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TOPIC PAPER

The role of BTA *stat* Test in follow-up of patients with bladder cancer: results from FinnBladder studies

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Abstract
Objectives To summarize the results of the FinnBladder studies of the BTA *stat* Test in follow-up of bladder cancer and more importantly to provide guidelines for daily clinical practise.
Methods Voided urine samples of 501 patients were obtained prior to cystoscopy and split for culture, cytology and BTA *stat* testing. The overall sensitivity and specificity for the BTA *stat* Test were calculated, factors interfering with testing and the role of false positive test result were evaluated.
Results Out of 501 patients 133 (26.5%) had a bladder cancer recurrence at cystoscopy, of which BTA *stat* Test detected 71 (53.4%). In the remaining 368 patients, 96 (26.1%) had a positive BTA *stat* Test result. An additional 9 (16.4%) recurrences were detected at further examinations. The overall sensitivities and specificities for the BTA *stat* Test and cytology were 56.0, 19.2 and 85.7%, and 98.3%, respectively. Urine infection and past BCG instillations and present instillations of any type caused false positive test result. Out of 79 patients with positive BTA *stat* Test and negative cystoscopy, 6 (7.6%) had recurrence at next scheduled follow-up cystoscopy.
Conclusions Although BTA *stat* Test cannot replace cystoscopy in the follow-up of patients with bladder cancer, it could replace routine cytology especially in patients with low-grade disease. Test should not be used in patients with urine infection, in those having received BCG, or in those with present instillation of any type. In case of positive test result but negative cystoscopy, urine cytology should be obtained as the first line examination. Positive cytology is the indication for further examinations, whereas patients with negative cytology might wait until the next scheduled cystoscopy.

Keywords Non-invasive bladder cancer · Monitoring · BTA *stat* · Tumor marker

Introduction

Cystoscopy and urine cytology are the standard monitoring tools for superficial bladder cancer. Cytology exhibits variable sensitivity depending on tumor grade with lowest sensitivity for low-grade tumors [1, 2]. Interpretation of urine specimens is highly dependent on the skill of the examiner [2, 3]. Furthermore, the sensitivity of cystoscopy is limited to the tumor that can be visualised and therefore, recurrences in upper tract are often missed. Frequent follow-up cystoscopies are expensive and even with flexible instruments cause some discomfort for the patients. A non-invasive, objective and easy to perform diagnostic test that detects bladder tumors and has a high specificity could improve the follow-up of patients with superficial bladder cancer.

The BTA *stat*® Test (Polymedco Inc., Cortlandt Manor, NY, USA) is a one-step, rapid immunochromatographic assay that detects bladder tumor associated antigen in human urine [4]. Antigen detected by the BTA *stat* Test has been identified as human complement factor-H related protein (hCFHrp). The BTA *stat* Test can be performed at the point of care in 5 min and without pre-treatment of the

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voided urine sample, which is a clear advantage of this test compared to several other markers. The test can be performed on fresh, refrigerated, or frozen single urine voids.

According to the systematic review, the overall sensitivity of the BTA *stat* Test was 70% [5]. Sensitivity has been as high as 80–90% in primary tumors [6, 7], whereas lower sensitivity (54.0%) in recurrent tumors has been reported [8]. Although the specificity of the BTA *stat* Test in healthy individuals has been reported to be as high as 98% [9], it is decreased by a history of a foreign body in the urinary tract, bowel interposition segment, another genitourinary cancer, benign genitourinary conditions such as urinary tract infection, benign prostate hyperplasia (BPH), stone disease, genitourinary trauma and instrumented urinary sample [4, 10].

Although the BTA *stat* Test has been suggested to replace urine cytology and even number of cystoscopies in follow-up for superficial bladder cancer [4, 11], to date, only limited data are available regarding the outcome of patients with a positive test result but negative cystoscopy. Furthermore, no suggestions for the management of these patients have been introduced. This paper summarizes the results of FinnBladder studies evaluating the role of the BTA *stat* Test in the follow-up of patient with bladder cancer [12–14]. Factors interfering with testing are analyzed aiming to clarify the management of patients with a positive test result but negative cystoscopy, and most importantly to provide guidelines for daily clinical practice of this test.

Materials and methods

In 1997, the FinnBladder Group conducted a prospective, multi-center study at 18 medical institutions in Finland. Five hundred and one consecutive patients with a history of transitional cell carcinoma of the urinary bladder, who were under follow-up were recruited in accordance to the Declaration of Helsinki.

In 215 patients (48.3%), the primary tumor was classified as Ta, 171 patients (38.4%) as T1 and seven patients (1.6%) as T2-3. Fifteen patients (3.4%) had carcinoma in situ (CIS) as the primary tumor, whereas classification was unknown in 37 (8.3%) patients. Primary tumors were mostly well or moderately differentiated, in that, 196 (44.0%) were Ta and 192 (43.1%) were T1, whereas 42 (9.4%) were poorly differentiated and in the remaining 15 (3.4%), the grade was unknown. Majority of the patients (327, 66.7%) had no prior history of intravesical instillation treatments (Group A), whereas 97 (19.9%) patients had past (at least 3 months from the last instillation, Group B) and 66 (13.5%) patients had present instillations (Group C). When the effect of intravesical instillation on the test was analyzed, nine patients with missing instillation information and seven patients with urine infection were excluded, as infection was a known factor for false positive testing [4].

Freshly voided urine samples were obtained prior to follow-up cystoscopy and split for culture, cytological analysis and BTA *stat* testing. The BTA *stat* Test was performed by adding five drops of an untreated voided urine sample into the sample well of the disposable test device using the disposable pipette provided, according to the manufacturer's instructions. Five minutes after the addition of urine to the sample well, a qualitative interpretation was performed by a trained nurse. Tumors were graded according to the World Health Organization grading system and staged according to TNM classification at each of the participating hospitals.

Cytology specimens were available for central review in 445 patients and only these cases, i.e., both BTA *stat* Test and review cytology result available, were included in analysis comparing BTA *stat* Test and cytology. Sensitivities and specificities are given with their 95% confidence intervals (CI). The overall sensitivity and specificity were calculated only on those having recurrent tumors detected by routine cystoscopy and on whom proper further examinations were performed, if needed. Patients were chosen for further examinations on the basis of the negative cystoscopy but positive urine cytology or BTA *stat* Test. Proper further examinations were considered to have been done if at least random biopsies, and in the case of negative random biopsies, i.v. urography or renal ultrasound had been performed. The result of the next scheduled follow-up cystoscopy was recorded on those with positive BTA *stat* Test result and negative cystoscopy.

Mc Nemar's test was used to evaluate the statistical significance between BTA *stat* Test and cytology. Sensitivity and specificity were calculated and compared also according to the treatment history. The time since the last instillation and its effect on BTA *stat* Test was analyzed and χ^2 test was used.

Results

Out of the 501 patients (397 men, mean age 69 years, range 28–91; 104 women, mean age 69 years, range 38–92) under follow-up for bladder cancer, 166 (33.1%) had no prior recurrences, 202 patients (40.3%) had a range of 1–3 recurrences, and 131 patients (26.1%) had more than 3 prior recurrences. The mean time from the primary diagnosis was 4.7 years (range 2 months–35 years, median 3 years). In the past instillation group (Group B) including 97 patients, the mean time since last instillation was 31 months (range 4–159, median 24 months), whereas the mean time was 43 days (range 3–90, median 30 days) since the last instillation

in patients with present treatment. In the 42 patients having had past Bacillus Calmette-Guérin (BCG) instillation, the last treatment was given on an average of 18 months (range 4–86, median 14 months) before testing.

One hundred and thirty-three of 501 patients (26.5%) had a recurrence detected by routine cystoscopy, of which the BTA *stat* Test and voided urine cytology analysis detected 53.4% (71 out of 133) and 17.8% (21 out of 117), respectively ($p < 0.001$). The distribution of these recurrent tumors and BTA *stat* Test and cytology results are listed in Table 1.

Ninety-six (27.1%) of the remaining 368 patients without visible tumor at cystoscopy had a positive BTA *stat* Test. Out of the 96 patients, 55 (57.3%) underwent additional examinations; nine (16.4%) of the 55 patients had recurrent tumors, making the total number of patients with recurrence 142 (28.3%). Five of the additional tumors had become visible by the second look cystoscopy, whereas cytology was positive in three out of seven of these nine cases (review cytology not available in two cases). The overall sensitivities and specificities for the BTA *stat* Test and cytology were 56.0 (95% CI, 46.8–64.9), 19.2 (12.7–27.2) and 85.7% (81.1–89.5); and 98.3% (96.0–99.4), respectively ($p < 0.001$). The detection method, location, and stage and grade of the tumors, and the urine cytology results of these nine patients, are listed in Table 2.

Out of the 41 patients who did not undergo additional examinations after having a positive BTA *stat* Test and negative cystoscopic findings, 33 (80.5%) had the result of their next routine cystoscopy available. Thirty (90.9%) were free of recurrence, whereas only three (9.1%) had recurrence. Out of the 46 patients with a positive BTA *stat* Test but without concomitant recurrence at routine cystoscopy or additional examinations, only three (6.5%) had recurrence at the next follow-up cystoscopy.

Four (8.7%) of the 46 patients with false-positive BTA *stat* Test result had urine infections, significantly more than for patients with a true-negative test result (0%, $p < 0.001$). The specificity of this test decreased to 65.3% in patients with present instillations, and to 70.7% in those with past treatments. The difference between those never treated (Group A) and those with present instillations (Group C)

Table 1 Sensitivity of BTA *stat* Test and voided urine cytology by tumor stage and grade

	No. sensitivity/(%) (95% CI)			
	Total no. pts.	BTA *stat*	Cytology	*p*-value[1]
Grade				
1	48	23/47.9 (33.3–62.8)	6/12.5 (4.7–25.2)	<0.001
2	35	21/60.0 (42.1–76.1)	7/20.0 (8.4–36.9)	0.001
3	3	3/100 (29.2–100)	3/100 (29.2–100)	Not calculated
Unknown	32	16/50.0 (31.9–68.1)	5/15.6 (5.3–32.8)	<0.001
Stage				
Ta	54	28/51.9 (37.8–65.7)	8/14.8 (6.6–27.1)	<0.001
T1	20	11/55.0 (31.5–76.9)	4/20.0 (5.7–43.7)	0.008
T2	3	3/100 (29.2–100)	0/0 (0–70.8)	Not calculated
Carcinoma in situ	2	2/100 (15.8–100)	2/100 (15.8–100)	Not calculated
Tumor in situ	6	4/66.7 (22.3–95.7)	2/33.3 (4.3–77.7)	0.50
Unknown	33	15/45.0 (28.1–63.6)	5/15.2 (5.1–31.9)	<0.001
Total	118	63/53.4 (44.0–62.6)	21/17.8 (11.4–25.9)	<0.001

Table 2 Cystoscopy negative tumors detected by the BTA *stat* Test

Case no.	Detection method	Location	Cytology[a]	Stage	Grade
1	Ureteroscopy	Ureter	5	T1	3
2	Next cystoscopy	Bladder	2	Ta	1
3	Next cystoscopy	Bladder	5	CIS	3
4	Next cystoscopy	Bladder	2	Ta	1
5	i.v. Urography	Renal pelvis	2	Ta	1
6	Next cystoscopy	Bladder	1	Ta	1
7	Random biopsy	Bladder	5	CIS	3
8	i.v. Urography	Ureter	NA	CIS	3
9	Next cystoscopy	Bladder	NA	Ta	1

NA review cytology not available

[a] Papanicolaou 1–5

was significant ($p = 0.023$), whereas the notable difference between groups A and B (past treatments) (80.7 vs. 70.7%) quite did not reach the statistical significance ($p = 0.076$), and also the difference between groups B and C was not significant ($p = 0.558$). The sensitivity and specificity of the BTA *stat* Test according to treatment history are summarized in Table 3. The interval between the last instillation and testing was not of statistical significance, although the specificity decreased to 50.0% (18.7–81.3), if there were <3 weeks from the last instillation, compared to that of 69.7% (51.3–84.4), if the interval was more than that ($p = 0.281$). Accordingly, there was no difference in specificity whether the instillation series were completed 6, 9 or 12 months before.

Discussion

This prospective, multi-center study of 510 consecutive patients, of which 163 were treated with intravesical instillations represent the population well, of those being monitored for recurrence of superficial bladder cancer. Although 133 patients were found to have recurrent tumor at routine cystoscopy, additional nine tumors were found by further examinations prompted by a positive BTA *stat* Test. This rate of recurrent tumors undetected by cystoscopy but with positive BTA *stat* Test, equals to about one sixth of these patients. In agreement with what have previously been reported [4–7], the overall sensitivity for BTA *stat* Test was superior to that of voided urine cytology, whereas the latter had better specificity. Specificity of the BTA *stat* Test was decreased by urine infection, past BCG instillations and present instillations of any kind.

Patients with superficial bladder tumors need regular follow-up. Cystoscopy, however, is invasive, associated with patient discomfort, and limited to tumors that can be visualized. Cytology, on the other hand, exhibits variable sensitivity depending on tumor grade with the lowest sensitivity in low grade tumors [1, 2]. In addition, interpretation of a urine specimen being highly dependent on the skill of the examiner and a high inter- and intra-observer variation in sensitivity has been observed [2, 3]. Therefore, all efforts to try to find a non-invasive, low-cost method with high sensitivity for bladder cancer are justified. In addition, this method must have high specificity to avoid unnecessary examinations of patients without the disease. Although nearly 50% of the recurring tumors remained undetected by the BTA *stat* Test in our studies, the BTA *stat* Test and other antigen based tumor markers theoretically have the potential to reveal recurrence outside bladder and to respond to recurrence before detection by cystoscopy. Whether the marker is detecting an earlier recurrence compared to that of cystoscopy cannot be ascertained because a more reliable means of diagnosis does not exist. We found that it is essential for patients with positive marker status but normal cystoscopy will be further examined and hence the true specificity could be determined. Because of protocol violation, only 55 (57.3%) of the 96 patients with a positive test but negative cystoscopy underwent sufficient further examinations. However, 80.5% of the rest had the result of their next routine follow-up cystoscopy available, which reflected the state of the disease.

In one of the first papers of the BTA *stat* Test, Sarosdy et al. [4] found that the sensitivity of BTA *stat* Test for recurrent tumors was 67%, whereas in prospective reports by Pode et al. [7] and Leyh et al. [15], the sensitivities of this test and voided urine cytology for recurrent tumors were 73.7, 22.0 and 58.0%; and 33.0%, respectively. Even when the BTA *stat* Test detected all high grade lesions here, there were only three grade 3 tumors, and therefore no

Table 3 The sensitivity and specificity with 95% confidence intervals of the BTA *stat* Test according to intravesical instillation treatments

Treatment	Patients	BTA *stat* Test		Sensitivity	Specificity	*p* values[a]
	n	+	–	(%)	(%)	
No.	327	98	229	52.9 (43.3–62.5)	80.7 (75.5–85.9)	
Past	97	34	63	54.5 (32.2–75.6)	70.7 (59.0–80.6)	0.076
BCG	42	17	25	50.0 (18.7–81.3)	62.5 (43.7–78.9)	0.036
MMC	28	10	18	75.0 (19.4–99.4)	70.8 (48.9–87.4)	0.284
BCG + IFN	13	4	9	66.7 (9.4–99.2)	80.0 (44.4–97.5)	1.0
Other	14					
Present	66	31	35	82.4 (56.6–96.2)	65.3 (50.4–78.3)	0.023
BCG	33	15	18	88.9 (51.8–99.7)	70.8 (48.9–87.4)	0.284
MMC	11	9	2	100 (29.2–100)	25.0 (3.2–65.1)	0.001
BCG + IFN	12	2	10	50.0 (1.3–98.7)	90.0 (55.5–99.7)	0.692
Other	10					
Total	490	163	327	56.6 (48.5–64.8)	76.4 (71.9–80.8)	

BCG Bacillus Calmette-Guérin, *MMC* Mitomycin C, *IFN* Interferon

Past vs. present treatment: $p = 0.558$

[a] Compared to no treatment

clear conclusion of the BTA *stat* Test on these tumors can be made. Although the overall sensitivity of the BTA *stat* Test for recurrent tumors was not >56.0% in our study, we believe as suggested by Boman et al. [8], that the false-negative results might be due to the small tumor size and the number of low grade, low stage tumors at follow-up cystoscopies, since the sensitivity seems to increase according to these tumor characteristics [6, 8]. Thus, it is possible that if the interval between follow-up cystoscopies is prolonged, the sensitivity of this test could be higher, but the consequence of leaving small recurrent tumors in the bladder for a longer period remains uncertain. Accordingly, these factors might have had some influence on relatively low sensitivity of urine cytology (19.2%) as well, even the samples were reviewed by experienced cytopathologist.

Although the specificity of the BTA *stat* Test is high in healthy individuals [9], more disappointing results have occurred when patients with other genitourinary disease or those being monitored for bladder cancer have been observed, and low specificity of this test has been criticised. In a multicenter US study, the specificities of the test in the patients with BPH, kidney stones and urinary tract infections was 88.5, 50 and 76%, respectively [4]. Specificity of the BTA *stat* Test on patients being monitored for bladder cancer has been 68–78% [4, 7, 15]. Until now, there are only a very few reports on patients with positive BTA *stat* Test and negative cystoscopy. Leyh et al. [15] suggested that the false-positive results on 74 patients with a history of bladder cancer indicated an early detection of subclinical disease. We cannot, however, confirm this as <10% of the patients with positive BTA *stat* Test and negative cystoscopy had recurrence at next scheduled cystoscopy. In contrast to our study, the current status of the disease is usually evaluated only on the basis of cystoscopy and cytology [4, 15]. If we had limited our methods accordingly in the present studies, the specificity of the BTA *stat* Test would have been 75.2%, in accordance with previous reports [4, 7, 16].

In this study, 55 patients with a positive BTA *stat* Test and negative cystoscopy were sufficiently further investigated and recurrence was observed in nine. It is noteworthy that four of these additional nine tumors were poorly differentiated (three CIS and one T1G3), three of which were detected by cytology (cytology was not available in one case), whereas two tumors were found by i.v. urography alone (cases 5 and 8). Although more accurate cystoscopy at first examination might have revealed some of the five papillary lesions, cystoscopy is not 100% sensitive [17]. Although one-sixth of patients with positive BTA *stat* Test but apparently normal bladder at cystoscopy had recurrence, the majority (83.6%) did not. Even if three recurrences at the next scheduled cystoscopy in further examined patients were considered as true positive, the rate for false positive testing was 78.2%. In some patients, the explanation might have been intravesical instillation, coexisting urine infection, BPH or stone disease [4, 10, 14].

As part of the idea for the bladder cancer markers is to decrease the number of invasive follow-up cystoscopies, the test should have specificity high enough to cause no unnecessary costly and invasive (in some cases) further examinations due to false positive test result. The BTA *stat* Test has, as has been shown earlier [5, 9, 10], superior sensitivity to that of cytology detecting both primary and recurrent bladder cancer. Therefore, the usability of this test could be improved by the knowledge of the role of a false positive result. However, until now, no suggestions for the management of patients with positive BTA *stat* Test and negative cystoscopy have been introduced. To our knowledge, this is the largest prospective study of consecutive bladder cancer patients under follow-up, evaluating the BTA *stat* Test.

To avoid false positive test result, the BTA *stat* Test should not be used in patients with conditions potentially interfering with testing such as urinary tract infections, intravesical instillations, BPH and kidney stones, which makes the use of BTA *stat* Test more complicated. If further investigations are planned due to positive testing, urine cytology should be taken as a first line investigation and in the case of positive cytology, i.v. urography and random biopsies are recommended as the second and the third line methods, respectively. Since all subsequent recurrences located in the bladder, that were missed by cytology, were of low grade and low stage, and since they were detected at second look cystoscopy, we suggest to undertake invasive measures only in cases of subsequent positive cytology, and otherwise waiting until the next scheduled cystoscopy. This proposal reduces the number of unnecessary, costly and often invasive further investigations in patients without the disease.

If the goal for the use of a marker is only to detect a high-grade recurrence, the use of urine cytology could be preferred because of its high specificity. However, the BTA *stat* Test seems to have potential not only to detect these aggressive recurrences but also tumors of lower stage and grade. According to our previous finding the BTA *stat* Test seems to have also some prognostic properties, as in patients with positive test result, the time to first recurrence was shorter compared to those with negative testing [18]. This might help in determining individual follow-up policy. This and the effect of prolonged cystoscopy interval on sensitivity of the test remain to be studied. As the BTA *stat* Test missed nearly half of the recurrences, it is clear that this test cannot replace cystoscopy in the follow-up of patients with bladder cancer. However, as the BTA *stat* Test seems to have the potential to detect recurrences that are missed by routine cystoscopy and urine cytology, it

could replace routine cytology especially in patients with low-grade disease.

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Appendix

Member list of The FinnBladder Group is given below.

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