BC Monitor is a fairly specific test (77%) even if our study did not reach the excellent results of the two previous studies.<sup>8 14</sup> It is, furthermore, an easy-to-handle, non-invasive test, which can be introduced easily into the daily routine. This suggests the combination of both cytology and Xpert BC Monitor in follow-up patients in order to achieve a higher sensitivity, thus maintaining the high specificity of cytology. The use of both tests in combination may have the potential to reduce the cystoscopies during the follow-up for low-risk bladder cancer.

## CONCLUSION

In the present study, we report our first experience with the new mRNA-based Xpert BC Monitor Test. Its sensitivity was significantly higher than for cytology in a LG predominant BC group. The test performed very well in terms of specificity but could not reach the high value of cytology, while PPV and NPV performed approximately the same for both tests.

This new mRNA-based test is promising as a urinary marker but needs further optimisation and evaluation in a more mixed LG and HG setting. It may be used in combination with cytology to reduce invasiveness in the follow-up of NMIBC, decreasing discomfort for the patients and related costs.

## Take home message

- ▶ The Xpert BC Monitor Test is a promising urinary marker that may be used in combination with cytology to reduce invasiveness in the follow-up of non-muscle invasive bladder cancer.

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## REFERENCES

- Amin MB, Smith SC, Reuter VE, *et al*. Update for the practicing pathologist: The International Consultation on Urologic Disease—European Association of Urology consultation on bladder cancer. *Mod Pathol* 2015;28:612–30.
- 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;49:466–77.
- Babjuk M, Burger M, Compérat E. *EAU guidelines on non-muscle-invasive bladder cancer*, 2017.
- Raitanen MP, Leppilähti M, Tuhkanen K, *et al*. Routine follow-up cystoscopy in detection of recurrence in patients being monitored for bladder cancer. *Ann Chir Gynaecol* 2001;90:261–5.
- Leyh H, Marberger M, Conort P, *et al*. Comparison of the BTA stat test with voided urine cytology and bladder wash cytology in the diagnosis and monitoring of bladder cancer. *Eur Urol* 1999;35:52–6.
- van Rhijn BW, van der Poel HG, van der Kwast TH. Urine markers for bladder cancer surveillance: a systematic review. *Eur Urol* 2005;47:736–48.



- 7 Reid MD, Osunkoya AO, Siddiqui MT, *et al.* Accuracy of grading of urothelial carcinoma on urine cytology: an analysis of interobserver and intraobserver agreement. *Int J Clin Exp Pathol* 2012;5:882–91.
- 8 Pichler R, Fritz J, Tulchiner G, *et al.* Increased accuracy of a novel mRNA-based urine test for bladder cancer surveillance. *BJU Int* 2018;121:29–37.
- 9 Epstein JI, Amin MB, Reuter VR, *et al.* The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. *Am J Surg Pathol* 1998;22:1435–48.
- 10 Brierley JD, Gospodarowicz M, Wittekind C. UICC International Union Against Cancer. In: *TNM classification of malignant tumors*. 8th edn. Wiley-Blackwell, 2017.
- 11 Rosenthal DL, Wojcik EM, Kurtycz DFI. *The Paris system for reporting urinary cytology*. Springer International Publishing Switzerland, 2016.
- 12 AUA. Advancing urology, 2018. [www.Auanet.org](http://www.Auanet.org)
- 13 Mian C, Pycha A, Wiener H, *et al.* Immunocyt: a new tool for detecting transitional cell cancer of the urinary tract. *J Urol* 1999;161:1486–9.
- 14 Van Valenberg FJP, Bridge JA, Mayne D, *et al.* Performance characteristics of a mRNA-based urine test for the detection of bladder cancer recurrence. *European Urology Supplements* 2017;16:e193–e194.
- 15 Barkan GA. Enough is enough: adequacy of voided urine cytology. *Cancer Cytopathol* 2016;124:163–6.
- 16 Mian C, Lodde M, Comloj E, *et al.* The value of the ImmunoCyt/uCyt+ test in the detection and follow-up of carcinoma in situ of the urinary bladder. *Anticancer Res* 2005;25:3641–4.
- 17 Lodde M, Mian C, Negri G, *et al.* Effect of intravesical instillation on performance of uCyt+ test. *Urology* 2004;63:878–81.
- 18 Xylinas E, Kluth LA, Rieken M, *et al.* Urine markers for detection and surveillance of bladder cancer. *Urol Oncol* 2014;32:222–9.
- 19 Hajdinjak T. UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. *Urol Oncol* 2008;26:646–51.
- 20 Tilki D, Burger M, Dalbagni G, *et al.* Urine markers for detection and surveillance of non-muscle-invasive bladder cancer. *Eur Urol* 2011;60:484–92.
- 21 Grossman HB, Soloway M, Messing E, *et al.* Surveillance for recurrent bladder cancer using a point-of-care proteomic assay. *JAMA* 2006;295:299–305.