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·*Clinical Experience*·

Comparisons of voided urine cytology, nuclear matrix protein-22 and bladder tumor associated antigen tests for bladder cancer of geriatric male patients in Taiwan, China

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Abstract

Aim: To compare the results of bladder tumor associated antigen (BTA TRAK), nuclear matrix protein 22 (NMP 22) and voided urine cytology (VUC) in detecting bladder cancer. **Methods:** A total of 135 elderly male and 50 healthy volunteers enrolled in this study were classified into three groups: (i) 93 patients with bladder cancer; (ii) 42 patients with urinary benign conditions; and (iii) 50 healthy volunteers. BTA TRAK and NMP 22 kits were used to detect bladder cancer. Voided urine cytology was used to compare the sensitivity and specificity of the screening tests. **Results:** The sensitivity and specificity of cytology, BTA TRAK and NMP 22 were 24% and 97%, 51% and 73%, 78% and 73%, respectively. The level of NMP 22 increased with tumor grading. The BTA TRAK kit has the lowest sensitivity among the screening tests. The NMP 22 with the best sensitivity can be an adjunct to cytology for evaluating bladder cancer. **Conclusion:** The NMP 22 test has a better correlation with the grading of the bladder cancer than BTA TRAK. As cytology units are typically not available in hospitals or in outpatient clinics, NMP 22 might be a promising tool for screening bladder cancer. *(Asian J Androl 2007 Sep; 9: 711–715)*

Keywords: bladder neoplasm; cytology; bladder tumor associated antigen; nuclear matrix protein 22

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1 Introduction

Bladder cancer is a common cancer in Taiwan, China [1]. It is the fourth most common cancer in men and the eighth in women [2]. Aging male have a high incidence of bladder cancer which is up to five times more

common than that in women and has prompted many urologists to screen this population more vigorously than others. Because bladder cancer appears to be more prevalent and more insidious in this population, testing the efficacy of bladder cancer biomarkers in geriatric patients is imperative [3].

Cystoscopy and cytology are routinely used for evaluation of bladder cancer. Previously, bladder cancer was only detected by cytology and cytoscopy. Both techniques have particular limitations: cytology has low sensitivity, and cystoscopy is a surgical intervention causing patient discomfort. There has been increasing interest in the development of more accurate tests ranging from flow cytometry to monoclonal antibodies and cell surface antigens. Portable screening tests have been developed to detect bladder cancer, and among them, two portable non-invasive diagnostic tools, bladder tumor associated antigen (BTA TRAK) [4] and nuclear matrix protein 22 (NMP 22) [5] are simple and rapid procedures.

Bladder tumor associated antigen detected by the BTA TRAK assay has been identified as a human complement factor H-related protein (hCFHrp) [6]. It is similar, but not identical, to the human complement factor H (hCFH), which plays an important role in the complement system. BTA TRAK is produced by bladder tumor cells in cell cultures and is shed into urine of bladder cancer patients. It can be detected using a BTA TRAK assay. NMP 22 is an RNA protein network forming the structural framework of the nucleus [7]. The matrix provides the functional organization of deoxyribonucleic acid. The proteins bound to the RNA in the matrix are nuclear matrix proteins (NMP). These NMP differ between normal and cancer cell lines. NMP 22 is released from the cell during cell death. Both BTA TRAK and NMP 22 levels are measured in freshly voided spot urine.

By applying an immunoassay principle, BTA TRAK and NMP 22 test kits provide results in a few minutes and in 1 day, respectively. The present study compares the results of the BTA TRAK test and the NMP 22 test in detecting bladder cancer, using a variety of controls, in relation to urinary cytology. The final diagnosis of bladder cancer is confirmed by cytoscopy (voided urine cytology [VUC]) and biopsy.

2 Materials and methods

All studies were approved by the Institutional Review Board for the Protection of Human Subjects and informed consent was obtained from each patient. A total of 135 elderly male and 50 healthy volunteers (range: 60–86 years; mean age: 68 years) were enrolled in this prospective study. Patients were accepted between April 2001 and November 2003. The subjects were classified into three groups: (i) 93 elderly patients with bladder cancer; (ii) 42 patients with urinary benign conditions; and (iii) 50 healthy volunteers. Using intravenous pyelography and ultrasound, all patients with hematuria produced negative urinary tract results. In the study, 45 and 48 patients were examined by BTA TRAK and NMP 22, respectively. No patient management decisions were based on both test results. The thresholds for BTA TRAK and NMP 22 corresponding to 97% specificity were 12 U/mL and 10 U/mL, respectively. The two screening tests were performed by two doctors. The cytology was examined by one pathologist only. Malignant transitional cell is defined by alterations of the ratio of cytoplasm and nucleus and morphology.

A diagnosis of bladder malignancy was made when a bladder tumor was observed during cystoscopic evaluation and was confirmed by a positive histology result from a biopsy sample. Tumors were graded according to the World Health Organization (WHO) grading system [8]: grade I (low), slight anaplasia; grade II (medium), moderate anaplasia; and grade III (high), severe anaplasia. Sensitivity is defined as the ratio of the number of true positive test results to the number of confirmed bladder cancer samples tested. Specificity is defined as the ratio of the number of true negative test results to the number of non-bladder cancer samples tested. Exact 95% confidence intervals (CI) indicate the precisions of sensitivity and specificity.

3 Results

The screening test results showed that the NMP 22 test had the highest sensitivity (Table 1). Cytology had

Table 1. Comparison of the screening tests for bladder cancer. BTA TRAK, bladder tumor associated antigen; NMP22, nuclear matrix protein 22; VUC, voided urine cytology.

	Sensitivity (%)	Specificity (%)
VUC	24	97
BTA TRAK	51	73
NMP 22	78	73

Table 2. The sensitivity of BTA TRAK, NMP 22 and VUC. BTA TRAK, bladder tumor associated antigen; NMP22, nuclear matrix protein 22; VUC, voided urine cytology; CI: confidence intervals.

Grade	BTA TRAK (95% CI)	NMP 22 (95% CI)	VUC	*P* value
I	28.6	70	23.8	< 0.001
II	60	83	0	< 0.001
III	73	91	23.5	< 0.001

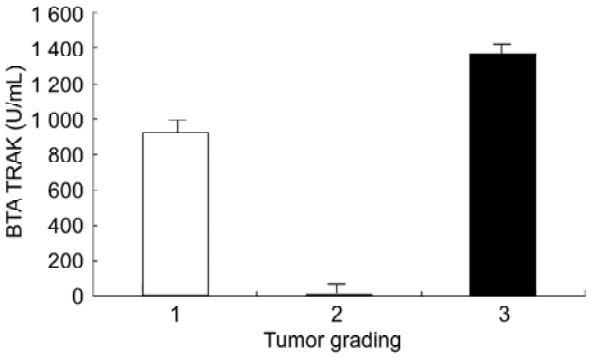

Figure 1. The relationship between bladder tumor associated antigen (BTA TRAK) levels and clinical tumor grade. BTA TRAK levels were significant predictors for high tumor grade.

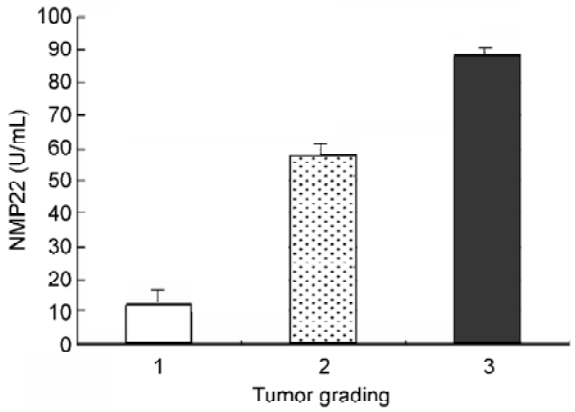

Figure 2. The relationship between the nuclear matrix protein 22 (NMP22) levels and clinical tumor grade. The high tumor grade was significantly correlated with NMP22 levels.

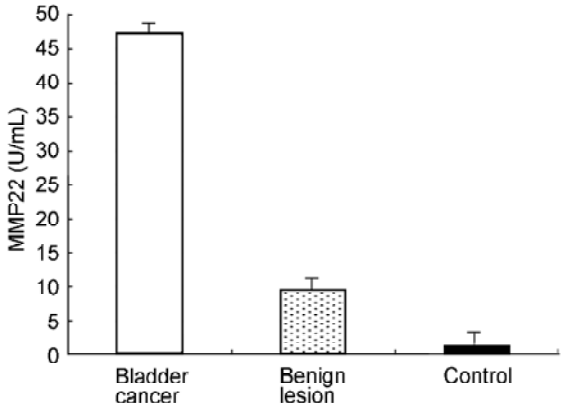

Figure 3. Urinary nuclear matrix protein 22 (NMP22) levels according to transitional cell carcinoma (TCC) stage, disease status and health status.

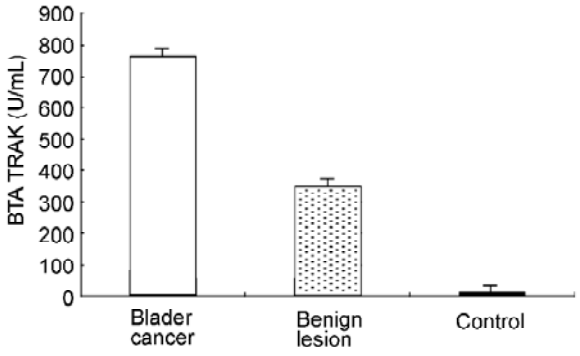

Figure 4. Bladder tumor associated antigen (BTA TRAK) levels according to transitional cell carcinoma (TCC) stage, disease status and health status.

the highest specificity. The specificity of BTA TRAK was similar to that of NMP 22 (Table 1). The sensitivity of both BTA TRAK and NMP 22 increased with bladder tumor grade (Table 2). In BTA TRAK immunoassay, the sensitivity for grade II bladder tumor was 60% (Table 2). The BTA TRAK level did not increase with bladder cancer grading (Figure 1), however the level of NMP 22 increased with bladder tumor grading. The sample sizes of tumor grading for the BTA TRAK screening test were 16 for grade I, 14 for grade II and 15 for grade III, respectively. In contrast, the sample sizes for NMP 22 were 17, 16 and 15, respectively. The two screening tests were performed by two doctors. The specificity of cytology was clearly superior to that of the BTA TRAK

assay. The NMP 22 immunoassay showed that the higher the grade of tumor, the higher the value of NMP 22 (Figure 2). Tukey–Krammer multiple comparison tests showed that the difference between the low grade (grade I) and high grades (grades II and III) was statistically significant ($P < 0.05$). Among the different groups of patients, bladder cancer had the highest NMP 22 and BTA TRAK levels (Figures 3 and 4). The NMP 22 kit test results for the benign lesions were less than the cut-off value of 10 U/mL (Figure 3). The BTA TRAK kit test results for benign lesions were greater than the cut-off value of 14 U/mL (Figure 4).

4 Discussion

Many institutions routinely use screening cystoscopy, urine cytology and random bladder biopsy to find pre-clinical bladder cancer in elderly patients. Considerable efforts have been made to improve the ability of urologists to detect bladder cancer. Urine cytology is the gold standard for non-invasive bladder cancer examination; however, cytologists might not be available in all hospitals. Furthermore, the cytological approach requires training to achieve low sensitivity evaluation and results for superficial tumors [9]. Some tumor markers have been proposed for the detection of bladder tumors, but each test has its drawbacks and limitations. The present study assessed the accuracy of non-invasive tests for detection of bladder cancer. Our analytical results show that NMP 22 is superior to all other tests in overall accuracy. The result is the same as that reported by Sözen *et al.* [10]. Although the sensitivity of the NMP 22 test is higher than that of the BTA TRAK test kit, a 1-day period is required for the results from the NMP 22 test kit. The BTA TRAK assay is a one-step test performed in 5 min with a high sensitivity (57%–83%) [11–14]. Both NMP 22 and BTA TRAK tests are better than VUC for detecting bladder cancer but have significantly low specificity. These results are similar to those reported by Poulakis *et al.* [14]. The NMP 22 test identified a significant difference in sensitivity between patients compared with the BTA TRAK test and cytology. The higher the tumor grade, the higher the sensitivity for NMP22 and BTA TRAK. Sözen *et al.* [10] also found that NMP 22 and BTA TRAK consistently showed superior sensitivities to VUC. There were also significant differences in degree of differentiation: sensitivities of BTA TRAK and NMP 22 tests were higher in undifferentiated tumors [9, 15–17] in contrast to other studies [18, 19]. These differing results could be attributed to the different thresholds used in these studies and differences in the number of patients in each series. These differences might also be attributed to the well-known difficulty in grading tumors [20]. Acceptable specificity and sensitivity of a test depends on its clinical utility, associated intervention and patient-care costs. Tests are useful in a clinical situation if they have a significantly high sensitivity and specificity. Required degrees of sensitivity and specificity also depend on the prevalence of the disease, whether an elderly patient is being screened for the first time, or evaluated for recurrence. If the objective is to ensure an elderly patient from disease and to avoid diagnostic cystoscopy, a test with a high specificity and negative predictive value is the most suitable to ensure that tumor is not missed. However, if the goal is to use cystoscopy to detect hematuria in an elderly patient, the sensitivity and positive predictive value must be high enough to assure no clinically relevant tumors are missed. The future risk of developing bladder cancer developted in elderly patients when a biomarker is expressed during carcinogenesis. Biomarkers expressed early in carcinogenesis will have a poor specificity but might indicate that a patient requires close follow-up in case of clinical recurrence.

The incidence of bladder cancer in patients, especially in aging men, has increased [2]. Those screening programs have not been well established for detecting bladder cancer in elderly patients. The present study analyzed the application of biomarkers, as an adjunct or a supplant to cystoscopy, as a screening test for bladder cancer in elderly patients, and demonstrated that BTA TRAK and NMP 22 are simple and convenient tests for screening bladder cancer. Although neither test can replace invasive cystoscopy, they provide extra information about bladder cancer status. Both BTA TRAK and NMP 22 tests can contribute significantly to bladder tumor diagnoses as an adjunct to cystoscopy. Although photodynamic diagnosis is another option for screening bladder cancer, some kinds of photosensitizers have to be introduced to the bladder, which means that the test is not as convenient as NMP 22 or BTA TRAK [20]. Separation of inflammatory urothelial cells from malignant variations is more reliable by cytology than with either NMP 22 or BTA TRAK tests. The combination of NMP 22, BTA TRAK and VUC can improve the diagnosis of bladder cancer. However, this combination involves an increase in cost. The use of one or several

urinary diagnostic tests to reduce the frequency of cystoscopic follow-up for elderly patients with bladder cancer remains an interesting concept worthy of prospective evaluation.

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