The influence of haematuria on the utility of the BTA stat test in the diagnosis of bladder cancer: a prospective study

Xiong Xiong (szbzbb@163.com)
Chongqing Medical University First Affiliated Hospital
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

Posted Date: October 27th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-64771/v2

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. Thus, this test should not be used for the diagnosis of bladder cancer in patients with haematuria.

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 [2]. Therefore, timely detection and diagnosis of BC are essential. Currently, cystoscopy and urine cytology are the best tests for the diagnosis of BC [3]. However, there are some limitations in practical clinical applications. Cystoscopy is invasive and costly [4]. Urine cytology has ideal specificity, but the interpretation of its results are highly dependent on the skill of the examiner, and it has low sensitivity in low-grade tumours [5].

Molecular markers have been proposed for the detection of bladder tumours within recent years. Some of them have been developed as commercial reagents. The advantage of these markers over cystoscopy and urine cytology is that they are simple to use, less pain and low-grade tumors can be found. In contrast, their disadvantages are mainly suboptimal accuracy and high inter-observer variability [6]. Hence, to be widely used, the specificity and sensitivity of these molecular markers need to be tested more in clinical practice to determine their suitable diagnostic conditions.

The bladder tumour antigen (BTA) stat test is the most concerned BC marker detection method and it has been approved by the US Food and Drug Administration for diagnosing and monitoring of BC [7, 8]. 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 [9]. Previous studies have suggested that the sensitivity of the BTA stat test is superior to urine cytology. However, more evidences are needed for its efficacy and utility in clinical, especially when it is interfered by some pathological factors.

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 [10, 11]. On the other hand, there are many causes of haematuria other than BC, including many benign urologic diseases, urinary tract infection, ureteric and renal stones etc. Even between 9% and 18% of normal individuals also have some degree of haematuria [12]. Hence, if haematuria has a serious effect on the specificity of the BTA stat test, using it to screen and monitor BC can lead to unnecessary investigation and anxiety.

Although there have been many studies regarding the diagnosis of BC with BTA stat test, its use in daily practice for patients presenting with hematuria is still not reach a consensus. Hence, we designed a prospective clinical cohort's study to investigate the influence of hematuria on BTA tests.

Material And Methods

Patients

After approval of the local institutional ethics committee and after informed consent, the study prospectively collected urine samples from 1,478 patients who visited the Urology Department of the First Affiliated Hospital of Chongqing Medical University from January 2018 to December 2019. All patients who provided urine samples were numbered after urine collection, and each sample was tested for routine urine test and BTA test respectively. None of the participants had mechanical manipulation of the urethra or bladder within the last two weeks before specimen collection. No one received any medical therapy (ie instillation therapy or systemic chemotherapy) before collecting urine samples.

Then we collected the clinical data from patients and enrolled completed related inspections as needed, including urinary ultrasound, CT, PSA screening etc. Of these, Cystoscopy biopsy were performed in patients suspected of having BC (due to painless gross or microscopic haematuria, or irritative voiding symptoms). For patients suspected of renal cancer on CT, postoperative pathological confirmation was performed. Patients who were suspected of prostate cancer by digital rectal examination and PSA test was confirmed by prostate puncture cytology or postoperative pathology. Benign diseases of the urinary system were diagnosed according to the corresponding diagnostic criteria. All diagnoses were confirmed by two senior urologists.

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 diseases 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 diseases group, patients with a recent history of urolithiasis in the other groups were excluded.

Finally, the study group consisted of 124 patients with BC. The benign urologic diseases 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. According to the previous numbering, the urine routine and BTA results of the initial test are retrieved for further analysis.

In addition, we recruited a group of healthy volunteers without any clinical symptoms from the physical examination centre during the study period. They completed routine physical examinations at the physical examination centre, including urinary occult blood test, urinary ultrasound, CT and PSA screening. Those who had normal physical examination results and agreed to complete the follow-up tests were included in the study. Finally, the healthy volunteers group had 50 individuals. The urine was also collected for BTA stat test and urine analysis.

Haematuria detection

In our study, the routine urinalysis includes dry chemical analysis, flow cytometry analysis and microscopic examination of urine sediment. Haematuria was included gross haematuria and microhaematuria. Gross haematuria defined that the color change was observed in the appearance of urine, and confirmed by demonstration of red blood cells in the urinary sediment as shown by microscopy [13]. Microhaematuria defined that 3 or more red blood cells per high-power microscopic field in urinary sediment [14].

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 [15]. 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 [7]. Groups were compared using and Fisher's exact test or Pearson χ^2 test. A P value <0.05 was considered significant.

Results

BTA stat test in patients with BC

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, 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, and the difference was statistically significant (P < 0.05). (Table 2).

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 diseases, 52.62% patients had false-positive BTA stat test results. The incidence of false positive results was related to the observed condition: 68.11% in patients with urolithiasis, 84.48% in patients with UTI, 43.97% in patients with BPH, 37.33% in patients with BAT and 46.43% in patients with RH had false-positive BTA stat test results. 19.7% in patients with renal cysts had false-positive BTA stat test results. In addition, in patients with prostatic cancer and renal cancer, the false-positive rates of the BTA stat test were 55.12% and 51.61%, 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 diseases and patients with other urologic cancers) of the BTA stat test was 50.84% (Table 3).

Effect of haematuria on the BTA stat test

In patients with benign urologic diseases, patients with haematuria account for a relatively high proportion. Haematuria in patients with BPH, RH and urolithiasis were 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 diseases with haematuria, the specificity of the BTA stat test was 11.34%, which was significantly lower than 74.6% that without haematuria who had the same conditions ($P < 0.05$). 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 to 73.21-98.08% ($P < 0.05$). In prostatic cancer patients with haematuria, the specificity of the BTA stat test was 28.95%, while the specificity increased to 60% after removing patients with haematuria from this analysis ($P < 0.05$). In all groups of patients with haematuria, the specificity of the BTA stat test was 15.82%, and the overall specificity of the BTA stat test was 70.5% in patients without haematuria. The difference was statistically significant ($P < 0.05$). (Table 4).

In addition, our study found that the specificity of BTA stat test was correlated with the severity of haematuria. The specificity of BTA stat test in patients with haematuria under microscope was significantly higher than that of gross haematuria ($P < 0.05$).

Discussion

Among non-invasive urine tests, urine tests with molecular biomarkers are promising tools for diagnosing BC [16]. 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. Considering that BC patients are often associated with haematuria, which also is the most common manifestation of other urological diseases [11, 12]. Hence, it is important to evaluate the usefulness of molecular biomarkers of BC in the context of haematuria interference.

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 [9]. 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 [17-19]. However, there is limited data on its effectiveness in real-world clinical situations. Especially its specificity under haematuria interference situations, needs further observations. In this study, we investigated the effect of haematuria on the sensitivity and specificity of BTA stat test in the clinical cohort.

Previous studies have shown that, the sensitivity of BTA stat test is between 57% and 83% [20]. In our study, the sensitivity of BTA stat test to detect patients without haematuria bladder cancer was 80.77%, that is consistent with previous studies. If you analyse only patients with haematuria, the overall sensitivity of the BTA stat test was 97.22%. This illustrates the effect of haematuria on its sensitivity. Because of false-positives, haematuria patients may exhibit higher sensitivity. Our research shows that haematuria had a significant effect on the specificity of the BTA stat test in each group. In Non-bladder cancer with haematuria patients, the specificity of the BTA stat test was significantly lower than that without haematuria who had the same conditions. (The data show that except UTI and renal cancer group. the reason maybe is that UTI Urinary tract infections also significantly affect the specificity of BTA stat test and the sample size of renal carcinoma group was too small.) Furthermore, compared with other studies, our study showed more lower specificity. 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 [21]. In this study, 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 in these data. In addition, our study found that the specificity of BTA stat test was correlated with the severity of haematuria. The specificity of BTA stat test in patients with haematuria under microscope was significantly higher than that of gross haematuria.

Our results suggested that haematuria leads to high positive rates. Considering that hCFHrp is a serum factor, the BTA stat test can possibly concomitantly detect serum proteins that cause haematuria. A 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 [22]. These evidences confirmed that the BTA stat test is very unsatisfactory for the diagnosis of BC. On the one hand, the majority of patients with BC will present with haematuria, gross or microscopic haematuria is often the first symptom in BC patients [11, 12]. 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 [23]. Even 9% to 18% of normal individuals also have some degree of haematuria [12]. 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, especially for patients with microscopic haematuria. Previous studies have shown that only about 1% of patients with microscopic haematuria actually have BC [10,24,25]. 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 not only identifying a method with high sensitivity in a population with significant disease prevalence, but also requires this method with reasonable specificity [26]. 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. Therefore, BTA stat test is not recommended for screening or diagnosing BC in patients with haematuria.

In addition, in case of follow up of urinary tumour markers, negative predictive value is also very important if our goal is to avoid a more invasive cystoscopy based on the urinary tumour marker report. In our study, the negative predictive value of BTA stat test was 97.09%, which is similar to the results of previous studies. Our study also showed that the negative predictive value of BTA in patients with haematuria was significantly higher than that in patients without haematuria, and the difference was statistically significant (P 0.05). Nevertheless, we must note that because the negative predictive value is affected by the prevalence, even if the diagnostic performance is very poor, this value can be as high as 90% or more when the prevalence is relatively low. At this time, it is not objective for us to use the negative predictive value to measure the diagnostic value of diagnostic experiments. After identifying high-risk groups, negative predictive value can play its greatest role, but this requires large-scale prospective studies to determine the actual prevalence in high-risk groups. If the prevalence is still low in high-risk groups, then the cost and actual benefits of screening need to be considered. 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.

A variety of biomarkers for evaluating bladder cancer have been developed, including protein-based and gene-based biomarkers. As with BTA, the use of the protein marker NMP22 also has been hampered by haematuria [27-28]. It is worth noting that these results did not indicate that the high false positive of protein-based makers in patients with haematuria are directly caused by red blood cells in the urine. It may also be that the factors that promote haematuria may also promote tumour cell shedding, thereby increasing the positive detection rate of these makers. Hence, for protein-based biomarkers, it needs to be tested under specific circumstances. Gene-based detection of bladder cancer is much better for performance

under haematuria interference, such as UroVysio test and Cxbladder test [29-30]. But these testing methods are far more complex, and the high cost is the biggest obstacle to their widespread use.

There are some limitations to our study. There is too less a number of patients in T3, T4 category, undifferentiated cancer groups. The sensitivity of 100% in these patient groups may not be statistically significant. the sample size of Renal cancer group is small, which may also cause some bias. We Providing and retaining these data so that readers allow readers to evaluate the sample size and clinical data.

Conclusions

Our research shows that the specificity of the BTA stat test was very low in haematuria condition, thus this test should not be used for the diagnosis of BC in patients with haematuria. False positive results can be decreased if patients with haematuria conditions were excluded from the test.

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):103-109.
3. 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):447-461.
4. Sievert, K.D., et al., Economic aspects of bladder cancer: what are the benefits and costs? World J Urol, 2009. 27(3): p. 295-300.
5. 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):21-27.
6. Vrooman, O.P.J. and J.A. Witjes, Urinary markers in bladder cancer. European Urology, 2008. 53(5): p. 909-916.
7. Tsui KH, Chen SM, Wang TM, Juang HH, Chen CL, Sun 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):711-715.
8. 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):4073-4085.
9. 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):181-184.
10. 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):289-292.
11. 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.
12. 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.
13. Bolenz, C., et al., The Investigation of Hematuria. Dtsch Arztebl Int, 2018. 115(48): p. 801-807.
14. Horstmann, M., T. Franiel and M.O. Grimm, Differential diagnosis of hematuria. Der Urologe, 2014. 53(8): p. 1215-1226.

15. 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):443-446.
16. Lodewijk I, Duenas 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).
17. 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):882-887.
18. 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, 161(2):388-394.
19. D'Hallewin MA, Baert L: Initial evaluation of the bladder tumor antigen test in superficial bladder cancer. J Urol 1996, 155(2):475-476.
20. Noon, A.P. and A.R. Zlotta, Urothelial Bladder Cancer Urinary Biomarkers. EJIFCC, 2014. 25(1): p. 99-114.
21. 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):349-353.
22. 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):869-873.
23. Avellino GJ, Bose S, Wang DS: Diagnosis and Management of Hematuria. Surg Clin North Am 2016, 96(3):503-515.
24. 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):788-790.
25. 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.
26. Lotan Y, Elias K, Svatek 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):52-57, 58.
27. Miyake, M., et al., Influencing factors on the NMP-22 urine assay: an experimental model. BMC Urol, 2012. 12: p. 23.
28. Huber, S., et al., Nuclear matrix protein-22: a prospective evaluation in a population at risk for bladder cancer. Results from the UroScreen study. BJU Int, 2012. 110(5): p. 699-708.
29. Todenhöfer, T., et al., Impact of different grades of microscopic hematuria on the performance of urine-based markers for the detection of urothelial carcinoma. Urol Oncol, 2013. 31(7): p. 1148-54.
30. Kavalieris, L., et al., A segregation index combining phenotypic (clinical characteristics) and genotypic (gene expression) biomarkers from a urine sample to triage out patients presenting with hematuria who have a low probability of urothelial carcinoma. BMC Urol, 2015. 15: p. 23.

Tables

Table 1
Patient demographics

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

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

riable	Negative	Positive	Total	Sensitivity (%)	95% CI
adder cancer	12	112	124	90.32	83.36-94.68
thologic subtype					
TCC	11	107	118 (95.16%)	90.68	83.57-95.02
SCC	1	4	5 (4.03%)	80	29.88-98.95
Undifferentiated carcinoma	0	1	1 (0.81%)	100	5.46-100
ade					
PUNLMP	1	4	5 (4.03%)	80	29.88-98.95
Low-grade	9	21	30 (24.19%)	70	50.44-84.59
High-grade	2	87	89 (71.77%)	97.75	91.35-99.61
thologic T stage					
Ta	10	52	62 (50%)	83.87	71.87-91.59
T1	1	23	24 (19.35%)	95.83	76.88-99.78
T2	1	27	28 (22.58%)	96.43	79.76-99.81
T3	0	4	4 (3.23%)	100	39.57-100
T4	0	6	6 (4.84%)	100	51.68-100
ine analysis					
Haematuria	2	70	72 (58.06%)	97.22*	89.42-99.52
No haematuria	10	42	52 (41.94%)	80.77	67.03-89.92
Statistical analysis				*P□0.002 vs No haematuria	

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

Table 3
Specificity of the BTA stat test in 50 healthy subjects and patients in eight disease categories

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.

Table 4 Specificity of the BTA stat test in patients with or without haematuria in eight disease categories

Categories	Haematuria				No haematuria				Statistical analysis*
	Total	BTA-positive	BTA-negative	Specificity (95% CI)	Total	BTA-positive	BTA-negative	Specificity (95% CI)	*P* value
Benign urologic diseases	238	211	27	11.34% (7.74-16.24)	315	80	235	74.6% (69.35-79.24)	0.001
BPH	55	50	5	9.09% (3.4-20.71)	86	12	74	86.05% (76.50-92.27)	0.001
Renal cyst	14	12	2	14.29% (2.51-43.85)	52	1	51	98.08% (88.42-99.9)	0.001
BAT	19	13	6	31.58% (13.56-56.50)	56	15	41	73.21% (59.46-83.77)	0.001
RH	12	10	2	16.67% (2.94-49.12)	16	3	13	81.25% (53.69-95.03)	0.001
Urolithiasis	123	112	11	8.94% (4.78-15.49)	62	14	48	77.42% (64.72-86.68)	0.001
UTI	15	14	1	6.67% (0.35-33.97)	43	35	8	18.6% (8.92-33.92)	0.272
Other urologic cancers	59	39	20	33.9% (22.41-47.49)	50	20	30	60% (45.2-73.27)	0.006
Prostate cancer	38	27	11	28.95% (15.99-46.11)	40	16	24	60% (43.39-74.72)	0.006
Renal cancer	21	12	9	42.86% (22.59-65.56)	10	4	6	60% (27.37-86.31)	0.458
Total	297	250	47	15.82% (11.96-20.59)	365	100	265	72.6% (67.67-77.05)	0.001

BPH, benign prostatic hyperplasia; RH, renal hamartoma; UTI, urinary tract infection; BAT, benign adrenal tumour; CI, confidence interval; * Specificity in Haematuria vs no haematuria.

Table 5 The specificity of the BTA stat test in microscopic haematuria and gross haematuria conditions

	Total	BTA-positive	BTA-negative	specificity	95%CI
o haematuria aematuria	415	100	315	75.9%	71.44-79.88
Microhaematuria	248	203	45	18.15%*	13.67-23.63
Gross haematuria	49	47	2	4.08%#△	0.7-15.14
tatistical analysis	*P < 0.0001 vs no haematuria #P < 0.0001 vs no haematuria △P = 0.014 vs microhaematuria				

Figures

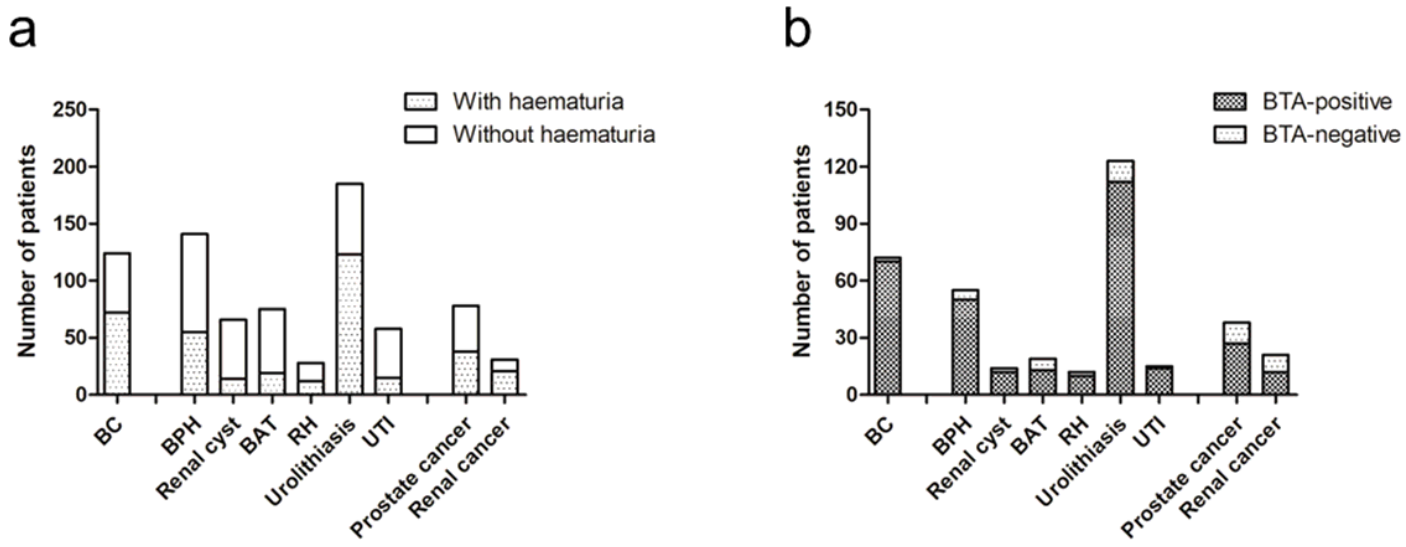

Figure 1

The proportion of haematuria in various diseases and the positive rate of BTA in haematuria patients (Figure1 had uploaded as additional file) Fig. 1 a. The proportion of haematuria in patients with BC, prostatic cancer, renal cancer and benign urologic diseases. Data are from 786 patients. b. The positive rate of BTA in patients with BC, prostatic cancer, renal cancer and benign urologic diseases. Data are from 353 patients.