A comparative analysis of rapid urine tests for the diagnosis of upper urinary tract malignancy

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Objectives: *To compare the effectiveness of two rapid urine tests fibrinogen/fibrin degradation products FDP (Aura Tek FDP, PerImmune Inc., Rockville, Maryland, USA) and bladder tumor antigen BTA (Bard BTA, Bard Canada Inc., Mississauga, Ontario, Canada) to urinary cytology in establishing the diagnosis of transitional cell carcinoma (TCC) in patients with suspected upper tract malignancy.*

Materials and methods: *In a prospective study, urine samples were collected from 29 patients with abnormalities of the upper tracts highly suspicious for malignancy. Sensitivity and specificity of the BTA and FDP tests were determined and compared to those of cytology. All persons interpreting the individual tests were blinded to the other test results.*

Results: *Of the 29 patients evaluated, 14 were found to have upper tract TCC. The overall sensitivity of FDP, BTA and urinary cytology was found to be 100%, 50% and 29%, respectively. The accuracy of the FDP test was 83% as compared to 62% for BTA and 59% for cytology.*

Conclusion: *Urinary cytology lacks sensitivity in the diagnosis of upper tract TCC. This preliminary study suggests that the point-of-care test, FDP, has better test characteristics than urinary cytology. Further evaluation of these tests is warranted for their potential to improve and facilitate the diagnosis of upper tract tumors.*

Key Words: kidney neoplasm, cytology, immunoassay

Introduction

The diagnosis of filling defects in the upper urinary tract has relied to a large extent upon selected urinary cytology specimens to identify those lesions due to transitional cell carcinoma (TCC). Unfortunately, cytological diagnosis for upper tract TCC is known to have low accuracy, primarily due to poor cellular harvest of low grade lesion.[1] Location, small size and method of collection of the urine for analysis have also been implicated in the poor sensitivity of cytology.[2,3] Ureteropyeloscopy has increased the diagnostic accuracy of upper tract lesions; however, it is an invasive procedure with reported complication rates of up to 10%.[4,5] A rapid, point-of-care test that could confirm radiological suspicions of upper tract TCC and, subsequently, direct further intervention would be advantageous.

Numerous alternative biological markers have been described reporting variable efficacy for the diagnosis and follow-up of TCC of the bladder.[6-24] A recent, excellent review[24] of these urine-based markers has pointed-out consistently superior sensitivity over urinary cytology, although at the expense of yielding more false-positive results. Unfortunately, few urine-based markers have been evaluated for their utility in diagnosing TCC of the upper tracts.

Ho and Kuo[25] compared urinary glucoronidase

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activity to routine cytology for upper tract TCC and found an improved sensitivity of 90%. However, urinary glucoronidase is not very specific for urothelial malignancies (renal cell carcinoma would test positive), as well, benign conditions such as stones were excluded from the study. In their study of the usefulness of fibrin/fibrinogen degradation products (FDP) to detect urothelial carcinoma, Jayachandran et al found elevated levels in one patient with an upper tract TCC after a previous cystectomy.[26] Zimmerman et al evaluated the bladder tumor antigen (BTA) test in detecting upper tract TCC and found a sensitivity and specificity of 60% and 40%, respectively.[27] They concluded that the test had no clinical value for detecting upper tract lesions based on these test characteristics. Lodde et al have recently reported the use of ImmunoCyt in the detection of upper urinary tract TCC. They found that this immunocytochemical test was additive to cytology in 16 patients with upper tract TCC.[28] We report on a prospective study comparing FDP, BTA and urinary cytology in establishing the diagnosis of patients with suspected upper tract malignancy.

Materials and methods

This was a prospective, single center equivalence study comparing the diagnostic accuracy of the rapid urine tests, BTA and FDP, to urinary cytology for the diagnosis of upper tract lesions suspicious for TCC. Included into the study were those patients found to have upper tract lesions that were clinically suspicious for upper tract TCC. This included filling defects on intravenous pyelogram or retrograde pyelogram as well as suspicious lesions on other radiological investigations such as ultrasound and CT scan. Exclusions to the study were those patients with an active history of TCC of the bladder.

Ureteral wash urine samples from patients undergoing retrograde pyelogram or ureteroscopy were collected after saline barbotage. Some patients did not undergo these investigations in the course of their investigations and, therefore, had only voided urine samples collected. All samples were then split into two aliquots and one sent to an experienced cytopathologist. The pathologists were blinded to the possible clinical diagnosis as well as the results of the rapid urine tests. Cytology was considered positive if it was reported as positive (diagnostic for TCC) or suspicious (highly suspicious for TCC). Cytology was considered negative if reported as atypical (non-diagnostic for TCC) or negative for malignancy.

The other urine sample was used to perform the BTA and AuraTek FDP tests. The research coordinator performing these tests was also blinded as to the clinical diagnosis and cytology results. A second blinded evaluator then confirmed the results of the rapid urine tests. These tests are dichotomous and are read as either negative or positive for each urine sample run.

The standard used for the final diagnosis of these lesions was histopathological examination of surgical specimens. For those who were not surgical candidates or whose pathology was not due to malignant disease, the final diagnosis was made after completion of traditional investigations for upper tract lesions, including biopsy results. By comparing the results of the rapid urine tests and urinary cytology against the standard in a four by four table, we computed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of these tests.

Statistical analysis

Test characteristics are presented with the 95% confidence interval. The McNemer test (the version based on the binomal test) was used to compare discordance of results between: 1) FDP versus BTA, 2) FDP versus cytology, and 3) BTA versus cytology.

Results

Thirty-two patients were evaluated over a 14-month period. The results of three of the enrolled patients were not included into the study as two were subsequently found to have TCC of the bladder and one patient died of unrelated disease prior to establishing a definitive diagnosis. Twenty-two men and seven women were enrolled and the mean age of these patients was 63 years.

Transitional cell carcinoma of the upper tract was found in 14 of the 29 patients (48%). The pathologic stages assigned to these specimens ranged from T1 to T3 (1997 TNM system). Tumor grade was assigned as either grades 2 and 3, with no grade 1 tumors present (1973 World Health Organization classification). Five of the patients found to have upper tract TCC were not surgical candidates; one because of concurrent health problems and the others were found to have metastatic disease prior to definitive management.

Of those 15 patients who did not have upper tract TCC, 10 were determined to have malignant disease and five had benign disease processes. On pathologic examination, nine patients had renal cell carcinoma, and one had a primary renal lymphoma, all of which did involve or encroach upon the renal collecting system. The five patients without malignant disease

TABLE 1. **Test characteristics of rapid urine tests and urinary cytology in the diagnosis of upper tract transitional cell carcinoma**

	Sensitivity (% [CI])	Specificity (% [CI])	PPV (% [CI])	NPV (% [CI])	Accuracy (% [CI])
FDP	14/14 (100 [78,100])	10/15 (67 [38,88])	14/19 (74 [49,91])	10/10 (100 [69,100])	24/29 (83 [64,94])
BTA	7/14 (50 [23,77])	11/15 (73 [45,92])	7/11 (64 [31,89])	11/18 (61 [36,83])	18/29 (62 [42,79])
Cytology	4/14 (29 [8,58])	13/15 (86 [60,98])	4/6 (66 [22,96])	13/23 (57 [34,77])	17/29 (59 [39,76])

FDP, fibrinogen/fibrin degradation products; BTA, bladder tumor antigen;
PPV, positive predictive value; NPV, negative predictive value; CI, 95% confidence interval

included two with radiolucent ureteric calculi, two with benign periureteral fibrosis (biopsy proven). One patient was found to have no upper tract abnormality after re-evaluation.

The overall sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy are displayed in Table 1. The sensitivity for FDP was 100%, significantly better than for both BTA and cytology. Combining the positive results for the BTA and urinary cytology tests did improve sensitivity to 64%. As demonstrated in previous trials of urine-based markers for TCC, urinary cytology was found to have the greatest specificity of 86%. The test characteristics of FDP, BTA and urinary cytology were also analyzed based on the type of urinary specimen collected Table 2. The sensitivity of urinary cytology appeared to improve when site directed urine was collected, although no statistically significant difference was established, most likely due to the small number of samples in this subset.

TABLE 2. **Sensitivity and specificity of rapid urine tests and urinary cytology based on method of sample collection**

	Sensitivity (%)	Specificity (%)
FDP		
Voided	7/7 (100)	9/11 (82)
Ureteral	7/7 (100)	1/4 (25)
BTA		
Voided	3/7 (43)	9/11 (82)
Ureteral	4/7 (57)	2/4 (50)
Cytology		
Voided	1/7 (14)	10/11 (91)
Ureteral	3/7 (43)	3/4 (75)

FDP, fibrinogen/fibrin degradation products; BTA, bladder tumor antigen;
Ureteral, ureteral wash specimen after saline barbotage; Voided, voided urine sample

All of the upper tract lesions that were found to be TCC were identified with the FDP test. However, it did produce five false positive results including two patients with ureteric stones, two with benign ureteral fibrosis and one with a primary renal lymphoma. The BTA failed to identify seven patients with upper tract TCC and there were four false positive results.

Using McNemar's test (two-tailed), there was a statistically significant difference between the FDP and BTA tests (p= 0.0391), as well as FDP and urinary cytology (p= 0.0042). There was no statistical difference found between BTA and cytology (p= 0.2668).

Discussion

The use of flexible ureteroscopy, as well as ureteral brush and cup biopsies, has ensured that fewer situations arise where a suspicious upper tract lesion goes undiagnosed prior to definitive management. Unfortunately, the reported poor sensitivity of urinary cytology, especially of voided samples, has resulted in its inability to reliably direct the use of the more invasive procedures. The advent of a rapid urine test to give a noninvasive and accurate diagnosis in the clinic setting would certainly be a welcome addition to the diagnostic investigations.

Although the majority of urine based markers for TCC require a specialized laboratory,[24] fibrinogen/fibrin degradation products (FDP) and bladder tumor antigen (BTA) are rapid urine tests that provide a point-of-care diagnosis. The Bard BTA is a latex agglutination test that can detect the presence of basement membrane complexes lost in the urine of patients with TCC.[15,29] Original reports suggested an

improved sensitivity over urinary cytology[9,10,15] although specificity may be decreased by other genitourinary diseases.[13,30] The AuraTek FDP, is a lateral flow immunoassay using monoclonal antibodies that has been evaluated to detect the elevated urinary fibrin/fibrinogen degradation products previously described in patients with bladder cancer.[11-13] This original test has been replaced by the Accu-Dx (Mentor Corporation, Santa Barbara, California, USA) test; however, marketing of this test has been temporarily suspended due to issues of test formulation. The FDP and BTA tests have been prospectively compared to urinary cytology in the detection of bladder cancer.[13] In that report, the FDP test was found to have a much higher overall sensitivity (81%) as compared to either BTA (28%) or urinary cytology (35%). There have not been any comparative evaluations of these point-of-care tests for malignancies of the upper tracts.

This study does suggest the inadequate performance of urinary cytology to aid in the diagnosis of TCC of the upper tracts. Cytology was found to have an overall sensitivity of only 29%. Possible explanations for the poor sensitivity of urinary cytology include the low cellular harvest of lower stage and grade tumors, as well, the inclusion of both voided and ureteral wash samples in this study will have decreased the overall sensitivity of urinary cytology. This was confirmed by analyzing our data based on the method of urine collection and subsequently showing an improved sensitivity of urinary cytology (43%) when ureteral wash samples were sent to the cytopathologist. One other possible reason for the lower sensitivity of urinary cytology was that no concommitent carcinoma in situ was detected in those patients with TCC. However, it should be pointed out that FDP was detected in all urine samples of those patients with upper tract TCC, even voided urine specimens. The rapid urine tests were found to be simple, inexpensive and give immediate results. They were found to improve on the sensitivity of urinary cytology without losing much specificity. The sensitivity of the FDP test was remarkable as it picked up every identified case of upper tract TCC, resulting in a negative predictive value of 100% in this population. The avoidance of a false negative result, if confirmed in larger studies with voided urine samples, would make the FDP test very useful in directing further investigation of upper tract abnormalities. There were five false positive results with this test, two of which were due to radiolucent renal calculi. This is consistent with previous reports linking elevated urinary FDP with urolithiasis.[13] The BTA test was found to be more operator dependent than FDP, although the new BTA Stat version has been reported to be easier to use and possibly more reliable.[16]

The availability of a rapid, point-of-care test for the detection of TCC of the upper tracts would provide a significant contribution to the diagnosis of suspicious lesions of the renal pelvis and ureter. A simple urine test available in the clinic and possessing an excellent diagnostic accuracy would be valuable in directing further invasive investigations, such as ureteroscopy. In this preliminary study, the FDP test appeared to have excellent test characteristics; in particular with voided urine samples, although the role of alternative biological markers in detecting upper tract TCC remains to be established. □

References

1. Farrow GM, Utz DZ, Rife CC. Morphological and clinical observations of patients with early bladder cancer treated with total cystectomy. *Cancer Res* 1976;36:2495-2501.
2. Blute ML, Segura JW, Patterson DE. Impact of endourology on diagnosis and management of upper urinary tract urothelial cancer. *J Urol* 1989;141:1298-1301.
3. Zincke H, Aguilo JJ, Farrow GM et al. Significance of urinary cytology in the early detection of transitional cell cancer of the upper urinary tract. *J Urol* 1976;166:781-783.
4. Streem SB, Pontes JE, Novick AC et al. Ureteroyeloscopy in the evaluation of upper tract filling defects. *J Urol* 1986;136:383-385.
5. Huffman JL. Ureteroscopic injuries of the urinary tract. *Urol Clin North Am* 1989;16:249-254.
6. Soloway MS, Briggman JV, Carpinto GA et al. Use of a new tumor marker: Urinary NMP22 in the detection of occult or rapidly recurrent transitional cell carcinoma of the urinary tract following surgical treatment. *J Urol* 1996;156:363-367.
7. Cordon-Cardo C, Wartinger DD, Melamed MR et al. Immunopathologic analysis of human urinary bladder cancer. Characterization of two new antigens associated with low-grade superficial bladder tumors. *Am J Pathol* 1992;140:375-385.
8. Keeley FX, Bibbo M, McCue P et al. Use of p53 in the diagnosis of upper-tract transitional cell carcinoma. *Urology* 1997;49(2):181-186.
9. Sarosdy MF, devere White DMA, Soloway MS et al. Results of a multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. *J Urol* 1995;154:379-383.
10. D'Hallewin M, Baert L. Initial evaluation of the bladder tumor antigen test in superficial bladder cancer. *J Urol* 1996;155:475-476.
11. Schmetter, BS, Habicht KK, Lamm DL et al. Results of a multicenter trial evaluation of AuraTek FDP: an aid in the management of bladder cancer patients. *J Urol* 1997;158:801-805.
12. McCabe RP, Lamm LD, Haspel MV et al. A diagnostic-prognostic test for bladder cancer using a monoclonal antibody-based enzyme-linked immunoassay for detection of urinary fibrin(ogen) degradation products. *Cancer Res* 1984;44:5886-5893.
13. Johnston B, Morales A, Emerson L et al. Rapid detection of bladder cancer: a comparative study of point of care tests. *J Urol* 1997;158:2098-2101.
14. Stampfer DS, Carpinito GA, Rodriguez-Villanueva J et al. Evaluation of NMP22 in the detection of transitional cell carcinoma of the bladder. *J Urol* 1998;159:394-398.

15. Ianari A, Sternberg CN, Rossetti A et al. Results of Bard BTA test in monitoring patients with a history of transitional cell carcinoma of the bladder. *Urology* 1997;49:786-789.
16. Sarosdy MF, Hudson MA, Ellis WJ et al. Improved detection of recurrent bladder cancer using the Bard BTA Stat test. *Urology* 1997;50:349-353.
17. Van der Poel HG, Van Balken MR, Schamhart DH et al. Bladder wash cytology, quantitative cytology, and the qualitative BTA test in patients with superficial bladder cancer. *Urology* 1998;51:44-50.
18. Ellis WJ, Blumenstein BA, Ishak LM et al. 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;50:882-887.
19. Landman J, Chang Y, Kavaler E et al. Sensitivity and specificity of NMP-22, telomerase and BTA in the detection of human bladder cancer. *Urology* 1998;52:398-402.
20. Lee DH, Yang SC, Hong SJ et al. Telomerase: a potential marker of bladder transitional cell carcinoma in bladder washes. *Clin Cancer Res* 1998;4:535-538.
21. Mian C, Pycha A, Wiener H et al. Immunocyt: a new tool for detecting transitional cell cancer of the urinary tract. *J Urol* 1999;161:1486-1489.
22. Pode D, Golijanin D, Sherman Y et al. Immunostaining of Lewis X in cells from voided urine, cytopathology and ultrasound for noninvasive detection of bladder tumors. *J Urol* 1998;159:389-392.
23. Sarosdy MF, Hudson MA, Ellis WJ et al. Detection of recurrent bladder cancer using a new one-step test for bladder tumor antigen (A 337). *J Urol* 1997;157:1318.
24. Konety BR, Getzenberg RH. Urine based markers of urological malignancy. *J Urol* 2001;165:600-611.
25. Ho K-J, Kuo S-H. Urinary beta-glucoronidase activity as an initial screening test for urinary tract malignancyin potential high risk patients. Comparison with conventional urine cytologic evaluation. *Cancer* 1995;76:473-478.
26. Jayachandran S, Unni Moopan MM, Wax SH, Kim H. The value of urinary fibrin/fibrinogen degradation products as tumor markers in urothelial carcinoma. *J Urol* 1984;132:21-23.
27. Zimmerman RL, Bagley D, Hawthorne C, Bibbo M. Utility of the Bard BTA test in detecting upper urinary tract transitional cell carcinoma. *Urology* 1998;51:956-958.
28. Lodde M, Mian C, Wiener H, Haitel A, Pycha A, Marberger A. Detection of upper urinary tract transitional cell carcinoma with ImmunoCyt: a preliminary report. *Urology* 2001;58:362-366.
29. Conn IG, Crocker J, Wallace DMA, Hughes MA, Hilton CJ. Basement membranes in urothelial carcinoma. *Brit J Urol* 1987;60:536-542.
30. Murphy WM, Rivera-Ramirez I, Medina CA et al. The bladder tumor antigen (BTA) test compared to voided urine cytology in the detection of bladder neoplasms. *J Urol* 1997;158:2102-2106.