

Measurements of Complement Factor H-Related Protein (BTA-TRAK™ Assay) and Nuclear Matrix Protein (NMP22 Assay) – Useful Diagnostic Tools in the Diagnosis of Urinary Bladder Cancer?

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Between 1997 and 2000 we investigated in a prospective study the voided urine samples of all consecutive patients undergoing cystoscopy independent from their clinical background (n = 705) with the BTA-TRAK™ assay (Bard Diagnostics, Redmont, USA) detecting a complement factor H-related protein (CFHRP) and the NMP22 assay (Matritech, Newton, USA) measuring a nuclear matrix protein, which is supposed to be specific for bladder cancer. The individuals were divided into three groups concerning the clinical background: 233 patients had urological diseases, 268 patients had urinary bladder cancer and 150 patients had other urological malignancies. Based on the clinical findings we compared our results with well established diagnostic methods for urinary bladder cancer such as cytology and the detection of hematuria. In addition, we investigated urine samples from 30 healthy individuals and 24 patients with urinary tract infection without performing cystoscopy. Following the recommendations of the European Group on Tumor Markers we used 95% specificity for benign urological diseases and urinary tract infections, which resulted in a sensitivity of 17% for active bladder cancer for the BTA-TRAK™ assay and 31% for NMP22. We compared these results with the detection of hematuria (specificity: 72%) and cytology, which had a sensitivity of 64% and 89%, respectively. Subsequently, we calculated sensitivity and specificity for the detection of relapse of the disease. Again using 95% specificity, in this case for patients with no evidence of disease (NED), in patients with recurrent disease the BTA-TRAK™ assay showed 8% sensitivity as compared to 12% for the NMP22 assay. Due to an insufficient specificity and sensitivity, both tests can neither be clinically useful in screening of high risk patients, nor in primary diagnosis of bladder cancer. They cannot replace neither cystoscopy nor cytology. In the follow-up care more investigations may be necessary to prove the benefit of existing diagnostic strategies for the discrimination between active and inactive bladder cancer. Clin Chem Lab Med 2003; 41(1):104–110

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Abbreviations: BTA, bladder tumor antigen; NMP22, nuclear matrix protein; CFHRP, complement factor-H related protein; NE, no evidence of disease; NuMA, nuclear mitotic apparatus.

Introduction

Bladder cancer is the most common tumor of the deferent urinary tract corresponding to 2–3% of all malignant tumors. The male to female ratio is 3:1. The annual incidence of this disease amounts to approximately 20/100000 inhabitants, the death rate accounts for 5000 individuals per year in Germany. The major problem caused by bladder cancer is the recurrent disease. It is vitally important to detect bladder cancer at an early stage of disease (pTa-pT1 according to TNM staging following the UICC-classification) as once it grows invasive (pT2-4, N1-3, M1) the prognosis of the 5-year survival decreases from 65–79% to 40–0% (1). Between 50 and 80% of the patients with superficial bladder cancer will relapse within the first year (2–4). In up to 80% of patients the most prominent and initial symptom is hematuria (5). In case of suspicious symptoms cystoscopy is performed, which includes exfoliative urinary or irrigation cytology. Currently, for primary diagnosis as well as for follow-up care only the invasive technique of cystoscopy is helpful. Additionally, in most cases patients undergo irrigation cytology to search for superficial and not visible bladder cancer. So, firstly, a non-invasive sensitive method would be desirable to replace cystoscopy and, secondly, a more objective and standardized method is needed instead of the subjective cytology. Over the last years it has been reported (6–9) that the bladder tumor antigen (BTA)-TRAK™ assay as well as the nuclear matrix protein (NMP)22 assay could be clinically useful in mass screening and in primary diagnosis to detect bladder cancer in voided urine. We investigated these two assays to see whether they can replace cystoscopy or cytology, respectively and whether they can improve existing diagnostic strategies.

Materials and Methods

Between 1997 and 2000 we investigated in a prospective study the voided urine samples of 651 consecutive patients undergoing cystoscopy. Clinical diagnosis was unknown at

this time. As a control group we also examined urine of 24 patients with urinary tract infections and 30 healthy individuals, without performing cystoscopy. We divided the collective into seven groups: a) 30 healthy individuals, b) 24 patients with urinary tract infections, c) 233 patients with benign urological diseases (prostatic adenoma, benign prostatic hypertrophy, condyloma, phimosis, incontinence, abscess, renal insufficiency, urethral stricture, urolithiasis, nephrolithiasis) d) 36 patients with urinary bladder cancer at primary diagnosis, e) 134 patients with relapse of the urinary bladder cancer (positive cytology or histology), f) 98 patients with bladder cancer history but at the stage of no evidence of disease (NED) and g) 150 patients with other urological malignancies (111 with prostate cancers, 29 with carcinoma of the kidney, 3 with colorectal cancers, 3 with testicular tumors, 3 with cancers of the deferent urinary tract and 1 with cancer of the vagina). We used the BTA-TRAK™ (Bard Diagnostics, Redmont, USA) and NMP22 (Matritech, Newton, USA) enzyme-linked immunoassays. BTA-TRAK™ detects a complement factor H-related protein (CFH-rP) and the test itself is conducted in a series of small wells (microtiter plates). The BTA test is an immunoassay that uses two monoclonal antibodies to detect bladder tumor antigen in urine samples. One antibody is used to capture the analyte and the second antibody, which is conjugated to colloidal gold, serves as the reporter molecule, allowing the visual detection of the analyte in the urine specimen. The nuclear matrix is a three-dimensional web of RNA and proteins that provides the structural foundation for a cell's nucleus. By serving as an anchoring point for enzymatic machinery, nuclear matrix participates in DNA replication, transcription, RNA processing and gene expression. NMPs undergo alteration at different stages of cell replication. Various NMPs are organ-specific. NMP22 is found in human epithelial cells and is a component of a large complex known as the nuclear mitotic apparatus (NuMA), the function of which seems to be related to the distribution of genetic material during mitosis.

NuMA is known to be concentrated in urothelial cancer cells with levels up to 25 times those of normal cells. Given this, NMP22 was recognized as a potential urothelium-specific cancer marker. The NMP22 analyte is detected in a stabilized, voided urine sample using an enzyme immunoassay in microplate format. Calibrators, controls and patients' specimens are detected in the microtiter plate using two monoclonal antibodies specific for the NMP22 protein. Quantitative results are reported as units of NMP22 per milliliter (U/ml).

Results

Methodological observations

The BTA-TRAK™ test method was quite satisfactory with an intraassay variation of 6% (n = 8) and an interassay variation between 4 and 18% (n = 5) for reagent kit controls. We also found acceptable results for NMP22 with an intraassay variation of 11% (n = 8) and an interassay variation of 8.9% (n = 8) also for reagent kit controls.

Clinical findings

Distribution of bladder cancer patients with regard to grade and stage

In the active bladder cancer collective of 170 patients we distinguished between primary diagnosis (36 patients) and recurrent disease (134 patients). By subclassifying these groups into grades G1-3 we found 50% (13/26) of the patients with a low differentiation grade (G3) in the primary diagnosis category and only 28%

Table 1 Distribution of the values for BTA-TRAK™ and NMP22.

Marker	Group	n	Median	95% percentile	Range	Positive results [%] Manufacturer's cut-off	
						true positive	false positive
BTA-TRAK [U/ml]	Healthy individuals	30	2.3	4.3	0.4–4.5		0
	Urinary tract infection	24	95.8	1188	2.8–1863		75
	Benign urological diseases	233	10.2	1295	1.0–9370		43
	Bladder cancer primary diagnosis	36	131.0	8551	1.0–12318	78	
	Bladder cancer relapse	134	31.3	1866	1.0–10842	48	
	Bladder cancer NED	98	10.3	1600	0.9–3300	59	
						Cut-off BTA-TRAK: 14 U/ml	
NMP22 [U/ml]	Healthy individuals	30	4.2	7.72	2.1–8.6		0
	Urinary tract infection	24	10.5	87.4	0.01–206		54
	Benign urological diseases	233	5.3	71.5	0.001–650		28
	Bladder cancer primary diagnosis	36	13.7	761	1.4–826	50	
	Bladder cancer relapse	134	5.1	220	0.01–898	28	
	Bladder cancer NED	98	4.1	72.6	0.01–622		22
						Cut-off NMP22: 10 U/ml	

(21/76) in the recurrent disease category. For primary diagnosis, thus, high or moderate differentiation was shown to be less prevalent (G1–23%, G2–27%) than with recurrent disease (G1–24%, G2–49%). Tumor staging resulted in no carcinoma *in situ* present in the primary diagnosis category but 10 present in the relapse category. In both groups most of the patients had stage pTa (primary diagnosis category: 43%, relapse category: 45%). However, 30% of the patients had tumor stage > pT2 at the time of primary diagnosis compared to only 8% at the relapse stage (Table 1).

Both assays showed low values for healthy individuals (BTA-TRAK™: < 4.5 U/ml, NMP22: < 9 U/ml). Patients with urinary tract infection had a high concentration of both analytes. In this group we found values up to approximately 1900 U/ml for BTA; for NMP22 the highest measured value was 206.0 U/ml. There was a wide range

of values in all the groups for the BTA test (1.0–12318.0 U/ml). For NMP22, very high values (>650.0 U/ml) were measured for active bladder cancer at primary diagnosis or progressive disease. The median value for BTA, as an example of the statistical distribution, showed that all the groups investigated had considerably higher values compared to healthy individuals (2.2 U/ml). For NMP22, very high median values, compared to healthy individuals, were observed only in active bladder cancer at primary diagnosis (13.7 U/ml) and in urinary tract infection (10.5 U/ml) (Table 2, Figures 1, 2, 3). Moreover, the BTA-TRAK™ did not show any relationship to pT-stages. We found widely spread values from tumor *in situ* stage to pT4. NMP22 displayed the same pattern with regard to staging with the exception of carcinoma *in situ* where only low values (< 10 U/ml) were observed. Regarding disease grade, the BTA-TRAK™ assay did not reveal any relationship, with high values present in stages G1-3. The NMP22 test showed that all patients with grade G1 had values below 22 U/ml; for grade G2 and G3, the values were widely distributed.

Table 2 Distribution of hematuria, BTA-TRAK™ and NMP22 for urinary bladder cancer at active stage.

		[%] NMP22 +	[%] NMP22 -	[%] BTA- TRAK +	[%] BTA- TRAK -
Hematuria +	Benign urological disease	17	19	28	5
	Active bladder cancer	29	19	46	2
Hematuria -	Benign urological disease	11	56	16	51
	Active bladder cancer	12	40	22	30

Cut-off: NMP22: 10 U/ml,
BTA-TRAK: 14 U/ml

Primary diagnosis

Seventy one percent of the bladder cancer patients were referred to our clinic with hematuria being the principal symptom at primary diagnosis but 72% of our collective had hematuria, but no bladder cancer, when they came to the hospital and thus were shown to be false-positive. Also, 34% (69/203) of the patients with benign urological diseases had hematuria. In 89% of the patients cytology was positive at the time of primary diagnosis and in 52% at the relapse stage. Furthermore, we compared the sensitivity of hematuria with the sensitivity of both assays for the detection of the active stage of disease and the specificity in benign urological diseases by using a simple crosslab and the manufacturers' recommended cut-off values (NMP22: 10 U/ml, BTA-TRAK™: 14 U/ml). Forty eight percent of

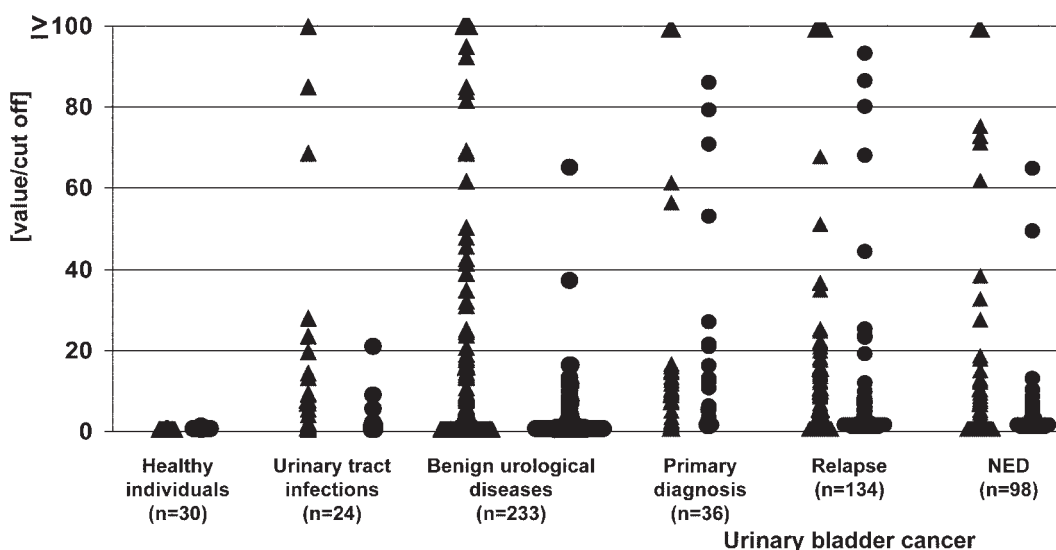


Figure 1 Dot plot: distribution of the values for the BTA-TRAK™ test and the NMP22 test in various groups.

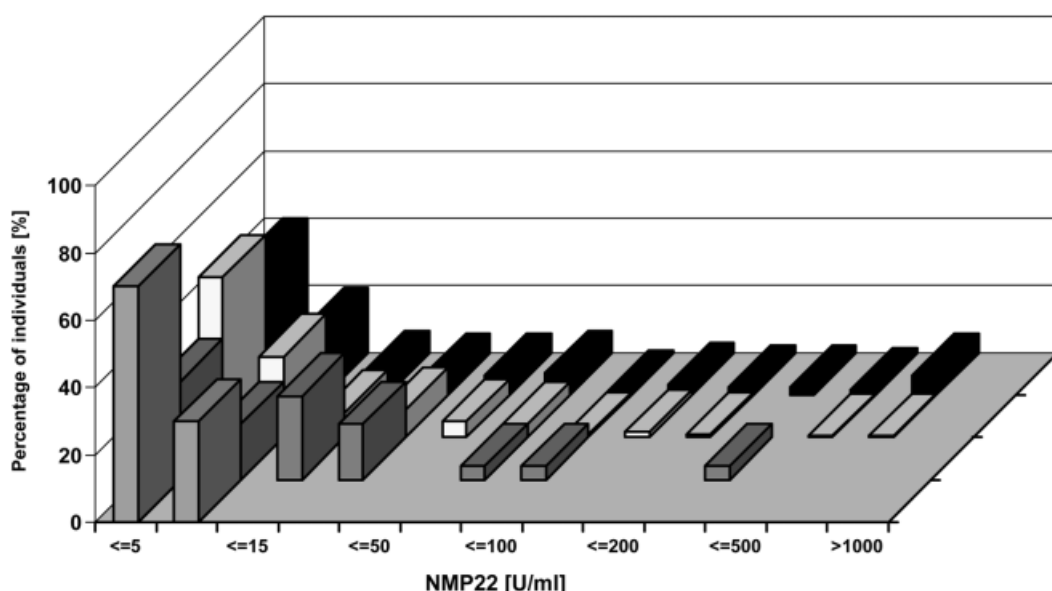


Figure 2 Distribution of the values for NMP22. ■ healthy individuals (n=30); ■ urinary tract infection (n=24); □ benign urological disease (n=233); ■ urinary bladder cancer (n=135).

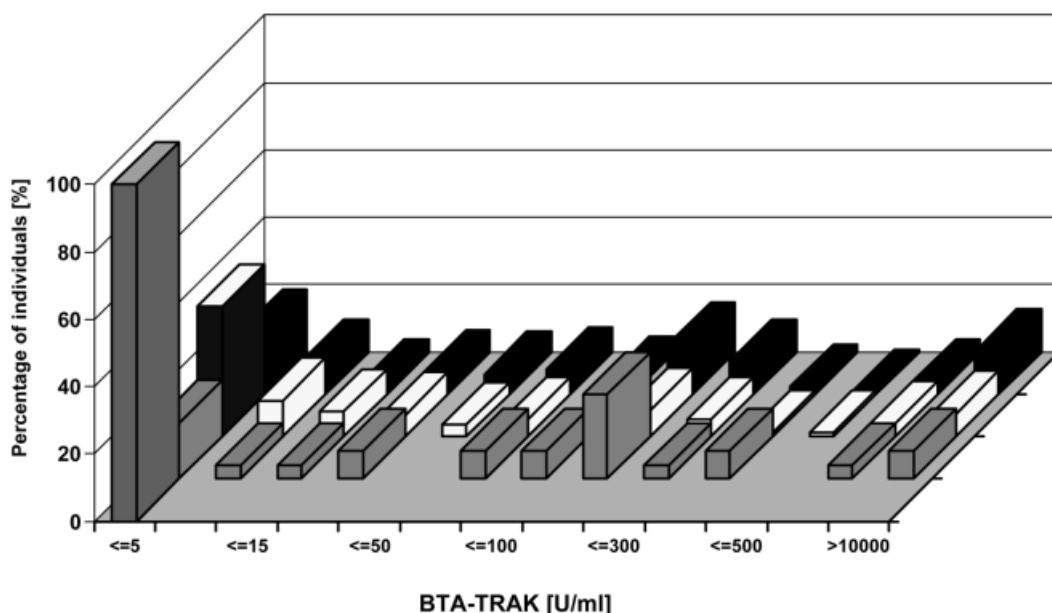


Figure 3 Distribution of the values for BTA-TRAK™. ■ healthy individuals (n=30); ■ urinary tract infection (n=24); □ benign urological disease (n=233); ■ urinary bladder cancer (n=135).

patients with active bladder cancer (either primary diagnosis or relapse) had micro- or macroscopic hematuria at the time of hospitalization. NMP22 result was true-positive in 41% and the BTA-TRAK™ test in 68% of the cases. However, these results also mean that there were 59% false negatives for NMP22 and 32% for BTA-TRAK™, respectively (Table 3). By maintaining this procedure but considering patients with benign urological diseases 36% came to the hospital with hematuria. At this time, NMP22 was false-positive in 28% and BTA-TRAK™ in 44% of the cases (Table 3). Following the guidelines of the European Group on Tumor Markers (10), as is usually done in our studies, we fixed specificity at 95% (6). First we used patients with benign uro-

logical diseases and urinary tract infections as one reference group and found 17% sensitivity for the BTA-TRAK™ test (cut-off: 1200.0 U/ml), corresponding to 31% for the NMP22 test (cut-off: 75 U/ml) (Figure 4).

We then used healthy individuals as reference group. With the same postulation (95% specificity) we obtained a sensitivity of 89% for the BTA-TRAK™ test (cut-off: 4.3 U/ml) compared to 61% for NMP22 test (cut-off: 7.7 U/ml) for patients with bladder cancer at primary diagnosis (Figure 5). In the next step we wanted to find out whether sensitivity increases with pT-staging. We used again the manufacturers' cut-off for BTA-TRAK™ (14 U/ml) and NMP22 (10 U/ml) and calculated the true-positive results. Finally, we also

compared these findings with cytology (only malignant cells were considered as positive results). The BTA-TRAK™ test showed a high sensitivity for carcinoma *in situ* with 80% and for pT1 81% but only 50% of the superficial bladder cancers pTa were detected. Worse sensitivity was found with the NMP22 assay: carcinoma *in situ* 0%, pTa 18%, pT1 65% and > pT2 23%. With cytology we gained quite acceptable results: carcinoma *in situ* 56%, pTa 74%, pT1 67% and > pT2 73% (Table 3). Finally, we took a look at a possible dependency on grade (G1-3). BTA-TRAK™ as well as NMP22 and cytology had an increasing sensitivity for decreasing cell differentiation grade (Table 4). The best sensitivity for high differentiation (G1) was found with cytology (53%).

Follow-up care and relapse

We next investigated whether the assays are able to discriminate between patients with no evidence of dis-

ease and patients with a recurrent bladder cancer in follow-up care. Using 95% specificity for NED-patients, we obtained 8% sensitivity for BTA-TRAK™ corresponding to 12% with NMP22 in patients with relapse (cut-off value: BTA-TRAK™: 1530.0 U/ml, NMP22: 58.0 U/ml).

To show the real power of discrimination between patients with NED and patients with a relapse we used

Table 3 Distribution of BTA-TRAK™, NMP22 and cytology values with regard to stage and grade.

	[%]	[%]	[%]	[%]	[%]	[%]	[%]
Stage/grade	Tis	TA	T1	T≥2	G1	G2	G3
n	10	44	31	13	24	44	34
NMP22 >10 U/ml	0	18	65	23	13	28	52
BTA-TRAK >14 U/ml	80	50	81	77	50	60	82
Cytology +	56	74	67	73	53	78	70

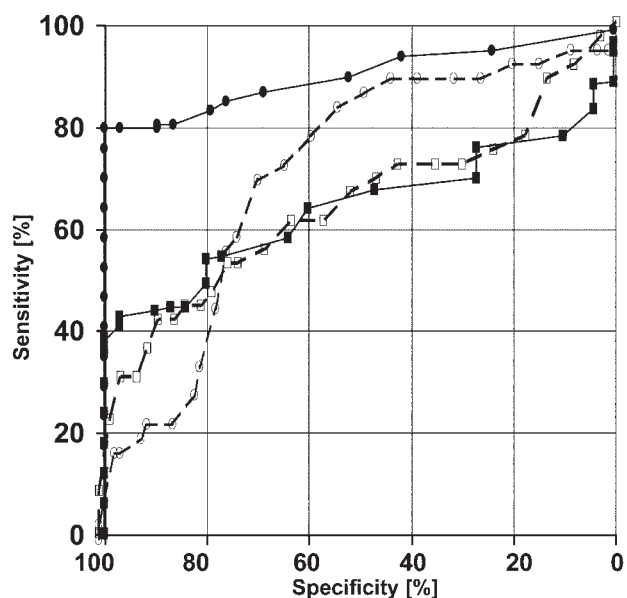


Figure 5 Receiver operating characteristic curves (ROC). Benign urological diseases vs. bladder cancer at primary diagnosis and healthy individuals vs. bladder cancer at primary diagnosis. BTA-TRAK: ● healthy individuals vs. urinary bladder cancer; ○ benign urological disease vs. urinary bladder cancer; ■ healthy individuals vs. urinary bladder cancer; □ benign urological disease vs. urinary bladder cancer.

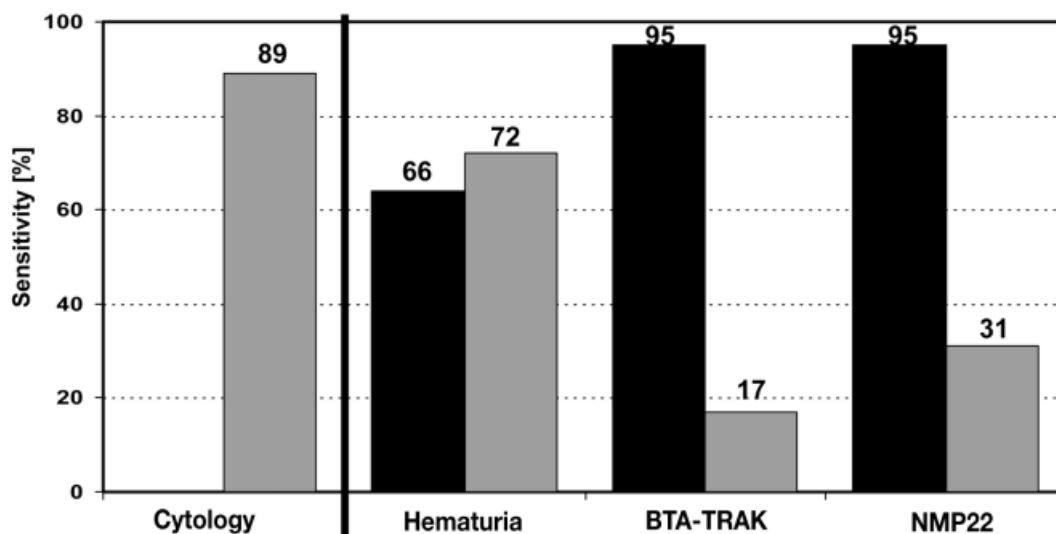


Figure 4 Specificity and sensitivity of hematuria, cytology, BTA-TRAK™ and NMP22 for bladder cancer at primary diagnosis. Specificity vs. benign urological disease and urinary

tract infection, cut-off: BTA-TRAK 1200 U/ml, NMP22 75 U/ml. ■ specificity; □ sensitivity.

the median value of healthy individuals (BTA-TRAK™: 2.3 U/ml, NMP22: 4.2 U/ml). By this means, we found for patients with recurrent disease 92% true positives with the BTA-TRAK™ assay and 63% with NMP22 but still 8% false-negative results for BTA corresponding to 37% with the NMP22 test. For NED patients the BTA-TRAK™ test revealed 85% true-negative results compared to 49% with the NMP22 test, which also means in turn 15% false-positive results were measured with BTA-TRAK™ corresponding to 51% with NMP22.

Discussion

In our investigation we compared the BTA-TRAK™ assay with the NMP22 test both of which are supposed to detect urinary bladder cancer in voided urine. The BTA-TRAK™ test measures a CFHRp and thus revealed – as also reported by other investigators – high values in all the various groups whenever patients presented with the symptom hematuria (11). NMP22 did not show this phenomenon so often, although there have been publications which show an impact on urinary NMP22 (12). Concerning bacterial infections, the BTA test showed widely spread values especially in case of a urinary tract infection with micro- or macroscopic hematuria. In our collective NMP22 was strongly influenced by infectious diseases of all kinds, although other investigating groups suggested excluding all these patients (5, 13). After extensive discussions with the specialists of the Urological Department in our hospital we came to the conclusion that if this was done, then not a lot of patients would be left for the testing, since once a bladder has been treated with bladder cancer therapy (radiation, chemotherapy, transurethral resection, bacillus Calmette-Guerin instillation) then it will no longer be a healthy organ anymore. Almost all of these patients suffer from chronic or active cystitis. Boman *et al.* (14) excluded these groups from their study (comparison of four bladder tumor markers) and thus revealed a better sensitivity (BTA stat 75%, NMP22 65%) than we did (BTA-TRAK™ 17%, NMP22 31%). They used the qualitative BTA stat test to obtain a specificity (75%) that was also used for the other tests to have a comparable situation for the calculation of sensitivity. Due to this specificity, NMP22 had a rather low cut-off value (4 U/ml). On the basis of such influences and the difficult matrix – urine – itself, it is not surprising that a discrimination between the different diseases is impossible independent of whether the sickness is benign or malignant. For these reasons neither of these tests can never be of clinical use in mass screening of high-risk patients. For this indication we would have to postulate 100% specificity and 100% sensitivity for an assay to exclude false-positive, but above all completely exclude false-negative results completely. With such an assumption we would find results for both tests to be of no real value.

In a next step we wanted to show if the assays could bring any help to already existing diagnostic methods in primary diagnosis. In order to be able to compare both tests' findings with the gold standard "histology"

(specificity and sensitivity 100% each) we had two different reference groups at our disposal. Following the guidelines of the European Group on Tumor Markers, we fixed specificity at 95% (cut-off: BTA-TRAK™ 4.3 U/ml, NMP22 7.7 U/ml) for healthy individuals first. The BTA test then had a sensitivity of 89% corresponding to 61% with NMP22. These results seemed to be very promising especially for the BTA assay but we used – in order to be comparable with other investigators – healthy individuals as a reference group (15, 16). This though does not reflect reality, it only shows the daily routine to appear in a more positive light – as patients present with symptoms! For this reason, patients with benign urological diseases and/or urinary tract infections have to be the real reference group to determine specificity for primary diagnosis. Using this as a basis we found a completely different situation. In our collective cytology had 89% sensitivity. Understandably we could not carry out specificity for it, as the urologists do not take irrigation cytology from all patients (benign urological diseases or urinary tract infections). But we determined specificity for hematuria, the BTA-TRAK™ assay and NMP22 test; 66% of the patients had hematuria, the cardinal symptom of bladder cancer, at the time of primary diagnosis, but 34% of the reference group also had hematuria and thus were false positive. By using now 95% specificity we found a sensitivity of 17% for the BTA test and for NMP22 31%. These results can be demonstrated again with receiver operating characteristic (ROC) curves to show the power of discrimination between two comparative groups (Figure 5). Boman *et al.* maintained that pT-stage is different when we compare primary diagnosis to recurrent disease (14). With our investigation we can affirm this statement. In our collective 57% of the patients were staged with > pT1 at primary diagnosis compared to 41% at recurrence. This fact can be expected if patients have regular follow-up care (*e.g.* every 3 months). In our study we could not find any dependency on pT-stage for the BTA-TRAK™ test with high values for all stages. NMP22 showed the same pattern for the distribution of the values with the exception of carcinoma *in situ* with only low values (< 10 U/ml). It is desirable that a diagnostic method should detect bladder cancer at an early stage of disease before it grows invasive. Therefore, we calculated sensitivity (true positive results) for the BTA-TRAK™ test, NMP22 and cytology in dependency on pT-staging for active bladder cancer (primary diagnosis and recurrent disease). We used the manufacturers' cut-off (BTA-TRAK™: 14 U/ml; NMP22: 10 U/ml). NMP22 had the worst sensitivity for low stages with 0% for carcinoma *in situ* and 18% for pTa. The BTA-TRAK™ test was superior with 50% for pTa and the best sensitivity was found with cytology (74% for pTa). Thus, we may ascertain as have other investigators (14) that both assays are not useful for the detection of early pT-stages for bladder cancer. Too many false-negative results would be measured. On this account, neither the BTA-TRAK™ assay nor NMP22 can replace cystoscopy or cytology.

Further we tested the two assays as to whether they

are able to distinguish between patients with NED and relapsed disease. Again, we used 95% specificity for NED patients and found a sensitivity for the BTA test of 8% and for NMP22 12% in relapsed disease (cut-off value: BTA-TRAK™: 1530.0 U/ml, NMP22: 58.0 U/ml). As the results were not satisfactory at all, we used the median value of healthy individuals (BTA-TRAK™: 2.3 U/ml, NMP22: 4.2 U/ml) to show specificity and sensitivity for the two assays. As NED patients are free of disease, we have to consider them as healthy individuals. In 92% of patients with relapsed disease, BTA-TRAK™ amounts to true positive results compared to 63% with the NMP22 test. On the other hand, though we had false-positive results in 15% for BTA and in 51% for NMP22 and that in turn could mean that unnecessary cystoscopies are carried out. These results are unsatisfactory and this has also been ascertained by other investigators (9). On the basis of our results, we conclude that there is not much hope that both tests can be applied in the follow-up care of bladder cancer due to the low specificity of the BTA-TRAK™ test (hematuria) and the low sensitivity of NMP22.

Conclusion

Due to an insufficient specificity and sensitivity, both tests cannot be of clinical utility neither in mass screening of high-risk patients nor in primary diagnosis of bladder cancer. They cannot replace neither cystoscopy nor cytology. In the follow-up care more investigations may be necessary to prove a potential benefit in existing diagnostic strategies, although we are not really hopeful for it because of too many false-positive results in inactive bladder cancer (NED) and, what is more important, because of false-negative results in the active stage of disease.

References

- Block T, Kriegsmair M, Busch M, Weiss M. Harnblasenkarzinom. Empfehlungen zur Diagnostik, Therapie und Nachsorge, Tumorzentrum München.
- Catalona WJ. Bladder cancer. In: Gillenwater JY, Graychuck YT, Howards SS, Duckett JW, editors. Adult and pediatric urology, 2nd ed. St. Louis: Mosby Year Book 1991, vol 1:1135–84.
- Loening S, Narayana A, Yoder L, Slymen D, Weinstein S, Penick G, *et al.* Factors influencing the recurrence rate of bladder cancer. *J Urol* 1980; 123:29–31.
- Ianari A, Sternberg CN, Rossetti A, Van Rijn A, Deidda A, Giannerelli D, *et al.* Results of Bard BTA test in monitoring patients with a history of transitional cell cancer of the bladder. *Urology* 1997; 49:786–9.
- Ponsky LE, Sharma S, Pandrangi L, Kedia S, Nelson D, Agarwal A, *et al.* Screening and monitoring for bladder cancer: refining the use of NMP22. *J Urol* 2001; 166:75–8.
- Sarosdy MF, deVere White RW, Soloway MS, Sheinfeld J, Hudson MA, Schellhammer P F, *et al.* Results of a multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. *J Urol* 1995; 154:379–83.
- Leyh H, Mazeman E, Hall RR, Bennett A. Results of a European multicentre trial comparing the BARD BTA test to urine cytology in patients suspected of having bladder cancer [abstract]. Proceedings of the American Urological Association 1996, abstract no 726.
- The United Kingdom and Eire Bladder Tumor Antigen Study Group. The use of the bladder tumour-associated analyte test to determine the type of cystoscopy in the follow-up of patients with bladder cancer. *Br J Urol* 1997; 79:362–6.
- Murphy WM, Rivera-Ramirez I, Medina CA, Wright NJ, Wajsman Z. The bladder tumor antigen (BTA) test compared to voided urine cytology in the detection of bladder neoplasms. *J Urol* 1997; 158:2102–6.
- Van Dalen A. Quality control and standardization of tumour marker tests. *Tumor Biol* 1993;14:131–5.
- Sözen S, Biri H, Sinik Z, Küpeli B, Alkibay T, Bozkirli I. Comparison of the Nuclear Matrix Protein 22 with voided urine cytology and BTA stat test in the diagnosis of transitional cell carcinoma of the bladder. *Eur Urol* 1999; 36:225–9.
- Atsü N, Ekici S, Öge Ö, Ergen A, Hascelik G, Özen H. False-positive results of the NMP22 test due to hematuria. *J Urol* 2002; 167:555–8.
- Sanchez-Carbayo M, Herrero E, Megias J, Mira A, Soria F. Evaluation of nuclear matrix protein 22 as a tumor marker in the detection of transitional cell carcinoma of the bladder. *Br J Urol Int* 1999; 84:706–13.
- Boman H, Hedelin H, Holmäng S. Four bladder tumor markers have a disappointingly low sensitivity for small size and low grade recurrence. *J Urol* 2002; 167:80–3.
- Johnston B, Morales A, Emerson L, Lundie M. Rapid detection of bladder cancer: a comparative study of point of care tests. *J Urol* 1997; 158: 2098–2101.
- Layfield L. Editorial comment to Sarosdy MF, deVere White RW, Soloway MS, Sheinfeld J, Hudson, MA, Schellhammer PF, *et al.* Results of a multicenter trial using the BTA test to monitor for and diagnose recurrent bladder cancer. *J Urol* 1995; 154:379–84.

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