Interference From Haematuria Renders the Urinary BTA Test Not Specific Enough for Diagnosing Bladder Cancer: Evidence From Clinical Practice

Xiong Xiong (szbzbb@163.com)
Department of Urology, The First Affiliated Hospital of Chongqing Medical University. 1st You Yi Road, Yu Zhong District, Chongqing, China, 400016

Wei He
Chongqing Medical University First Affiliated Hospital

Bin Xiong
People's Hospital of Nanchuan

Feng Yao
Banan People's Hospital

Research article

Keywords: BTA stat test, bladder cancer, haematuria, diagnostic

DOI: https://doi.org/10.21203/rs.3.rs-64771/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Background The urinary bladder tumour antigen (BTA) stat test has already been used for the diagnosis and monitoring of bladder cancer (BC). However, more evidence is needed regarding its efficacy and utility in the clinic. In this study, we investigated the influence of haematuria on the performance of the BTA stat test in a clinical cohort.

Methods Urine samples from 836 subjects, including 50 healthy volunteers, 553 patients with benign urologic disorders, 124 patients with histologically proven BC, and 109 patients with other histologically proven urologic cancers, were analysed by the BTA stat test and urinalysis. We detected the sensitivity and specificity of the BTA stat test in each group, and analysed the effect of haematuria on the specificity.

Results Our data showed that 58.06% of patients had haematuria in the BC group. Haematuria with benign prostatic hyperplasia (BPH), renal hamartoma (RH) and urolithiasis were identified in 39.01%, 42.86% and 66.49% of patients with benign urologic disorders, respectively. Haematuria was identified in 48.72% of prostatic cancer patients and 67.74% of renal cancer patients. The overall sensitivity of the BTA stat test was 90.32%. The sensitivity was 97.22% in BC patients with haematuria and 80.77% in BC patients without haematuria. The overall specificity in healthy individuals, patients with benign urologic disorders and other urologic cancers was 50.84%. In all patients with haematuria, the specificity of the BTA stat test was 15.82%, while it was 72.6% in patients without haematuria.

Conclusions Haematuria has a significant influence on the BTA stat test. Our study suggested that the BTA stat test is not an ideal diagnostic tool for BC.

Background

Bladder cancer (BC) is the ninth most common cancer disease worldwide, and the incidence is particularly high in men [1]. Early BC has a better prognosis, but the progression of the disease greatly increases the rate of metastasis and mortality. The data showed that BC is likely to recur in 50–70% of patients within 5 years [2]. Approximately 15–25% of recurrent BC can be expected to progress to a higher tumour stage [3], and 50% of patients diagnosed with muscle-invasive BC succumb to the disease within five years [4]. Therefore, early screening and long-term close follow-up with repeated examinations are essential.

Currently, cystoscopy and urine cytology are the best tests for the diagnosis of BC [5]. However, there are some limitations in practical clinical applications. Although cystoscopy is the gold standard, it is an invasive test and is not suitable as the first choice for early screening and long-term follow-up. On the other hand, urine cytology has ideal specificity, but its sensitivity varies depending on the grade of the tumour, as urine cytology has low sensitivity in low-grade tumours. In addition, the interpretation of urine cytology results is highly dependent on the skill of the examiner, which varies greatly in different studies and observations [6]. Therefore, finding and evaluating a non-invasive, inexpensive, highly sensitive and specific test for BC is important. Molecular markers have been proposed for the detection of bladder tumours within recent years. However, to be widely used, the specificity and sensitivity of these molecular markers need to be tested more in clinical practice.

The bladder tumour antigen (BTA) stat test is a qualitative enzyme immunoassay that detects a bladder tumour-associated antigen in urine. This antigen has been recognized as a human complement factor H-related protein (hCFHrp) and inhibits the complement pathway to cause cytolysis in cells with a resulting selective advantage for the tumour [7]. Previous studies have suggested that the sensitivity of the BTA stat test is superior to urine cytology, and it has been approved by the US Food and Drug Administration for diagnosing and monitoring of BC [8, 9]. However, the specificity of the BTA stat test is often disturbed by some factors, such as infection and intravesical treatment [10–12]. Few studies have mentioned the impact of haematuria, and relevant clinical data are limited. In the clinic, haematuria is a symptom of BC that cannot be ignored. The initial diagnosis of BC most often occurs after an episode of gross or microscopic haematuria. Over 80% of patients with BC have some degree of haematuria [13, 14]. On the other hand, there are many causes of haematuria other than BC, including many benign urologic disorders. Studies have shown that only 0.7–1.3% of patients with haematuria actually have BC [13, 15–16]. Even between 9 and 18% of normal individuals also have some degree of haematuria [17]. Hence, if haematuria has a serious effect on the specificity of the BTA stat test, using it to screen and monitor for BC can lead to unnecessary investigation and anxiety.

In this study, we analysed the specificity and sensitivity of the BTA stat test in BC. The effect of haematuria on the specificity of the BTA stat test in patients with BC, patients with benign urologic disorders, patients with other urologic cancers and healthy volunteers was also determined. Our study aims to evaluate the utility of the BTA stat test in the diagnosis of BC in clinical practice.
Material And Methods

Patients

We recruited patients with BC, urinary benign diseases and other urinary cancers who visited the Urology Department of the First Affiliated Hospital of Chongqing Medical University from January 2018 to December 2019 (Table 1). Patients enrolled completed related inspections as needed. All diagnoses were confirmed by two urologists. Cystoscopy biopsy was the gold standard for BC diagnosis. Benign diseases of the urinary system were diagnosed according to the corresponding diagnostic criteria. Other urinary cancers were diagnosed based on the results of the corresponding pathological examination. In addition, we recruited a group of healthy volunteers without any clinical evidence of disease. All participants underwent BTA stat test and urinalysis, and none of the participants had mechanical manipulation of the urethra or bladder within the last two weeks before specimen collection. Participants who ultimately did not belong to one of the diagnostic categories or refused further tests to confirm a diagnosis were excluded. Except for the urinary tract infection (UTI) group in the benign urologic disorders group, patients with active UTIs in the other groups, indicated either by symptom reports or by pyuria, were excluded. Except for the urolithiasis group in the benign urologic disorders group, patients with a recent history of urolithiasis in the other groups were excluded.

| Group                      | Total | Male | Female | Mean age | Range age |
|----------------------------|-------|------|--------|----------|-----------|
| Bladder cancer             | 124   | 103  | 21     | 66.7     | 26–89     |
| Healthy volunteers         | 50    | 30   | 20     | 50.2     | 25–72     |
| Benign urologic diseases   | 553   | 378  | 175    | 62.4     | 22–95     |
| BPH                        | 141   | 141  | 0      | 70.7     | 51–95     |
| Renal cyst                 | 66    | 36   | 30     | 64.1     | 47–87     |
| BAT                        | 75    | 27   | 48     | 53.3     | 22–74     |
| RH                         | 28    | 6    | 22     | 55       | 31–70     |
| Urolithiasis               | 185   | 129  | 56     | 60       | 26–90     |
| UTI                        | 58    | 39   | 19     | 63.3     | 48–88     |
| Other urologic cancers     | 109   | 101  | 8      | 68.9     | 35–93     |
| Prostatic cancer           | 78    | 78   | 0      | 72       | 52–93     |
| Renal cancer               | 31    | 23   | 8      | 61       | 35–76     |

BPH, benign prostatic hyperplasia; BAT, benign adrenal tumour; RH, renal hamartoma; UTI, urinary tract infection

Finally, the study group consisted of 124 patients with BC that was first confirmed histologically. The benign urologic disorders group consisted of benign prostatic hyperplasia (BPH, n = 141), renal cyst (n = 66), benign adrenal tumour (BAT, n = 75), renal hamartoma (RH, n = 28), urolithiasis (n = 185), and UTI (n = 58). The other urologic cancer group consisted of 78 patients with prostate cancer and 31 patients with renal cancer. The healthy volunteers group had 50 individuals.

BTA stat test

The BTA stat test (Polymedco, NY, USA) is a one-step qualitative assay. We assayed all samples according to the manufacturer's instructions [18]. Three drops of urine are placed on the BTA stat test device, which contains a small lateral flow immunochromatographic assay. The urine reacts with colloidal gold-conjugated anti-bladder tumour-associated antigen antibody to form an immune complex. Then, when the immune complex flows through the detection area, it binds to another anti-bladder tumour-associated antigen antibody and forms a visible colour band. If there is no hCFHrp in the urine, no visible line will form in the detection area. Regardless of the presence or absence of hCFHrp in the urine sample, the control area can bind to the detection antibody to form a visible line. Therefore, when both target and control zones form two visible lines, the reading is positive; when only the control line is formed, the reading is negative. The absence of a visible control line means that the test is invalid, and the test needs to be repeated.

Statistical analysis
Statistical analysis was performed using SPSS software, version 20.0 (Chicago, IL, USA). Analysis of the study population characteristics used descriptive statistics. Qualitative variables were expressed as proportions. Sensitivity is defined as the ratio of the number of true positive test results and the number of subjects with confirmed BC. Specificity is defined as the ratio of the number of true-negative test results and the number of subjects without BC. Exact 95% confidence intervals (CIs) indicate the precision of the sensitivity and specificity [8].

**Results**

**BTA stat test in patients with BC**

Urinary specimens of 124 patients with histologically proven BC were examined prior to therapy. Histologically, 118 patients had transitional cell carcinoma (TCC), five had squamous cell carcinoma (SCC) and one had undifferentiated carcinoma. Of these patients, 112 patients were BTA-positive, and 12 were BTA-negative with the BTA stat test. According to the histologic stage, 62 patients had a pTa tumour; 24 patients had a pT1 tumour; 28 patients had a pT2 tumour; and 10 patients had a higher tumour (Table 2). The overall sensitivity of the BTA stat test for the detection of BC was 90.32%. The sensitivity of the BTA stat test was 83.87% in pTa tumours, 95.83% in pT1 tumours, and 96.43% in pT2 tumours. None of the patients with pT3-pT4 tumours had negative BTA stat test results. For histologic grades, the sensitivity was 80% for papillary urothelial neoplasm of low malignant potential (PUNLMP) tumours, 70% for low-grade tumours, and 97.75% for high-grade tumours. In the BC group, 72 (58.06%) patients had haematuria. The sensitivity of the BTA stat test was 97.22% for BC patients with haematuria and 80.77% for BC patients without haematuria (Table 2).

| Variable                        | Negative | Positive | Total   | Sensitivity (%) | 95% CI          |
|---------------------------------|----------|----------|---------|-----------------|-----------------|
| Bladder cancer                  |          |          |         | 90.32           | 83.36–94.68     |
| Pathologic subtype              |          |          |         |                 |                 |
| TCC                             | 11       | 107      | 118     | 90.68           | 83.57–95.02     |
| SCC                             | 1        | 4        | 5       | 80              | 29.88–98.95     |
| Undifferentiated carcinoma      | 0        | 1        | 1       | 100             | 5.46–100        |
| Grade                           |          |          |         |                 |                 |
| PUNLMP                          | 1        | 4        | 5       | 80              | 29.88–98.95     |
| Low-grade                       | 9        | 21       | 30      | 70              | 50.44–84.59     |
| High-grade                      | 2        | 87       | 89      | 97.75           | 91.35–99.61     |
| Pathologic T stage              |          |          |         |                 |                 |
| Ta                              | 10       | 52       | 62      | 83.87           | 71.87–91.59     |
| T1                              | 1        | 23       | 24      | 95.83           | 76.88–99.78     |
| T2                              | 1        | 27       | 28      | 96.43           | 79.76–99.81     |
| T3                              | 0        | 4        | 4       | 100             | 39.57–100       |
| T4                              | 0        | 6        | 6       | 100             | 51.68–100       |
| Urine analysis                  |          |          |         |                 |                 |
| Positive for RBC                | 2        | 70       | 72      | 97.22           | 89.42–99.52     |
| Negative for RBC                | 10       | 42       | 52      | 80.77           | 67.03–89.92     |

**Table 2** Sensitivity of the BTA stat test by tumour stage and grade

TCC, transitional cell carcinoma; SCC, squamous-cell carcinoma; PUNLMP, papillary urothelial neoplasm of low malignant potential; RBC, red blood cell; CI, confidence interval

**BTA stat test in healthy volunteers and patients with other urologic disorders**
In urinary specimens from 50 healthy individuals, none of the specimens had positive BTA stat test results. Of the 553 patients with benign urologic disorders but without clinical evidence of BC, 262 patients had negative BTA stat test results; in 291 patients, the BTA stat test had false-positive results. The rate of false-positive results varied in correlation with the condition observed: 126 of 185 (68.11%) patients with urolithiasis and 49 of 58 (84.48%) patients with a UTI had false-positive results. A total of 62 of 141 (43.97%) patients with BPH, 28 of 75 (37.33%) patients with BAT and 13 of 28 (46.43%) patients with RH had positive BTA stat test results. In addition, 13 of 66 (19.7%) patients with renal cysts had positive BTA stat test results. Urinary specimens were obtained from 109 patients with other urologic cancers. Of the 78 patients with prostatic cancer, 43 had false-positive BTA stat test results. Of the 31 patients with renal cancer, 16 had false-positive BTA stat test results. The specificity of the BTA stat test for prostatic cancer and renal cancer was 44.87% and 48.39%, respectively. The specificity of the BTA stat test was 100% if only healthy individuals were considered. The overall specificity (healthy individuals, patients with benign urologic disorder and patients with other urologic cancers) of the BTA stat test was 50.84% (Table 3).

### Table 3

| Category                  | BTA-negative | BTA-positive | Total | Specificity (%) | 95% CI      |
|---------------------------|--------------|--------------|-------|----------------|-------------|
| Healthy volunteers        | 50           | 0            | 50    | 100            | 91.11–100   |
| Benign urologic diseases  | 262          | 291          | 553   | 47.38          | 43.16–51.63 |
| BPH                       | 79           | 62           | 141   | 56.03          | 47.43–64.29 |
| Renal cyst                | 53           | 13           | 66    | 80.3           | 68.32–88.7  |
| BAT                       | 47           | 28           | 75    | 62.67          | 50.69–73.34 |
| RH                        | 15           | 13           | 28    | 53.57          | 34.21–71.99 |
| Urolithiasis              | 59           | 126          | 185   | 31.89          | 25.35–39.2  |
| UTI                       | 9            | 49           | 58    | 15.52          | 7.77–27.93  |
| Other urologic cancers    | 50           | 59           | 109   | 45.87          | 36.38–55.66 |
| Prostatic cancer          | 35           | 43           | 78    | 44.87          | 33.74–56.51 |
| Renal cancer              | 15           | 16           | 31    | 48.39          | 30.56–66.60 |
| Total                     | 362          | 350          | 712   | 50.84          | 47.11–54.57 |

BPH, benign prostatic hyperplasia; BAT, benign adrenal tumour; RH, renal hamartoma; UTI, urinary tract infection; CI, confidence interval.

**Effect of haematuria on the BTA stat test**

In patients with benign urologic disorders, patients with haematuria account for a relatively high proportion. Haematuria in patients with BPH, RH and urolithiasis was 39.01%, 42.86% and 66.48%, respectively. In urologic cancers, 58.06% of BC patients were accompanied by haematuria, and 48.72% of prostatic cancer patients and 67.74% of renal cancer patients were also accompanied by haematuria (Fig. 1). Haematuria had a significant effect on the specificity of the BTA stat test in each group. In benign urologic disorders with haematuria, the false-positive rate of the BTA stat test was 211 of 238 patients, which was significantly higher than that of 80 of 315 patients without haematuria who had the same conditions. The false-positive rate of the BTA stat test increased correspondingly in patients with BPH, RH and urolithiasis, as a high proportion of these patients had haematuria (Fig. 1). The specificity of this test decreased to 8.94%-31.58% in patients with BPH, renal cyst, BAT, RH and urolithiasis associated with haematuria. When we removed the patients with haematuria from this analysis, and the specificity of the BTA stat test increased correspondingly in patients with BPH, RH and urolithiasis, as a high proportion of these patients had haematuria (Fig. 1). The specificity of this test decreased to 8.94%-31.58% in patients with BPH, renal cyst, BAT, RH and urolithiasis associated with haematuria. When we removed the patients with haematuria from this analysis, and the specificity of the BTA stat test increased correspondingly in patients with BPH, RH and urolithiasis, as a high proportion of these patients had haematuria (Fig. 1). The specificity of this test decreased to 8.94%-31.58% in patients with BPH, renal cyst, BAT, RH and urolithiasis associated with haematuria. When we removed the patients with haematuria from this analysis, and the specificity of the BTA stat test increased correspondingly in patients with BPH, RH and urolithiasis, as a high proportion of these patients had haematuria (Fig. 1). The specificity of this test decreased to 8.94%-31.58% in patients with BPH, renal cyst, BAT, RH and urolithiasis associated with haematuria. When we removed the patients with haematuria from this analysis, and the specificity of the BTA stat test increased correspondingly in patients with BPH, RH and urolithiasis, as a high proportion of these patients had haematuria (Fig. 1). The specificity of this test decreased to 8.94%-31.58% in patients with BPH, renal cyst, BAT, RH and urolithiasis associated with haematuria.
Discussion

The high recurrence rate of BC requires long-term regular follow-ups [1]. Non-invasive urine testing is ideal for both the patient and the healthcare system [19]. Among non-invasive urine tests, urine tests with molecular biomarkers are promising tools for diagnosing BC. However, these tests are still not well established in daily clinical routine and in the standard diagnostic workup of BC. Further evaluation of the usefulness of these biomarkers in complex clinical situations is needed before they are recommended for widespread clinical use.

The BTA stat test is a molecular urine test for BC that that is currently subject to the most attention. The BTA stat test specifically recognizes bladder tumour-associated antigen (hCFHrp) in urine through monoclonal antibodies. This antigenic protein is considered to be isolated from the urine of patients with BC but cannot be detected in the urine of most healthy individuals [7]. Previous studies have compared the sensitivity of the BTA stat test and cytology detection in patients with BC diagnosed by cystoscopy, and the sensitivity of the BTA stat test was found to be superior to urine cytology and bladder irrigation cytology [20–22]. However, its effectiveness in real-world clinical situations, especially its specificity under different interference situations, needs further observations. In this study, we investigated the sensitivity and specificity of the BTA stat test in patients who were diagnosed with BC, and more importantly, we investigated the influence of haematuria on the performance of the BTA stat test in a clinical cohort.

Our overall sensitivity results were similar to those published by Dov Pode et al. for the sensitivity of the BTA stat test in detecting bladder tumours [18]. In a European multicentre study of 107 patients with a final diagnosis of BC confirmed by cystoscopy and biopsy, the overall sensitivity of the BTA stat test was 72% [23]. In the Rüdiger Heicappell et al. study, they reported sensitivities for grade I, II, and III disease to be 59.1%, 50%, and 77.3%, respectively [7]. By contrast, we demonstrated higher sensitivities of 80%, 70 and 97.75% for PUNLMP, low-grade and high-grade tumours, respectively. These results may be related to different tumour grading methods. More importantly, our analysis of the overall sensitivity included patients with haematuria, which may show higher sensitivity due to false positives. When patients with haematuria were removed from this analysis, the overall sensitivity of the BTA stat test was 80.77%. If you analyse only patients with

---

Table 4

| Categories                  | Positive for RBCs | Negative for RBCs |
|-----------------------------|-------------------|-------------------|
|                             | Total             | BTA-positive | BTA-negative | Specitivity (%) | 95% CI | Total | BTA-positive | BTA-negative | Specitivity (%) | 95% CI              |
| Benign urologic diseases    | 238 (43.04%)      | 211 27       | 11.34        | 7.74–16.24     | 315 (56.96%) | 80   | 235         | 74.6         | 69.35–79.24     |
| BPH                         | 55 (39.01%)       | 50 5         | 9.09         | 3.4–20.71      | 86 (60.99%)  | 12   | 74          | 86.05        | 76.50–92.27     |
| Renal cyst                  | 14 (21.21%)       | 12 2         | 14.29        | 2.51–43.85     | 52 (78.79%)  | 1    | 51          | 98.08        | 88.42–99.9      |
| BAT                         | 19 (25.33%)       | 13 6         | 31.58        | 13.56–56.50    | 56 (74.67%)  | 15   | 41          | 73.21        | 59.47–83.77     |
| RH                          | 12 (42.86%)       | 10 2         | 16.67        | 2.94–49.12     | 16 (57.14%)  | 3    | 13          | 81.25        | 53.69–95.03     |
| Urolithiasis                | 123 (66.49%)      | 112 11       | 8.94         | 4.78–15.49     | 62 (33.51%)  | 14   | 48          | 77.42        | 64.72–86.68     |
| UTI                         | 15 (25.86%)       | 14 1         | 6.67         | 0.35–33.97     | 43 (74.14%)  | 35   | 8           | 18.6         | 8.92–33.92      |
| Other urologic cancers      | 59 (54.13%)       | 39 20        | 33.9         | 22.41–47.49    | 50 (45.87%)  | 20   | 30          | 60           | 45.2–73.27      |
| Prostate cancer             | 38 (48.72%)       | 27 11        | 28.95        | 15.99–46.11    | 40 (51.28%)  | 16   | 24          | 60           | 43.39–74.72     |
| Renal cancer                | 21 (67.74%)       | 12 9         | 42.86        | 22.59–65.56    | 10 (32.26%)  | 4    | 6           | 60           | 27.37–86.31     |
| Total                       | 297 (44.86%)      | 250 47       | 15.82        | 11.96–20.59    | 365 (55.14%) | 100  | 265         | 72.6         | 67.67–77.05     |

BPH, benign prostatic hyperplasia; RH, renal hamartoma; UTI, urinary tract infection; BAT, benign adrenal tumour; CI, confidence interval.
haematuria, the overall sensitivity of the BTA stat test was 97.22%. In our study, the specificity of the BTA stat test was 100% in healthy volunteers, and this result was within the ranges reported by other authors, thus excluding the possibility of technical or analytical errors [10]. Although the specificity of the BTA stat test is high in healthy individuals, disappointing results have nevertheless occurred in patients with other urologic diseases. In a multicentre US study, the specificity of the BTA stat test in patients with BPH, urolithiasis and UTI was 88.5%, 50% and 76%, respectively [24]. These variations may be explained by inherent influencing variables such as infection, trauma, and calculi [10, 23]. Our study showed even lower specificity; the specificity of the BTA stat test decreased to only 31.89% and 15.52% in patients with urolithiasis and UTI, respectively. In patients with prostatic cancer and renal cancer, the specificity of the BTA stat test was only 44.87% and 48.39%, respectively. This may be because we did not exclude patients with haematuria from our total enrolment.

Our results suggested that haematuria leads to high positive rates. On the one hand, the majority of patients with BC will present with haematuria [13, 14]. Considering that hCFHrp is a serum factor, the BTA stat test can possibly concomitantly detect serum proteins that cause haematuria. Another study supports our conclusion; the Makito Miyake group built an experimental haematuria model. They added whole blood to BTA-negative urine samples and found that spiking BTA-negative urine samples with as little as 1 µl whole blood /10 ml urine was enough to produce a positive BTA stat test result [25]. On the other hand, haematuria is the most common symptom in urology patients. In many benign or tumorous urologic diseases, haematuria is often the only manifestation [26]. Even 9–18% of normal individuals also have some degree of haematuria [17]. This fact means that the BTA stat test may be prone to detect particularly high false positives in haematuria patients. We analysed patients with haematuria symptoms in a clinical setting and found that the specificity of the BTA stat test was only 15.82% for all groups of patients with haematuria. The meaning of these data is that when only patients with haematuria symptoms who were suspected of having BC were considered, the specificity of diagnosing BC with the BTA stat test was only 15%. In addition, the proportion of BC patients with haematuria is very small. Previous studies have shown that only 0.7–1.3% of patients with haematuria actually have BC [13, 15, 16]. In this case, if the BTA stat test has such a high false-positive rate for haematuria, it will lack further directivity, and its clinical utility and benefit will be low. The cost of screening includes the cost of labelling each patient and the cost of evaluating patients with false-positive results. Adding any test to the clinical assessment will increase costs; thus, the cost-effectiveness needs to be balanced by the benefits. Optimal screening strategies require identifying a method with high specificity and reasonable sensitivity in a population with significant disease prevalence [27]. The specificity of the marker plays a key role because more patients with false-positive results correlates with needing additional "unnecessary" tests. Our results suggest that the BTA stat test cannot achieve ideal effective specificity and that using it to screen and monitor for BC may lead to "unnecessary" costs. Of course, further studies can compare and analyse the specific costs involved in the clinical practice of qualitative urinary tract testing. However, there are no available data on this problem.

**Conclusions**

In patients with haematuria, there is a relatively low prevalence of BC. Our research shows that the BTA stat test appears to have a high false-positive rate and low specificity in patients with haematuria. Hence, using the BTA stat test to screen and monitor for BC is not ideal. Our data support the relative inadequacy of the BTA stat test as a diagnostic tool for BC.

**Abbreviations**

BTA: bladder tumour antigen

BC: bladder cancer

BPH: benign prostatic hyperplasia

RH: renal hamartoma

hCFHrp: human complement factor H-related protein

UTI: urinary tract infection

BAT: benign adrenal tumour

CI: confidence interval

TCC: transitional cell carcinoma

SCC: squamous cell carcinoma
PUNLMP: papillary urothelial neoplasm of low malignant potential

**Declarations**

**Ethics approval and consent to participate**

The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Our study protocol was approved by The Ethics Committee of The First Affiliated Hospital of The Chongqing Medical University (Approval number:2017-180). Informed consent was obtained from all patients prior to inclusion.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets analyzed during the current study is available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no conflict of interest.

**Funding**

Not applicable.

**Authors’ contributions**

XX was involved in data collection and data analysis and wrote the manuscript. WH was involved data collection and edited the manuscript. FY and BX were involved in data collection. All authors reviewed the manuscript.

**Acknowledgements**

Not applicable.

**References**

1. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. EUR UROL. 2017;71(1):96–108.

2. Abel PD. Prognostic indices in transitional cell carcinoma of the bladder. Br J Urol. 1988;62(2):103–9.

3. Heney NM, Ahmed S, Flanagan MJ, Frable W, Corder MP, Hafermann MD, Hawkins IR. Superficial bladder cancer: progression and recurrence. J Urol. 1983;130(6):1083–6.

4. Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, Skinner E, Bochner B, Thangathurai D, Mikhail M, et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J CLIN ONCOL. 2001;19(3):666–75.

5. Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, et al. EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2016. EUR UROL. 2017;71(3):447–61.

6. Yafi FA, Brimo F, Auger M, Aprikian A, Tanguay S, Kassouf W. Is the performance of urinary cytology as high as reported historically? A contemporary analysis in the detection and surveillance of bladder cancer. Urol Oncol. 2014;32(1):21–7.

7. Heicappell R, Muller M, Fimmers R, Miller K. Qualitative determination of urinary human complement factor H-related protein (hcfHrp) in patients with bladder cancer, healthy controls, and patients with benign urologic disease. UROL INT. 2000;65(4):181–4.

8. Tsui KH, Chen SM, Wang TM, Juang HH, Chen CL, Sin GH, Chang PL. Comparisons of voided urine cytology, nuclear matrix protein-22 and bladder tumor associated antigen tests for bladder cancer of geriatric male patients in Taiwan, China. ASIAN J ANDROL. 2007;9(5):711–5.

9. Chen A, Fu G, Xu Z, Sun Y, Chen X, Cheng KS, Neoh KH, Tang Z, Chen S, Liu M, et al. Detection of Urothelial Bladder Carcinoma via Microfluidic Immunoassay and Single-Cell DNA Copy-Number Alteration Analysis of Captured Urinary-Exfoliated Tumor Cells. CANCER RES. 2018;78(14):4073–85.
10. Raitanen MP, Tammela TL. Specificity of human complement factor H-related protein test (Bard BTA stat Test). Scand J Urol Nephrol. 1999;33(4):234–6.
11. Sharma S, Zippe CD, Pandirangi L, Nelson D, Agarwal A. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. J Urol. 1999;162(1):53–7.
12. Glas AS, Roos D, Deutekom M, Zwinderman AH, Bossuyt PM, Kurth KH. Tumor markers in the diagnosis of primary bladder cancer. A systematic review. J Urol. 2003;169(6):1975–82.
13. Messing EM, Young TB, Hunt VB, Roecker EB, Vaillancourt AM, Hisgen WJ, Greenberg EB, Kuglitsch ME, Wegenke JD. Home screening for hematuria: results of a multiclinic study. J Urol. 1992;148(2 Pt 1):289–92.
14. Messing EM, Young TB, Hunt VB, Newton MA, Bram LL, Vaillancourt A, Hisgen WJ, Greenberg EB, Kuglitsch ME, Wegenke JD. Hematuria home screening: repeat testing results. J Urol. 1995;154(1):57–61.
15. Britton JP, Dowell AC, Whelan P, Harris CM. A community study of bladder cancer screening by the detection of occult urinary bleeding. J Urol. 1992;148(3):788–90.
16. Murakami S, Igarashi T, Hara S, Shimazaki J. Strategies for asymptomatic microscopic hematuria: a prospective study of 1,034 patients. J Urol. 1990;144(1):99–101.
17. Grossfeld GD, Litwin MS, Wolf JS, Hricak H, Shuler CL, Agerter DC, Carroll PR: Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy–part I: definition, detection, prevalence, and etiology. UROLOGY 2001, 57(4):599–603.
18. Pode D, Shapiro A, Wald M, Nativ O, Laufer M, Kaver I. Noninvasive detection of bladder cancer with the BTA stat test. J Urol. 1999;161(2):443–6.
19. Lodewijk I, Dueñas M, Rubio C, Munera-Maravilla E, Segovia C, Bernardini A, Teijeira A, Paramio JM, Suarez-Cabrera C. Liquid Biopsy Biomarkers in Bladder Cancer: A Current Need for Patient Diagnosis and Monitoring. INT J MOL SCI 2018, 19(9).
20. Ellis WJ, Blumenstein BA, Ishak LM, Enfield DL. Clinical evaluation of the BTA TRAK assay and comparison to voided urine cytology and the Bard BTA test in patients with recurrent bladder tumors. The Multi Center Study Group UROLOGY. 1997;50(6):882–7.
21. Ramakumar S, Bhuiyan J, Besse JA, Roberts SG, Wollan PC, Blute ML, O’Kane DJ. Comparison of screening methods in the detection of bladder cancer. J Urol. 1999;162(2):388–94.
22. D’Hallewin MA, Baert L. Initial evaluation of the bladder tumor antigen test in superficial bladder cancer. J Urol. 1996;155(2):475–6.
23. Leyh H, Marberger M, Conort P, Sternberg C, Pansadoro V, Pagano F, Bassi P, Boccon-Gibod L, Ravery V, Treiber U, 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(1):52–6.
24. Sarosdy MF, Hudson MA, Ellis WJ, Soloway MS, DeVere WR, Sheinfeld J, Jarowenko MV, Schellhammer PF, Schervish EW, Patel JV, et al. Improved detection of recurrent bladder cancer using the Bard BTA stat Test. UROLOGY. 1997;50(3):349–53.
25. Miyake M, Goodison S, Rizwani W, Ross S, Bart GH, Rosser CJ. Urinary BTA: indicator of bladder cancer or of hematuria. WORLD J UROL. 2012;30(6):869–73.
26. Avellino GJ, Bose S, Wang DS. Diagnosis and Management of Hematuria. Surg Clin North Am. 2016;96(3):503–15.
27. Lotan Y, Elias K, Svatke RS, Bagrodia A, Nuss G, Moran B, Sagalowsky AI. Bladder cancer screening in a high risk asymptomatic population using a point of care urine based protein tumor marker. J Urol. 2009;182(1):52–7, 58.

Figures
Figure 1

The proportion of haematuria in various diseases and the positive rate of BTA in haematuria patients (Figure 1 had uploaded as additional file) Fig. 1 a. The proportion of haematuria in patients with BC, prostatic cancer, renal cancer and benign urologic disorders. Data are from 786 patients. b. The positive rate of BTA in patients with BC, prostatic cancer, renal cancer and benign urologic disorders. Data are from 353 patients.