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Comparison of the BTA *stat*™ Test with Voided Urine Cytology and Bladder Wash Cytology in the Diagnosis and Monitoring of Bladder Cancer

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Key Words

Bladder cancer · Bladder neoplasms · BTA *stat*™ test · Cytology · Tumor-associated antigen

Abstract

Objective: To compare the BTA *stat*™ test (BTA *stat*), a new one-step immunochromatographic assay that can be performed in the urologist's office or in the laboratory, to voided urine cytology and bladder wash cytology (cytology) in the diagnosis and monitoring of cancer of the bladder (BC). ***Methods:*** BTA *stat* and cytology were performed in a double-blinded, prospective, clinical study on specimens from 240 subjects (68 females; mean age of subjects: 64 years) suspected of having BC. ***Results:*** In 107 subjects with final diagnoses of BC confirmed by cystoscopy or cystoscopy and biopsy, the overall sensitivities of BTA *stat* and cytology were 65 and 33%, respectively. For tumor grades I, II, and III, the sensitivities of BTA *stat* were 39, 67 and 83%, respectively. Those of cytology were 4, 20 and 69%. Nine subjects had a diagnosis of 'suspicious for bladder cancer'. The specificities of BTA *stat* and cytology in the 124 subjects without BC were 64 and 99%, respectively. In the subjects with a history of BC (n = 74), the specificities of BTA *stat* and cytology were 72 and 99%, respectively. The specificity of BTA *stat* was lower in subjects with benign or malignant genitourinary disease other than BC (46%) than in subjects without genitourinary disease (71%). ***Conclusions:*** The BTA *stat* test is considerably more sensitive than cytology in the detection of BC and can replace cytology as an adjunct to cystoscopy in the diagnosis and follow-up of patients with BC. However, due to low specificity, BTA *stat* should not be used without first ruling out potential interferences such as infections, renal disease and cancer, or genitourinary trauma.

Introduction

A definitive diagnosis of cancer of the bladder (BC) is made by cystoscopic inspection of the interior of the bladder, usually accompanied by pathologic examination of a

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resected tumor or biopsy specimen. When bladder tumors are small or flat (carcinoma in situ), however, they cannot always be reliably visualized by cystoscopy [1, 2]. For this reason, some urologists perform an adjunctive diagnostic procedure, such as voided urine cytology or bladder wash cytology. Cytology, however, has certain drawbacks, including its subjectivity, its reliance on the skill of the pathologist, and its relative insensitivity to grade I tumors [3]. In addition, any kind of therapy for BC (e.g., surgery, radiotherapy, or intravesical chemotherapy) reduces the accuracy of cytology [4]. Other disadvantages of cytology include its high cost and the fact that results are usually not available for several days. Because of these disadvantages, a simple, sensitive, objective urine test that can be performed at the point of care has long been sought as an alternative adjunct to cytoscopy.

The original Bard® BTA® test (Bard Diagnostic Sciences, C.R. Bard, Inc., Redmond, Wash., USA) is a multiple-step latex-agglutination assay for the detection of bladder-tumor-associated analytes in voided urine that can be performed in the urologist's office. In multicenter studies conducted in the United States and Europe, the Bard BTA test has been found to be at least twice more sensitive in detecting bladder cancer than voided urine cytology [5–7]. The BTA *stat*™ test (BTA *stat*; Bard Diagnostic Sciences) is a new, one-step qualitative assay that detects a distinctly different bladder-tumor-associated antigen in voided urine. The BTA *stat* test can be performed at the point of care as well as in the laboratory. In a recent study involving 134 patients with recurrent BC, the sensitivities of voided urine cytology and the BTA *stat* test were 8 and 38% for grade I BC, 18 and 64% for grade II BC, and 38 and 72% for grade III BC, respectively [8].

The BTA *stat* test detects bladder-tumor-associated antigen within 5 min after the placement of five drops of untreated urine in the sample well of a disposable test device. The antigen detected by the BTA *stat* test is a human complement factor-H-related protein (hCFHrp), which is similar in structure and function to human complement factor H. In vivo, hCFHrp may confer a selective growth advantage to cancer cells by allowing them to evade the host immune system. In cell culture, hCFHrp has been found to be produced by human bladder cancer cell lines but not by normal epithelial cell lines [9, 10].

In the present multicenter study, we compared the sensitivity and specificity of the BTA *stat* test for BC with those of voided urine cytology or bladder wash cytology, using cystoscopy or cystoscopy and histology as the standard for definitive diagnosis.

Subjects and Methods

The study was conducted according to the current (1989) revision of the Declaration of Helsinki. The study protocol was approved by an ethics committee at each of six participating institutions and was conducted in accordance with the laws and regulations of the four countries in which it was performed.

Eligible for participation in the study were persons with or without a history of BC who were suspected of having BC on the basis of signs, symptoms, or recent intravenous urographic or cystoscopic results and who were scheduled to undergo routine cystoscopy and cytologic examination. Written or verbal informed consent was obtained from each subject before participation in any study-related activity.

All subjects provided a single midstream or catheterized urine specimen the same day as but prior to cystoscopy. A portion of the specimen was processed according to the instructions provided in the BTA *stat* test kit. The remainder was sent to the institution's cytology laboratory for examination according to standard procedures. When a bladder wash cytology was performed, the bladder wash was done at the time of cystoscopy and the wash also was sent to the cytology laboratory for processing. In the case of bladder wash cytology, the BTA *stat* test was performed on a voided urine sample before cystoscopy.

The BTA *stat* test is performed by placing five drops of untreated urine in the sample well of a disposable test device. In the well, the urine mixes with a colloidal gold-conjugated antibody. This mixture flows through a membrane to the test zone of the device that contains immobilized capture antibody. If bladder-tumor-associated antigen is present, an antibody-antigen-conjugate sandwich forms and a visible line appears in the test zone. If no antigen is present, no line appears. A colored line of any intensity indicates a positive test result. The control zone of the device contains an immobilized reagent that captures the conjugated antibody and forms a visible line whether or not bladder-tumor-associated antigen is present. Formation of the line in the control zone indicates that the device has functioned properly. The results are read 5 min after placement of the urine in the well.

The cytologic examinations were performed by pathologists blinded to the results of the BTA *stat* test. A positive cytology result was defined as a laboratory report of 'positive' for malignancy. Reports of 'suspicious' or 'atypical cells' were not considered positive but were reported.

A positive final diagnosis was defined as BC confirmed by cystoscopy or cystoscopy and biopsy. Tumors were graded and staged according to the classification of the International Union Against Cancer (UICC) [11], in which staging depends on the type of tumor and its extent of penetration into the bladder wall and grading is based on the degree of tumor differentiation. Grade I, II, and III tumors are well, moderately well, and poorly differentiated, respectively. A negative final diagnosis was defined as no BC ascertained by cystoscopy or cystoscopy and biopsy. If a definitive final diagnosis was not possible ('suspicious' results), the case was not included in the determination of sensitivity or specificity.

There were biopsies performed on 47/124 subjects with a final diagnosis of no bladder cancer to rule out CIS. All 47 biopsies were negative. Seventy-seven subjects did not get a biopsy because it was clear that the bladder was normal.

The primary measures of diagnostic efficacy were sensitivity to BC defined as the ratio of the number of true positive results to the

number of positive final diagnoses (based on cystoscopy or cystoscopy and biopsy), and specificity for BC defined as the ratio of the total number of true negative results to the total number of negative final diagnoses. The number of study participants required to detect a difference of 0.15 or greater between the BTA *stat* test and cytology sensitivities was computed using the method of Schork and Williams [12]. Sensitivities and specificities were calculated using combined data from the six study institutions. The precision of the sensitivity and specificity estimates is shown as exact 95% confidence intervals. An exact test analogous to the McNemar test was used to compare the sensitivities of the BTA *stat* test and cytology for the tumor grades and stages. Data are presented separately for the subjects with a history of BC and those without, because subjects with a history of BC had received treatment and were being monitored for recurrence.

Table 1. Number of subjects by site, patient type, and final diagnosis

	Site code					
	BOC	CON	LEY	MAR	PAG	STE
Type						
No history of BC	9	8	36	36	15	2
History of BC	9	36	23	12	19	35
Total	18	44	59	48	34	37
Final diagnosis[1]						
No BC	3	33	15	34	17	22
BC	15	7	44	14	17	10
Suspicion of BC	0	4	0	0	0	5
Total	18	44	59	48	34	37

[1] Based on cystoscopy or cystoscopy and biopsy.

Results

Two hundred fifty-eight subjects were enrolled in the study at six institutions in Austria, France, Germany, and Italy. The results from 18 subjects were excluded from evaluation due to protocol violations. Of the 240 evaluable subjects, 68 were females, 172 were males, 98% were Caucasians. The mean age of the evaluable subjects was 64 years (range 24–94 years). Table 1 shows that the final diagnoses and subject types were not equally distributed across the six centers. Nine patients had a final diagnosis of 'suspicion' and were therefore excluded from the sensitivity and specificity calculations. The BTA *stat* test results on these 9 patients were: BTA *stat* positive: 2/9 (22%). Bladder wash cytology was performed on 5/9 patients and was negative in all 5 patients. Voided urine cytology was performed on 4/9 patients. The VUC results were: negative: 2/4, atypical: 1/4, and suspicious: 1/4.

Of the 107 subjects with a final diagnosis of BC, 105 had transitional cell carcinoma of the bladder, 1 had adenocarcinoma, and 1 had bladder epidermal carcinoma. Hematuria (gross or microscopic) was the most frequently presenting sign of subjects with no history of BC. Among the subjects with a history of BC, suspicious cystoscopic results and microhematuria were the most common presenting examination results.

The overall sensitivity and specificity of the BTA *stat* test as compared to cytology are presented in table 2. The sensitivity of the BTA *stat* test was significantly higher than that of cytology, especially in low-grade and low-stage tumors (table 3).

Table 2. Sensitivity and specificity by subject type

Subject type	Test	Sensitivity			Specificity		
		n[1]	%	p value	n[2]	%	p value
No history of BC	BTA *stat*	55	73	<0.001	50	52	<0.001
	Cytology	55	33		50	100	
History of BC	BTA *stat*	52	58	0.018	74	72	<0.001
	Cytology	52	33		74	99	
All	BTA *stat*	107	65	<0.001	124	64	<0.001
	Cytology	107	33		124	99	
	BTA *stat* + cytology	107	71	0.463 vs. *stat*	124	64	<0.001 vs. *stat*

[1] Total number of positive final diagnoses of BC.
[2] Total number of negative final diagnoses of BC.

Table 3. Comparison of sensitivities by grade and stage

	Test	Sensitivity			
		n	%	95% CI	p
Grade					
I	BTA *stat*	26	39	20–59	0.005
	Cytology	26	4	0–20	
II	BTA *stat*	45	67	51–80	<0.001
	Cytology	45	20	10–35	
III	BTA *stat*	36	83	67–94	0.267
	Cytology	36	69	52–84	
Stage					
T_a	BTA *stat*	58	53	40–67	<0.001
	Cytology	58	14	6–25	
T_1	BTA *stat*	27	70	50–86	0.166
	Cytology	27	48	29–68	
T_2–T_4	BTA *stat*	17	88	64–99	0.118
	Cytology	17	59	33–82	
T_{is}	BTA *stat*	5	100	48–100	1.00
	Cytology	5	80	28–100	

CI = Confidence interval.

Table 4. BTA *stat* specificity by disease category

Patient type	n[1]	% Specificity	95% CI
No GU disease	89	71	60–80
GU disease	35	46	29–64
Benign renal disease	6	33	4–78
Tumors other than BC[2]	6	17	0.4–64
Urinary tract infection	8	50	16–84
Other GU disease	15	60	32–84
Total	124	64	55–72

GU disease: benign or malignant genitourinary disease other than BC; CI = confidence interval.

[1] Total number of negative final diagnoses of BC.

[2] 3/6 of these are benign bladder tumors: 1 is a hemangioma, 1 is a nephrogenic adenoma, and 1 is a benign papilloma. One is a prostate cancer, 1 is a renal cancer, and 1 is an osteosarcoma.

Of the 124 subjects whose final diagnosis was no BC, 35 were found to have other genitourinary cancers or genitourinary diseases such as renal calculi, urinary tract infection, or benign prostatic hyperplasia. Of these 35 subjects, 19 had a positive BTA *stat* test result (46% specificity) (table 4). Of the 89 subjects with no BC, and no other genitourinary disease, 26 had a positive BTA *stat* test result (71% specificity) (table 4).

Discussion

For many years, researchers have sought a tumor marker for BC. Only recently, however, have technological advances and discoveries in tumor biology revealed markers with practical diagnostic potential. Possible markers under investigation include DNA and genetic anomalies, tumor-suppressor genes, oncogenes, cell receptors, tumor products, tumor proteins, tumor-associated hyaluronic acid, and tumor-associated antigens defined by monoclonal antibodies [13–15]. During this clinical study, the original BTA test and the BTA *stat* test were the only commercially available point-of-care assay kits that can detect tumor-related proteins in a single untreated voided urine sample. The tests detect distinctly different proteins.

In this study, the overall sensitivity (65%) of the BTA *stat* test was considerably greater than that of cytology (33%) ($p < 0.001$).

The sensitivities of cytology in the subjects without and with a history of BC were identical (33%), whereas the sensitivity of the BTA *stat* test in the subjects without history of BC (73%) was higher than that in those with (58%). The specificity of the BTA *stat* test in subjects with a history of BC (72%) was significantly ($p = 0.036$) greater than that in subjects with no history (52%). The differences in sensitivity between the subjects without and with a history of BC possibly are attributable to an unequal distribution of tumor grades in the two subject types.

The BTA *stat* test was significantly more sensitive than cytology for grade I and II tumors ($p < 0.005$) and for stage T_a tumors ($p < 0.001$). The BTA *stat* test and cytology detected 100 and 80% of T_{is} tumors (carcinoma in situ), respectively. However, due to the small sample size (5 subjects), the confidence intervals were wide and the difference in sensitivity was not statistically significant.

The specificity of cytology (99%) was greater than that of the BTA *stat* test (64%) ($p < 0.001$). This is mainly due to the type of patients enrolled and used in the specificity calculations. There were no exclusions made for patients with infections and renal stones. These conditions are clearly stated in the BTA *stat* package insert to cause interference. Although the protocol required it, follow-up information was not available on non-BC patients that tested positive by the BTA *stat* test. Some of these patients had a history of bladder cancer (47%). These should not be considered falsely positive. Given the high tumor

recurrence rate in patients with a history of BC, it is possible that some of these positive tests may indicate early detection of subclinical disease. This is, however, an assumption and further studies with close follow-up are warranted.

The specificity of the BTA *stat* test in subjects with benign or malignant genitourinary diseases other than BC (46%) was lower than that in subjects without such disease (71%). This may be due to human complement factor H, which is similar in structure to the bladder-tumor-associated antigen detected by the BTA *stat* test, entering the bladder as a result of the disease process. Positive BTA *stat* results have been seen in conjunction with stone disease and urinary tract infection, as well as other urinary tract malignancies such as upper tract transitional cell carcinoma and cancer of the kidney and the prostate [8]. It has been shown by RT-PCR that cultured prostate and renal carcinoma cell lines have mRNA for human complement factor-H-related protein(s) [unpubl. results]. It is possible that in the case of these cancers, and because of their location in the urinary tract, the marker expressed by the tumor cells is released directly into the urine.

We conclude from our study that the BTA *stat* test is a sensitive, rapid test for BC that can easily be performed by the urologist or an assistant at the point of care. Although the sensitivity of the test is significantly better than cytology in both monitoring and diagnostic populations, the test should not be used without first ruling out the types of diseases that may cause potential interference such as upper tract abnormalities, genitourinary trauma, and infections. Because of its greater sensitivity, the BTA *stat* test can replace cytology as an adjunct to cystoscopy in the diagnosis and follow-up of patients with BC. The BTA *stat* test appears to be especially useful in the detection of low grade tumors. Our study population did not include enough subjects to draw conclusions regarding the sensitivity of the BTA *stat* test in patients with carcinoma in situ. Carcinoma in situ generally has a worse prognosis than papillary carcinoma [16], and until the BTA *stat* test can be further evaluated, we also recommend that cytology continue to be used to supplement cystoscopy in patients with a history or suspicion of carcinoma in situ.

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