The BTA-test is an agglutination assay that qualitatively detects the presence of complexes of basement membrane within the urine of patients with bladder cancer. However, the significance of BTA test in other urothelial tumours has not been extensively studied. Therefore, in the present research, we examined the validity of BTA-test in patients suffering from ureteral or renal pelvis cancer, and compare results with urincitology.

35 patients were included in this study (13 with ureteral and 22 with renal pelvis cancer). BTA-test and urincitology in each patient was performed on two urine specimens: morning voiding urine and separate urine. Separate urine was obtained by means of ureteral catheter. The results of BTA test were compared with urine cytology. All patients underwent routine clinical examination (biochemical analysis, ultrasonography, i.v. urography and retrograde urography). The presence of urothelial carcinoma was histopathologically proved in all cases. 35 patients with renal calculosis without malignancy were control group. Separate and voided urine was taken for examination with BTA test and urincitology from all these patients.

BTA test in urine specimen obtained by ureteral catheter were positive in 22 (62.9%) patients and in complete urine in 18 (51.4%) patients. Urine cytology was positive in 20 (57.1%) separate urine specimen and in complete urine in 17 (48.6%) patients. Characteristics of each patient, clinical findings and pathohistology findings take place in statistic evaluation of the results.

The sensitivity of BTA test depends directly on histopathology characteristics of tumours. Tumours with high grade and high stage more often have positive results of test and urincitology. The test had the highest sensitivity among the group of high risk patients, with T2, T3, T4 stadium, 77.8% in separate urine and 61.11% in voided urine with specificity of 80% for separate urine and 85.71% for voided urine. Sensitivity for cytology in the same group was 72.22% for separate and 55.56% for voided urine, with specificity of 97.14% in both specimens.

Based on the obtained results, we can conclude that simple and rapid BTA test can have significant position in the diagnostics of upper urinary tract tumours, but we still have to search for an ideal tumour marker for transitional cell carcinoma of upper urinary tract.

Key words: upper urinary tract carcinoma, BTA test

INTRODUCTION

At the beginning of 90s, more significant researches were initiated concerning potentials of the BTA test (the bladder tumour antigen) in diagnostics of transitional cell urinary bladder carcinoma. The increased number of studies and greater number of patients involved in researches provided more reliable results of the test’s values.

American authors were first to publish their results, but soon after that joint studies of groups of au-thors started to appear1,2,3. All the studies compared sensitivity and specificity of the test with urinary cytology, controlling the obtained values with pathological-histological results. The obtained values of almost all studies showed higher sensitivity of Bard BTA test, especially with tumours in lower stage and grade and slightly lower specificity of urinary cytology.

The principle of this procedure is based on the fact that during their expansive growth, urothelial tumours produce proteolytic enzymes that disintegrate a basal membrane into fragments. Polypeptidic fragments of the damaged basal membrane (comprising type IV collagen, laminin, fibronectin and proteoglycan) and tumour proteases are constantly released into urine whereby they mutually interconnect with high affinity, forming a complex of great molecular mass (16-165 kD), which has antigen characteristics.
The presence of this antigen is detected by latex agglutination test. As the very same epithelia lines the bladder and all the upper urinary tract, it is likely to expect a formation of complexes of basal membranes with tumours of upper urinary tract, and they may be detected by this test, the results of which are available straightaway, and thus may contribute to easier diagnostics of upper urinary tract tumours.

The objective of this study is to analyse positive results of BTA tests in separate urine and voided urine samples with the patients diagnosed with upper urinary tract tumours, and to analyse the relation between the obtained BTA test results and the previous clinical findings and patho-histological characteristics of tumours, as well as to establish the applicability of BTA tests in diagnostics of upper urinary tract tumours by means of determined values of sensitivity and specificity, and to compare them with urinary cytology.

MATERIALS AND METHODS

Material for this analysis were urine specimens from 35 patients (13 women and 22 men) diagnosed with transitional cell carcinoma of upper urinary tract at various stages of the illness. Mean age of patients was 63.4. Twenty two patients had renal pelvis and/or calyx carcinoma and 13 had ureteral carcinoma.

Two urine specimens were taken from each patient – voided urine and separate urine specimens, the latter being obtained by means of ureteral catheter of the affected upper urinary tract. Both samples were analysed by BTA tests and urinary cytology respectively.

The renal calculuses assays were performed on a control group comprising 35 patients of various ages (21 patient with calculi in renal pelvis and 14 with calculi in calyx) and they were prepared to undergo ESWL (extracorporal lithotripsy with shock waves) treatment. Calculi’s sizes varied from 1.5 to 2.5 cm.

Two urine specimens were obtained from the control group patients as well. Before that, the control group patients had been examined by ultrasound, Rtg nat and IVP whereby no malignancy had been detected. Both BTA tests and urinary cytology were performed respectively on each urine specimen obtained from the control group patients.

RESULTS

In the analysed group of 35 patients there were 13 (37.1%) patients with negative BTA results, and 22 (62.9%) with positive BTA results in separate urine specimens. Positive findings of BTA tests in voided urine samples were noted with 17 (48.6%) patients. Positive findings of urinary cytology in separate urine specimens were noted with 20 (57.1%) patients and in voided urine specimens with 16 (45.7%) patients.

Diagnostic accuracy of BTA v.s. Cytology tests in separate urine specimens is lower in the analysed group then in the control group but without statistical significance. Diagnostic accuracy of BTA v.s. Cytology tests in voided urine samples is statistically more significant in the analysed group then in the control group, p (Table 1)

Sensitivity of BTA tests in separate urine specimen is statistically more significant with stage T2 tumours in the analysed group than the sensitivity of cytology, for p. With the other levels of T stages, sensitivity of cytology in separate urine samples is slightly higher but without statistical significance in relation to the Sensitivity of BTA tests in separate urine specimen. Sensitivity of BTA tests in separate urine specimen is higher with tumour grades GI and GII in the analysed group than the sensitivity of cytology, but without statistical significance. With the tumour grade GII, sensitivity of cytology is slightly higher but without statistical significance in relation to the sensitivity of BTA test.

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Sensitivity of BTA tests in separate urine specimen is higher in respect to the level of risk with the low risk analysed group then the sensitivity of cytology, but without statistical significance. Sensitivity of BTA tests in separate urine samples is lower in respect of the risk level with the med. risk analysed group then the sensitivity of cytology, but without statistical significance. With the high risk analysed group the sensitivity of cytology is slightly equal to the sensitivity of BTA tests (Table 2).

Sensitivity of BTA test in voided urine samples is significantly higher with stage T2 tumour in the analysed group then the sensitivity of cytology, for p.

With the other levels of stage T tumours, sensitivity of cytology is slightly higher but without statistical significance in relation to the sensitivity of BTA test.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>BTA AND CYTOLOGY TESTS - DIAGNOSTIC ACCURACY</th>
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</thead>
<tbody>
<tr>
<td>BTA test and cytology</td>
<td>Analysed Group</td>
</tr>
<tr>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>BTA/Cyto separate specimens</td>
<td>85.00%</td>
</tr>
<tr>
<td>BTA/Cyto voided specimens</td>
<td>81.25%</td>
</tr>
</tbody>
</table>
Sensitivity of BTA test in voided urine specimens is lower with grades GI and GIII tumours of the analysed group then the sensitivity of cytology, but without statistical significance. With the other levels of grade GIII tumours, sensitivity of cytology is slightly lower but without statistical significance in relation to the sensitivity of BTA test.

Sensitivity of BTA test in voided urine specimens is lower in respect of the risk level with the low-level and med-level analysed group then the sensitivity of cytology, but without statistical significance. With the high-risk analysed patients, sensitivity of cytology is slightly lower but without statistical significance in relation to the sensitivity of BTA tests (Table 3).

**DISCUSSION**

General characteristics of the analysed group fully correspond to the characteristics of analysed groups of foreign authors. If location of tumours in the analysed group is taken into consideration, statistically significant number of tumours larger than 2cm occurs in an upper third section of a ureter and renal pelvis & calyx system as they are located in that segment in 62.86% cases.

The same reason may be found in a presence (in 34.38% of cases) that is in an absence of a renal passage. If a histological-pathological tumour stage of patients is considered in the analysed group, there are no statistical significant differences in occurrence among tumour stages. Eight (22.86%) of patients had a low-risk level, 9 (25.71%) of patients had a medium risk level and the high risk level was noted with 18 (51.43%) patients, which outnumbered the others, but here were no statistically significant differences between the patients in respect of the risk level.

Direct correlation of stage T stage tumours with results of BTA tests and cytology of urine specimens has been proved in many studies. The greatest number of positive results was found in the highest stage of illness, with sensitivity of cytology ranging from 20% to 67%, and for BTA test even up to 80%. The results of the analysed group correspond to the results published in literature showing statistically significant co-relation between positive results and the level of T stage. Statistically most often positive BTA test in separate urine specimens is found in stage T3 for $y^2=7.926$, $p<0.05$.

The most often negative results of BTA tests in voided urine specimens are found with the statistically significant number of patients in stage Ta. Spirman's R coefficient 0.401 proves a statistically significant direct correlation, for $p$, more frequent negative results of BTA tests correlate with stage Ta; with the rise of a tumour stage, positive results of BTA appear more frequent in voided urine specimens of patients.

Cytology results also show more frequent positive results $u$ stages T3 and T4. The obtained values of sensitivity for BTA tests range from 25.5% for stage Ta up to 63.1% in stage T3, i.e. 77.9% in stage T4. Sensitivity of cytology is within the range of 21.1% in stage Ta up to 67.3% in stage T3, i.e. 77.9% in stage T4.

Depending on patho-histological grade of tumours the statistically most significant positive results of BTA tests and cytology in separate and voided urine specimens respectively of the whole analysed group were found in the G III patients.

### TABLE 2

**SENSITIVITY OF BTA TESTS AND CYTOTOLOGY IN SEPARATE URINE SPECIMENS OF THE ANALYSED GROUP OF PATIENTS IN RESPECT OF CARCINOMA GRADE, T STAGE AND RISK LEVEL**

<table>
<thead>
<tr>
<th>T stage</th>
<th>Analysed group</th>
<th>Signific. BTA/Cyto</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BTA in separate urine specimen</td>
<td>Cyto in separate urine specimen</td>
</tr>
<tr>
<td>Ta</td>
<td>25.5%</td>
<td>5.4%-45.5%</td>
</tr>
<tr>
<td>T1</td>
<td>49.8%</td>
<td>26.7%-72.9%</td>
</tr>
<tr>
<td>T2</td>
<td>56.9%</td>
<td>27.8-85.9%</td>
</tr>
<tr>
<td>T3</td>
<td>62.1%</td>
<td>44.3%-81.9%</td>
</tr>
<tr>
<td>T4</td>
<td>69.1%</td>
<td>31.3%-79.6%</td>
</tr>
<tr>
<td>Grade</td>
<td>BTA in separate urine specimen</td>
<td>Cyto in separate urine specimen</td>
</tr>
<tr>
<td>GI</td>
<td>19.6%</td>
<td>2.1%-37.7%</td>
</tr>
<tr>
<td>GII</td>
<td>55.4%</td>
<td>30%-74.7%</td>
</tr>
<tr>
<td>GIII</td>
<td>65.6%</td>
<td>46.9%-84.3%</td>
</tr>
<tr>
<td>Risk level</td>
<td>BTA in separate urine specimen</td>
<td>Cyto in separate urine specimen</td>
</tr>
<tr>
<td>Low risk</td>
<td>25.5%</td>
<td>5.4%-45.5%</td>
</tr>
<tr>
<td>Med. risk</td>
<td>52.6%</td>
<td>30.6-74.6%</td>
</tr>
<tr>
<td>High risk</td>
<td>65.4%</td>
<td>46.8%-83.9%</td>
</tr>
</tbody>
</table>
When the sensitivity of BTA tests and cytology are concerned there are no statistically significant differences. Sensitivity values of BTA tests range from 19.6% for G1 tumours up to 65.6% for G III tumours while the sensitivity values for cytology range from 14.3% for GI up to 63.6% for G III tumours. Obtained results are in compliance with the sensitivity results published so far for BTA tests and urine cytology depending on tumour grade, ranging from 16-69% for BTA and from 17-58% for urine cytology.

Comparing the results obtained from three groups of patients, classified by the level of risk, it is noticed that the statistically most often positive results of both BTA test and cytology are found with the patients in the analysed group with a high risk level. The lowest sensitivity values for BTA tests are found in the group of patients with the low level of risk being 25.5%, while the highest values are found in the high-risk group (65.4%).

Sensitivity values for cytology are in co-relation with the results of BTA test, lowest values ranging from 21.1% for a low-risk level group up to 65.5% for a group with a high risk level. The results of the sensitivity of BTA tests (64%) and cytology (58%) obtained from the analysed group show slightly better sensitivity values but the same classification of groups as the study prepared by a group of American authors that included 499 subjects.

When diagnostic values of BTA tests and cytology of separate urine samples are analysed, the sensitivity result of testing is 85%. When a prognostic aspect is concerned, a positive predictive value of the testing is 77.27%. Diagnostic accuracy is 77.44%. If diagnostic values of BTA tests and cytology of voided urine specimens are analysed, the sensitivity result is 81.25%, and when a prognostic aspect is concerned, a positive predictive value of the tests is 76.47%, and a negative predictive value being 83.33%. Diagnostic accuracy is 80%. The results show that a combination of BTA tests with cytology provide an increased sensitivity, which is of a particular importance for superficial tumours of low grade, and decreases the frequency of endoscopic testing.

Comparison of patients’ distribution in respect of the results of BTA tests in separate and voided urine specimens proves a high statistically significant direct co-relation, that is, the patients with positive BTA test results in separate urine specimens most often have a positive BTA result in voided urine samples too. The obtained sensitivity values of BTA tests in separate urine specimens range of 25.5% - 65.4% and BTA tests in total urine samples of 26.6% - 62.9%, as well as the specificity of 80% that is of 85.7%, also give proof that the assays of any of these two urine specimens have almost identical diagnostic accuracy, in which case the invasive method of urine sampling by means of the insertion of a ureteral catheter through the urinary bladder may be avoided, while the BTA test is characterised as a quick and simple detection method of the upper urothelial tumours.

Specificity of BTA tests was 80% in separate urine specimens and 85.7% in voided urine specimens in contrast to 97.14% for cytology in both specimens. The obtained specificity of a BTA test in the control group was slightly lower but without significant difference in rela-
tion to the results published in literature up to the present, being around 90%2,6,11. If diagnostic values of BTA test and cytology are analysed together in separate urine specimens, specificity is 100%. When a prognostic aspect is concerned, a positive predictive value of these tests is 100%, while the negative predictive value is 82.35%.

Diagnostic accuracy is 82.86%. If diagnostic values of BTA test and cytology are analysed in voided urine specimens, specificity is 73.3%. When a prognostic aspect is concerned, a positive predictive value of these tests is 11.11%, while the negative predictive value is 84.62%. Diagnostic accuracy is 65.71%.

CONCLUSION

The occurrence of positive results of BTA tests in separate and void urine samples show a highly significant direct co-relation with stage T, grade and level of risk of patients with tumours, i.e. a probability of positive findings increases with the rise of the stage and grade of a tumour as well as a level of risk.

Distribution of patients in relation to the findings of BTA tests in separate and voided urine specimens show a highly statistically significant direct correlation, that is, the patients with positive results of BTA in separate urine specimens most often have a positive result of BTA in voided urine specimens as well.

The obtained sensitivity values of BTA tests ranging from 26.6% to 76.5%, and specificity values being 85.7% in relation to the sensitivity values of cytology ranging from 14.3% to 72.1%, and specificity being 97.14%, indicate that BARD BTA test may be successfully used in diagnostics of upper urothelial carcinoma, but an ideal marker for tumours still remains to be discovered.

SUMMARY

PROCENA VREDNOSTI BARD BTA TESTA U DJAGNOSTICI TUMORA GORNJEG UROTELIJUMA

Od 1994. godine objavljeno je više studija koje su procenjivale vrednost tumorskog markera BTA (antigen tumora mokračne bešike) u dijagnostici tumora mokračne bešike. Princip ove procedure zasniva se na činjenici da tumori urotelja u toku svog ekspanzivnog rasta produkuju proteolitičke enzime koji razgradjuju bazalnu membranu u fragmente.

Polipeptidi fragmenti otečene bazalne membrane (koje čine tip IV kolagen, laminin, fibronektin i proteoglikani) i tumorske proteze konstantno se oslobađaju u urin i pri tom medijusobno vezuju sa velikim afinitetom formirajući kompleks velike molekularne mase (16-165 kD) koji ima antigena svojstva. Prisustvo ovog antigena se dedektuje testom lateks aglutinacije.

S obzirom da isti epitel oblaže mokračnu bešiku i ceo gornji urinarni trakt za očekivati je da se kompleks bazalne membrane formiraju i kod tumora gornjeg urotelijuma, te da se mogu detektovati ovim testom čiji su rezultati dostupni odmah, što bi doprinosilo lakšem dijagnostikuju tumora gornjeg urotelijuma.

Ispitivanjem je obuhvaćeno 35 pacijenata kod kojih je dijagnostikovan tumor gornjih urinarnih puteva. BTA testom i urin citologijom analizirana su po dva uzorka urina. Prvi dobijen kateterizacijom obolele strane urotraka i drugi u vidu izmokrenog urina. Kontrolna grupa su bili pacijenti sa kalkulozom bubrega bez malignih oboljenja. Dobijeni nalazi statistički su obradjeni ukљučujući i nalaze kliničkih ispitivanja i patohistoloških karakteristika tumora.

Prema dobijenim rezultatima senzitivnost BTA testa direkto zavisi od patohistološkog stadijuma tumora. Najveća senzitivnost test je imao u grupi pacijenata visokog rizika u stadijumu T2, T3, T4 od 77,8% u separatnom urinu i 61,11% u izmokrenom urinu, sa specifičnošću od 80% odnosno 85.71%.

Senzitivnost citologije u istoj grupi bila je 72,22% separatnog, odnosno 55,56% ukupnog urina sa specifičnošću od 97,14% u oba uzorka. Uvidom u dobijene kompletne rezultate može se reći da BTA test može naći svoje mesto u dijagnostici tumora gornjeg urotelijuma, ali i da se za idealnim tumorskim markerom za tranziciocelularne karcinome i dalje mora tragati.

Ključne reči: gornji urinarni trakt, BTA test

BIBLIOGRAPHY


