0022-5347/02/1675-2001/0
THE JOURNAL OF UROLOGY®
Vol. 167, 2001–2006, May 2002
Printed in U.S.A.

A COMPARISON OF BTA STAT, HEMOGLOBIN DIPSTICK, TELOMERASE AND VYSIS UROVYSION ASSAYS FOR THE DETECTION OF UROTHELIAL CARCINOMA IN URINE

KEVIN C. HALLING,* WALTER KING,* IRINA A. SOKOLOVA,* R. JEFFREY KARNES, REID G. MEYER, ERIC L. POWELL, THOMAS J. SEBO, JOHN C. CHEVILLE, AMY C. CLAYTON, KELLY L. KRAJNIK, THOMAS A. EBERT, ROBERT E. NELSON, HALEH M. BURKHARDT,* SANJAY RAMAKUMAR, CHRISTOPHER S. STEWART, VERNON S. PANKRATZ, MICHAEL M. LIEBER, MICHAEL L. BLUTE, HORST ZINCKE, STEVEN A. SEELIG,* ROBERT B. JENKINS AND DENNIS J. O'KANE

From the Departments of Laboratory Medicine and Pathology, Urology and Biostatistics, Mayo Clinic and Foundation, Rochester, Minnesota, and Vysis, Inc., Downers Grove, Illinois

ABSTRACT

Purpose: We determine the sensitivity and specificity of various assays for the detection of urothelial carcinoma.

Materials and Methods: A total of 280 voided urine specimens from 265 patients were obtained immediately before cystoscopy for BTA stat, (Bard Diagnostic, Redmond, Washington) hemoglobin dipstick, (Bayer, Elkhart, Indiana) telomerase and UroVysion (Vysis, a wholly owned subsidiary of Abbott Laboratories, Abbott Park, Illinois) analysis.

Results: Of the 265 patients 75 had biopsy proven urothelial carcinoma, and the sensitivity of the assays was determined from these patients. From most sensitive to least sensitive, the overall sensitivity of UroVysion (73 cases), BTA stat (72), hemoglobin dipstick (73) and telomerase (70) was 81%, 78%, 74%, and 46%, respectively. Each of the first 3 tests was statistically significantly more sensitive than the telomerase assay ($p < 0.05$). However, the differences in overall sensitivity of UroVysion, BTA stat and hemoglobin dipstick were not statistically significant. The specificity of the tests was calculated for 80 of the 265 patients in this study who had no history of urothelial carcinoma and negative cystoscopy findings despite common urological complaints. From most specific to least specific, the specificity of UroVysion, telomerase, BTA stat and hemoglobin dipstick was 96%, 91%, 74% and 51%, respectively. UroVysion and telomerase were statistically significantly ($p < 0.01$) more specific than the BTA stat and hemoglobin dipstick assays, and all of the assays were more specific than hemoglobin dipstick testing ($p < 0.001$).

Conclusions: Our study reveals that UroVysion is the most sensitive and specific assay among those tested for the detection of urothelial carcinoma. Telomerase testing had good specificity but poor sensitivity. The BTA stat and hemoglobin dipstick tests had good sensitivity but relatively poor specificity. UroVysion is a promising new assay for the detection of urothelial carcinoma in urine specimens. However, further studies are needed to explore the role of the various assays in the treatment of patients with superficial urothelial carcinoma.

KEY WORDS: bladder neoplasms; carcinoma, transitional cell; in situ hybridization, fluorescence; immunoassay; urine

Cystoscopy and cytology are the standard modalities to monitor tumor recurrence and progression of "superficial" urothelial carcinoma.[1] Numerous studies have demonstrated that cytology has high specificity but poor sensitivity for urothelial carcinoma detection.[2–12] The sensitivity of cytology is lowest for low grade bladder tumors but is suboptimal even for high grade tumors. The poor sensitivity of urine cytology has encouraged the development of numerous tests for the detection of urothelial carcinoma in urine.[13] Some of the newly developed tests for urothelial carcinoma detection, such as BTA stat, NMP22 (Matritech, Newton, Massachusetts) and fibrin degradation products are based on the detection of antigens present at increased levels in the urine of patients with urothelial carcinoma.[13] Other tests such as digital image analysis,[14] fluorescence in situ hybridization (FISH)[12, 15–21] and microsatellite analysis[22–24] are based on the detection of exfoliated urothelial cells that have genetic alterations (for example aneuploidy or loss of heterozygosity) consistent with a diagnosis of urothelial carcinoma.

BTA stat is a qualitative point-of-care Food and Drug Administration (FDA) approved immunoassay for the "bladder tumor antigen" human complement factor H related protein.[6, 10, 25] FISH is a technique that uses fluorescently labeled centromeric and locus specific DNA probes to detect urinary cells with chromosomal abnormalities such as chromosomal gains.[12, 15–21, 26] The finding of such cells in the urine is consistent with a diagnosis of urothelial carcinoma or less likely other genitourinary cancer. We have recently developed a multi-target FISH assay that is tailored for the detection of urothelial carcinoma. This probe set is called Vysis UroVysion and has recently received FDA approval (July 2001) for use in monitoring urothelial carcinoma patients for tumor recurrence. Hemoglobin dipstick testing is used to detect hematuria. Hematuria is a frequent finding in

Accepted for publication November 2, 2001.
* Financial interest and/or other relationship with Vysis, Inc.

***Editor's Note*: This article is the second of 5 published in this issue for which category 1 CME credits can be earned. Instructions for obtaining credits are given with the questions on pages 2178 and 2179.**

patients with urothelial carcinoma but is more frequently associated with numerous nonneoplastic conditions.[27] Only a small percentage (less than 5%) of patients with hematuria have urothelial carcinoma. The telomerase assay is an assay for an enzyme known as telomerase that repairs the ends of telomeres.[28] Telomerase activity is present in a high percentage (greater than 90%) of bladder tumors but not in nonneoplastic bladder tissue.[29–32] In this study we examine the sensitivity and specificity of several assays, including BTA stat, hemoglobin dipstick, telomerase and UroVysion for the detection of urothelial carcinoma in urine.

MATERIALS AND METHODS

Patient population. A total of 280 voided urine specimens from 200 males and 65 females 36 to 94 years old (median age 71, mean 69.7) were obtained immediately before cystoscopy and/or cystoscopic intervention for BTA stat, hemoglobin dipstick, telomerase and UroVysion analysis. The urine specimens were processed within 4 hours and transported at ambient temperature. Of the 265 patients 146 (55%) had a history of urothelial carcinoma and 119 did not. The 119 patients without a history of urothelial carcinoma were being evaluated for a variety of genitourinary symptoms/signs, including obstructive/irritative voiding symptoms, microhematuria and incontinence.

A total of 131 biopsies or surgical resections were performed on 121 of the 265 patients (46%). A single biopsy was performed in 113 patients, 2 biopsies in 6 and 3 biopsies in 2. The surgical pathology diagnoses for these 131 biopsies or resections were classified as positive for urothelial carcinoma in 75 cases (57%), negative for urothelial carcinoma in 47 (36%), atypia/dysplasia in 6 (5%) and metastatic carcinoma in 3 (2%). The pathological T stage (current TNM staging system[33]) for the 75 biopsies/resections was pTa in 38 patients (51%), pTIS in 18 (24%), pT1 in 9 (12%), pT2 in 4 (5%), pT3 in 5 (7%) and pT4 in 1 (1%). Tumor grade was 1 in 12 (16%), 2 in 25 (33%) and 3 in 38 (51%).

Assays. Voided urine specimens for UroVysion analysis were processed and performed as described previously.[12,26] A probe mix consisting of directly labeled probes to the pericentromeric regions of chromosomes 3 (CEP3), 7 (CEP7) and 17 (CEP17), and to the band 9p21 locus (LSI 9p21) was hybridized to the cells on the slide.[12] The CEP3, CEP7, CEP17 and LSI 9p21 probes were labeled with red, green, aqua and gold fluorophores, respectively. The finding of 5 or more urinary cells with gains of 2 or more chromosomes, or 10 or more cells with gains of a single chromosome (for example trisomy 7) on the slide was scored as positive for urothelial carcinoma.[26] Homozygous deletion of 9p21 in greater than 20% of the epithelial cells was also considered a positive result.[26]

BTA stat and hemoglobin dipstick assays were performed according to manufacturer specifications. Telomere repeat amplification protocol assay for telomerase was performed as described previously.[9] In a previous study of telomerase for urothelial carcinoma detection at our institution urine specimens were transported under refrigerated conditions before processing.[9] However, for this study urine specimens were collected and transported according to typical urology laboratory practice (for example at room temperature).

Statistical analyses. The sensitivity of various tests was determined for the 75 patients with biopsy proven urothelial carcinoma. McNemar's test for correlated proportions was used to determine the difference in sensitivity among the various tests.[34] The specificity of the various tests was determined for 80 patients with no history of urothelial carcinoma and negative cystoscopic findings despite possible urological signs and symptoms of bladder malignancy. As not all assay results were available from each specimen, the specificity of the various tests was compared using a chi-square test, after adjusting for intra-individual correlations using generalized estimating equations.[35] This adjustment resulted in tests for differences in specificity that were essentially mixtures of McNemar's test and a standard chi-square test.

RESULTS

The surgical pathology, cystoscopy, BTA stat, hemoglobin dipstick, telomerase and UroVysion findings for the 75 patients with biopsy proven urothelial carcinoma are shown in table 1. BTA stat, hemoglobin dipstick, telomerase and UroVysion analyses were performed on 72, 73, 70 and 73 of these patients, respectively. Representative examples of positive UroVysion results from patients with biopsy proven urothelial carcinoma are shown in the figure.

Overall and stage specific sensitivity. Table 2 shows the overall, stage specific and grade specific sensitivities of BTA stat, hemoglobin dipstick, telomerase and UroVysion analyses. From most sensitive to least sensitive, the overall sensitivity of UroVysion (73 cases), BTA stat (72), hemoglobin dipstick (73) and telomerase (70) was 81%, 78%, 74% and 46%, respectively. The p values for the differences in overall sensitivities of the assays are shown in table 3. Each of the tests was significantly more sensitive than the telomerase assay ($p < 0.01$). However, the differences in overall sensitivity of the assays other than telomerase (for example UroVysion, BTA stat and hemoglobin dipstick) were not statistically significant.

From most sensitive to least sensitive, the sensitivity of UroVysion (37 cases), BTA stat (38), hemoglobin dipstick (38) and telomerase (36) for pTa tumors was 65%, 63%, 55% and 42%, respectively. The sensitivity of UroVysion and BTA stat assays was greater than telomerase for pTa tumors ($p < 0.05$). However, the difference in sensitivity of the other tests for pTa tumors was not statistically significant.

From most sensitive to least sensitive, the sensitivity of UroVysion (17 cases), hemoglobin dipstick (17), BTA stat (17) and telomerase (18) for pTis tumors was 100%, 100%, 94% and 67%, respectively. The UroVysion and hemoglobin dipstick assays were more sensitive than telomerase for pTis tumors ($p < 0.05$). However, the difference in the sensitivity of tests other than telomerase for pTis tumors was not statistically significant.

From most sensitive to least sensitive, the sensitivity of UroVysion (19 cases), BTA stat (17), hemoglobin dipstick (18) and telomerase (16) for pT1-pT4 tumors was 95%, 94%, 89% and 31%, respectively. Each of the assays was more sensitive than telomerase for pT1-pT4 tumors ($p < 0.01$). However, the difference in the sensitivity of tests other than telomerase for pT1-pT4 tumors was not statistically significant.

Grade specific sensitivity. From most sensitive to least sensitive, the sensitivity of BTA stat (12 cases), hemoglobin dipstick (12), UroVysion (11) and telomerase (10) for grade 1 tumors was 50%, 42%, 36% and 30%, respectively. The differences in sensitivity of these assays for grade 1 tumors were not statistically significant due to the small number of cases.

From most sensitive to least sensitive, the sensitivity of UroVysion (25 cases), BTA stat (25), hemoglobin dipstick (25) and telomerase (25) for grade 2 tumors was 76%, 72%, 60% and 48%, respectively. UroVysion was more sensitive than telomerase for the detection of grade 2 tumors ($p < 0.01$). However, the differences in the sensitivity of the other assays for grade 2 tumors were not statistically significant.

From most sensitive to least sensitive, the sensitivity of UroVysion (37 cases), hemoglobin dipstick (36), BTA stat (35) and telomerase (35) for grade 3 tumors was 97%, 97%, 91% and 49%, respectively. Each of the assays was more sensitive than telomerase for the detection of grade 3 tumors ($p < 0.001$). However, the differences in the sensitivity of the