Primerjava občutljivosti urinskih testov UBC ELISA in BTA Trak pri odkrivanju ponovitev tumorjev mehurja

Comparison of the UBC ELISA test and the BTA Trak test for the detection of bladder tumor recurrence

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Izvleček

Namen: *Primerjati natančnost pri odkrivanju ponovitev tumorja mehurja med uveljavljenim testom BTA Trak™ in novejšim testom UBC ELISA™ .*

Metode: *Testiranih je bilo 63 vzorcev urina, ki so bili odvzeti pred kontrolno cistoskopijo. V primeru pozitivnega izvida cistoskopije je bila opravljena operativna odstranitev tumorja. Rezultat izvida vzorca urina je bil ovrednoten glede na rezultat cistoskopije in histološke preiskave.*

Rezultati: *Mediana vrednost UBC je bila pri slabše diferenciranih tumorjih statistično pomembno višja. Mediane vrednosti UBC (µg/L) glede na stadij tumorja so bile: 22.1, 47.5, 95.7 in >150 za pTa, pT1, pT2/3 in CIS. Občutljivost testov UBC in BTA za vse vzorce je znašala 62 % in 90 % (p = 0.0049). Glede na stadije je primerjava občutljivosti med BTA in*

Abstract

Purpose: *To compare the sensitivity of the established BTA Trak™ test with the newer UBC ELISA™ test for the detection of bladder tumor recurrence.*

Methods: *Urine samples from 63 patients were collected prior to cystoscopy. In cases of positive cystoscopy findings, patients were scheduled for TUR and the histologic findings were correlated with the urine test results.*

Results: *There was a statistically significant increase in median UBC value with increasing tumor grade. Median UBC values (µg/L) for tumor stages were: 22.1, 47.5, 95.7 and >150 for pTa, pT1, pT2/3 and CIS, respectively. The overall sensitivity of the UBC and BTA was 62% and 90% (p=0.0049). Comparing different tumor stages, the sensitivity for the UBC vs. BTA was 52% vs.*

UBC pri pTa pokazala 52 % proti 86 % (p = 0.022), pri pT1 56 % proti 100 % (p = 0.029), 83 % za oba testa pri pT2/3 in 100 % pri CIS. Za tumorje nizkega gradusa je bila občutljivost UBC 47 %, BTA 84 % (p = 0.022), za tumorje visokega gradusa pa 75 % in 95 % (p = 0.085). Kombinacija obeh testov v primerjavi z uporabo samo testa BTA občutljivosti ni izboljšala . Specifičnost je na celotnem vzorcu za UBC znašala 71 % in za BTA 50 % (p > 0.1). Izračunana natančnost testa UBC je za preučevani vzorec znašala 65 %, natančnost testa BTA pa 75 %.

Zaključek: *Test UBC ELISA™ ni pokazal boljše občutljivosti v primerjavi s testom BTA Trak™, in sicer predvsem zaradi slabših rezultatov pri bolje diferenciranih tumorjih in tumorjih nižjega stadija.*

86% (p=0.022) in pTa, 56% vs. 100% (p=0.039) in pT1, 83% for both tests in pT2/3 and 100% for both in CIS. In low grade tumors, the sensitivity of the UBC vs. BTA was 47% vs. 84% (p=0.022); in high grade tumors it was 75% vs. 95% (NS - p=0.085). The combination of both tests did not increase sensitivity over BTA alone. Overall specificity for the UBC was 71% and for the BTA 50% (NS). Overall test accuracy for the UBC 65% was and for the BTA 75% (NS).

Conclusion: *The UBC ELISA™ test in its present form did not have better sensitivity than the BTA Trak™ test because of its lower validity in low grade/stage tumors.*

INTRODUCTION

The natural history of endoscopically (transurethrally) resected bladder tumors (pTa and pT1) is a recurrence rate in the first year of up to 70%. This necessitates regular follow-up cystoscopies, which are very demanding for patients and result in low compliance and high costs. To decrease the frequency of cystoscopies, many noninvasive diagnostic tests are under investigation(1) but we do not yet know whether we have a "PSA" for bladder cancer(2). Some tests have been in use for many years, others have come and gone(3). We compared the diagnostic value of two enzyme-linked immunosorbent assay tests (ELISA), the older BTA Trak™ test and the newly developed UBC ELISA™, test for the detection and follow up of bladder tumors.

The BTA test has been used as a bladder tumor marker for some time. It was developed from multiple monoclonal antibodies, raised against urine samples of patients with different stages of transitional cell carcinoma. Based on their ability to bind to plates coated with urine from patients compared to low binding to plates coated with normal urine, monoclonal antibody pairs were selected and incorporated into an ELISA immunoassay. It was later found that the antigen was very similar to complement factor H (a cofactor in the proteolysis of the complement component C3b), so it was named complement factor H related protein (CFHrp). Its role in tumor survival is thought to be in preventing complement mediated tumor cell lysis(4).

The UBC test is relatively newer investigation. Its development was based on studies of the cell cytoskeleton, which contains intermediate filaments composed of, among other elements, cytokeratins. More than 20 cytokeratins have been described in humans. Based on the differential expression of cytokeratins in normal and malignant urothelium, antibodies against their fragments were evaluated for detecting bladder tumors and different tests were developed. The UBC test detects urinary fragments of cytokeratines 8 and 18(5).

MATERIAL AND METHODS

Patients

Urine samples were collected at the Department of Urology, University Clinical Centre, Maribor. Patients were prospectively enrolled during times

when the investigator was available. The study included 63 patients (20 female and 43 male). The mean age was 68 years, range 31 to 91 (50% of patients were between 61 and 76 years). Patients were tested either during evaluation for dysuria or hematuria or during follow up for previously diagnosed and resected bladder tumor. All patients who had undergone instrumentation or intravesical therapy within the previous three months were excluded. All patients underwent cystoscopy in the three days following sample collection and in the case of positive findings were scheduled for TUR, from which the histopathological reports were evaluated. From 63 cystoscopies, 47 necessitated biopsy and in 39 cases the tumour histology proved positive.

Assays

Measurements were performed simultaneously on previously frozen urine samples, thawed according to each test's manufacturers instructions. The measuring equipment used was a properly adjusted Biomedica Open System automated analyser. The ETI BTA Trak™ test is produced by Bard Diagnostic Sciences, Inc, Redmond, USA, and distributed in Europe by Sorin Diagnostics, Saluggia, Italy. The UBC ELISA™ test is produced by IDL Biotech, Bromma, Sweden, and distributed by Biomedica. The analytical range for the BTA Trak™ was 0-100 U/mL and for the UBC ELISA™ 0-150 µg/L. The cut off value for the BTA Trak™ assay was 14 U/mL and for UBC ELISA™ assay 12 µg/L (both as suggested by manufacturers). For both tests, control low and high values were within the expected range for the kit lots.

Statistical methods

To compare the results of each test between different categories of patients, the Mann-Whitney test for two categories and the Kruskal-Wallis test for three categories were used. The significance of any differences between two results were calculated based on a two-sided t test. A p value ≤ 0.05 was considered statistically significant. For both the descriptive statistics and calculations, SPSS 10.0 software for Windows (SPSS Inc.) was used.

RESULTS

The median UBC value for patients with a negative control cystoscopy or benign histology was 3.8 µg/L and was significantly different from urine from patients with transitional cell carcinoma on histology (34.9 µg/L, p=0.025) (table 1). The median BTA value for patients with negative control cystoscopy or benign histology was 13.3 U/mL and was also significantly different from patients with transitional cell carcinoma (median ≥100 U/mL, p<0.0001) (table 2).

There was a statistically significant increase in median UBC value between low and high grade tumors. With increasing tumor grade, median UBC value increased (pTa 22.1 µg/L, pT1 47.5 µg/L, pT2/pT3 95.7 µg/L, CIS 150 µg/L) (table 3). The median BTA value for all tumours was ≥100 U/mL (table 4).

A comparison of the sensitivity of the BTA with that of the UBC is given in detail in table 5.

For low grade and low stage tumors the BTA consistently proved to be more sensitive than the UBC.

Table 1. *UBC ELISA™ values (µg/L) by disease category. a p = 0.025, Mann-Whitney's test*

	N	Median	Interquartile range	Mean	SD
Transitional cell ca on histology	39	34.9	3 – 115	56.1	58
No tumor or histology negative	24	3.8	1.2 – 16.5	23	43

Table 2. *BTA Trak™ values (U/mL) by disease category. a p < 0.0001, Mann-Whitney's test*

	N	Median	Interquartile range	Mean	SD
Transitional cell ca on histology	39	≥100	52.8 – ≥100	78.5	34.5
No tumor or histology negative	24	13.3	4.9 – 49.4	31.0	37.9

The sensitivity of the UBC ELISA™ and BTA Trak™ combined was almost the same as that for the BTA alone (92% vs 90%).

The overall specificity and predictive value of both tests are given in table 6. The UBC had much better specificity (71% vs 50%). Probably because of the relatively low number of cases, this difference was not statistically significant. Overall test accuracy was 65% for the UBC and 75% for the BTA.

DISCUSSION

In order to reduce the number of follow up cystoscopies, the sensitivity of tests used for patients with treated bladder cancer is arguably the most important criterion. High test sensitivity means a low number of false negatives, which implies that patients with negative test results are almost certainly free of disease (recurrence). High sensitivity incorporates some decrease in specificity, which

Table 3. *UBC ELISA™ test values (µg/L) for bladder tumor patients by stage and grade.*

	N	Median	Interquartile range	Mean	SD	p value (Kruskal-Wallis test)
All tumors	39	34.9	3 - 115	56.1	58.2	
pTa	21	22.1	2.5 – 74.1	40.9	50.2	-
pT1	9	47.5	2.3 – 70.4	41.0	41.2	-
pT2/pT3	6	95.7	13.2 - 150	84.9	72.5	0.37
CIS	3	150	150 – 150	150	0	
low grade	19	12.0	3 – 72.3	34.2	44.4	-
high grade	20	61.8	6.4 – 150	76.9	63	0.05

Table 4. *BTA Trak™ test values (U/mL) for bladder tumor patients by stage and grade.*

	N	Median	Interquartile range	Mean	SD	p value (Kruskal – Wallis test)
All tumors	39	≥100	52.8 - ≥100	79	34.5	
pTa	21	≥100	42.3 – ≥100	73.2	36.9	-
pT1	9	≥100	50.7 – ≥100	80.2	31.8	-
pT2/pT3	6	≥100	76 – ≥100	84.0	39.1	0.66
CIS	3	≥100	≥100 - ≥100	100	0	
low grade	19	≥100	43.2 – ≥100	73.4	37.6	-
high grade	20	≥100	73.2 – ≥100	83.4	31.6	0.31

Table 5. *Comparison of the sensitivity (95% CI) obtained with the BTA Trak™ and UBC ELISA™ and sensitivity obtained with both tests according to stage and grade (in %)*

	N	BTA Trak™	UBC ELISA™	p (BTA vs UBC)	BTA + UBC	p (both vs BTA)
All	39	90(80-100)	62(46-78)	0.0049	92(84-100)	NS(0.76)
pTa	21	86(69-100)	52(29-76)	0.0221	90(77-100)	NS(0.69)
pT1	9	100	56(16-95)	0.0386	100	NS
pT2/pT3	6	83(41-100)	83(41-100)	NS	83(41-100)	NS
CIS	3	100	100	NS	100	NS
low grade	19	84(66-100)	47(23-72)	0.0217	89(74-100)	NS(0.65)
high grade	20	95(85-100)	75(54-96)	NS(0.0854)	95(85-100)	NS

Table 6. *Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall test accuracy of the BTA Trak™ and UBC ELISA™ tests*

	BTA	BTA	UBC	UBC	p
Sensitivity	35/39	90 %	24/39	62 %	0.0049
Specificity	12/24	50 %	17/24	71 %	0.1435
PPV	35/47	75 %	24/31	77 %	0.8406
NPV	12/16	75 %	17/32	53 %	0.1487
Overall test accuracy	47/63	75 %	41/63	65 %	0.2230

means more false positives, but because confirmatory cystoscopy is routinely scheduled this is not of high importance. Being spared cystoscopy is a relief for the patient involved. For this reason we focused on comparing the sensitivities of the two tests.

It is understandable that relatively fewer studies on the UBC ELISA™ test are available in comparison to the BTA Trak™, because the latter has been on market for many years now. Some studies defined their own, higher cut off values, based on a 95% specificity and tested on their population (5). Since this higher cut off further reduced sensitivity and sensitivity was our main interest, we used the lower cut off value (12 µg/L) proposed by manufacturer.

The sensitivity of the UBC ELISA™ in our study (62%, 95%CI 46-78) is almost exactly the same as that reported in the study of Mian et al. (6), which was 64.8%. Our results also correlate well with those of Mian et al. for histologic stages. Specifically, our values for Ta and T1 were 52% and 56% and theirs were 62% and 53%; for T2 tumours or higher our figure was 83%, which is similar to their figure of 80%. We used new WHO terminology for histologic grades(7) which separated tumours only into low and high grades (which was at the time of the study and still is the standard used by the Maribor Department of Pathology). Mian et al (6), however, used three grades. Our sensitivities were 47% for low grade tumors and 75% for high grade tumors, compared to figures of 66%, 60% and 69% in their study.

The sensitivity of BTA Trak™ test has been studied many times and the results collected by Malkowicz (4). It is surprising that our sensitivity (90%) was better than figures from some other studies (range: 66-78%) (8)(4). We believe this can be explained by our lower cut off level of 14 U/mL, which resulted in lower specificity. Specificity in our study was

much lower (50%) than in the studies reported by Malkowicz (4), such as the study of Thomas et al. (8) which put the figure at 69%. However, a recent report by Khaled et al. (9) found a 96% sensitivity for the BTA Trak test for transitional cell carcinoma, which agrees with our results.

In the current study, the BTA Trak™ test was significantly more sensitive than the UBC ELISA™ test (+28%). We used ROC curves based on the same data to test other cut off values for the UBC ELISA™ test and did not find a value that would improve this test's results. Additionally, combining the UBC and BTA was not better than using the BTA alone, a finding that was supported by the multivariant logistic regression model we developed and by a comparison of the sensitivities for different histological stages and grades (data not shown).

CONCLUSIONS

In our hands the new test UBC ELISA™ test in its present format is not more sensitive in detecting the recurrence of bladder tumor (potentially reducing the number of follow up cystoscopies) than the BTA Trak™ test. A combination of the two tests is likewise not better than using the BTA Trak™ alone. The high sensitivity found in our study for the BTA Trak™ is promising and could, perhaps in combination with tests yet to be developed, help reduce the number of follow up cystoscopies for patients with resected low grade TCC.

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