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Evaluation of UroVysion and Cytology for Bladder Cancer Detection: A Study of 1,835 Paired Urine Samples with Clinical and Histological Correlation

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Abstract

Background—Urine cytology has been used for screening of bladder cancer but has been limited by its low sensitivity. UroVysion is a FISH assay that detects common chromosome abnormalities in bladder cancers. The present study evaluates the effectiveness of UroVysion and urine cytology in detecting urothelial cell carcinoma (UCC) in same urine sample.

Methods—1,835 cases with the following criteria were selected: valid results of both UroVysion and cytology from same urine sample; histological and/or cystoscopic follow up within 4 months of the original tests, or at least 3 year clinical follow up information. The results of the UroVysion and cytology were correlated with clinical outcome that was derived from combination of histological, cystoscopic and clinical follow up information.

Results—Of 1,835 cases, 1,045 cases were for surveillance of recurrent UCC, 790 cases were for hematuria. Overall sensitivity, specificity, PPV and NPV in detecting UCC were 61.9%, 89.7%, 53.9% and 92.4%, respectively for UroVysion, and 29.1%, 96.9%, 64.4% and 87.5%, respectively for cytology. The performance of both UroVysion and cytology was generally better in the surveillance population and in samples with high grade UCC. In 95 of 296 cases with atypical cytology that were proven to have UCC, 61 cases, mostly high grade UCC, were positive for UroVysion.

Conclusions—UroVysion was more sensitive than cytology in detecting UCC, but produced more false positive result. Our data suggest that the use of UroVysion as a reflex test following an equivocal cytological diagnosis may play an effective role for UCC detection.

Keywords

UroVysion; Urine Cytology; Bladder Cancers; Urothelial cell carcinoma

Introduction

Bladder cancer is one of the most common cancers in the United States, with an estimated 73,000 new cases and more than 14,000 deaths from bladder cancer in 2012[1]. Around 70% of bladder cancers are non-muscle invasive, most of which are low grade urothelial cell carcinomas (UCC) that will frequently recur with a possibility of grade and stage progression [2, 3]. The recurrent rate of bladder cancers has been reported ranging from 15–

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61% at one year and 31–78% at five year after initial treatment with fulguration or transurethral resection[4, 5]. Therefore, lifelong surveillance of recurrence of bladder cancer constitutes an important part of routine management of patients with bladder cancer. Urine cytology has been widely used for screening for bladder cancers. It is highly specific for the detection of high grade UCC, but suffers from low sensitivity, especially in cases of low grade UCC[6, 7, 8]. The multiprobe fluorescence *in situ* hybridization (FISH) assay, UroVysion (Abbott Molecular Inc., Ill., USA), was developed to be more sensitive for detection of bladder cancers[9]. The assay was designed to detect the common chromosome abnormalities seen in UCC, i.e. polysomy for chromosomes 3, 7, and/or 17, and homozygous loss of 9p21 in urine samples. UroVysion was approved by The US Food and Drug Administration initially for surveillance of recurrent UCC and later was extended to detect bladder cancer in patients with hematuria[9, 10,11]. Although the use of UroVysion as a supplement to urinary cytology has been generally reviewed favorably, the studies have shown great variation in its sensitivity (8 – 100%) and specificity (29 – 100%) in detecting UCC[12–18]. Therefore evidence based studies regarding effectiveness of UroVysion and cytology in detecting UCC are still encouraged[19].

The goal of the present study was to evaluate the sensitivity and specificity of UroVysion and cytology in same urine sample by correlating with histological, cystoscopic and clinical findings and to understand the clinical utility of UroVysion.

Material and Methods

The study cases were identified by retrospective analysis of data from the Department of Pathology and Laboratory Medicine clinical laboratory database and the electronic medical record, with the following criteria: 1) valid results of both UroVysion and cytologic examination from same urine sample, including voided, catheterized, and bladder washing; and 2) histological and/or cystoscopic follow up within 4 months from the original tests, or 3) at least 3 years clinical follow up post laboratory testing. For cytologic studies, urine samples were processed with liquid-based technology (ThinPrep, Hologic, Inc., MA) and read by two Cytopathology board certified pathologists who had a minimum of 10 years of experience in Cytopathology. The original slides were not reviewed and all cytologic diagnoses were obtained from the original cytology reports. For the study, cytologic diagnoses were classified into 3 categories: positive for UCC, atypical urothelial cells (AUC), and negative for malignant cells. The cases with the original diagnosis of “suspicious for UCC” were included in positive for UCC category because the rates of malignancy in these two categories were similar in our institution based on the initial analysis on the data collected (70.8% in positive vs. 69.6% in suspicious category).

UroVysion tests were performed following the manufacturer’s instructions as previously described[14]. A positive test result was defined as one of the following: the presence of 4 or more morphologically abnormal cells of 25 analyzed cells that showed polysomy of 2 or more chromosomes 3, 7, and 17 in the same cell; isolated gain of a single chromosome in 10% or greater cells; homozygous deletion of 9p21 in 12 or more cells. The tests with unsatisfactory or equivocal results were excluded from the study.

To evaluate the performance of UroVysion and cytology in detecting UCC in the different population, the cases were divided into Hematuria group and Surveillance group; the former included the patients with hematuria or symptoms of urinary tract obstruction but no history of UCC, and the later included the patients with history of UCC. The performance of these two tests was also evaluated based on tumor grade. High grade and low grade UCC were determined based on the patients’ history and/or follow up histological diagnoses. Patients with histological diagnosis of “carcinoma in situ” were included in high grade UCC.

The sensitivity, specificity, positive and negative predictive value (PPV and NPV) of UroVysion and cytology in detecting UCC were obtained by comparison to "gold standards", defined as Clinically Positive and Clinically Negative for UCC, which were derived from the combination of the histological, cystoscopic, and clinical follow up information. The criteria for Clinically Positive for UCC included: UCC confirmed by bladder biopsy performed concurrently or within 4 months of the tests; bladder lesions consistent with UCC in cystoscopic examination concurrently or within 4 months of the tests; and UCC diagnosed in outside institutions and referred to our institution for treatment. The criteria for Clinically Negative for UCC included: negative biopsy concurrently and/or within 6 months; or negative cystoscopic findings concurrently and no evidence of UCC within one year; or no evidence of UCC within 3 years by clinical follow up which were obtained from patients' electronic medical charts.

The measures of sensitivity and specificity were compared between UroVysion and cytology using McNemar's chi-square tests [20], and the positive and negative predictive values (PPVs and NPVs) were compared between UroVysion and cytology using Leisenring's technique [21]. Both methods are ideal for measuring agreement between paired data. Statistical analyses were conducted using SAS v9.3 (Cary, NC).

The study was approved by the Institution Review Board of the Medical University of South Carolina.

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Results

Between January 1, 2003 and December 31, 2006, 4,782 urine samples were cytologically examined and all had valid cytologic diagnosis. Two thousand eight hundred and seventy (2,870) UroVysion tests were requested in the same period, and among them, 256 (8.9%) cases were not run due to low cellularity, and 38 (1.3%) cases received inclusive results. A total of 1,835 cases from 957 patients(347 female and 610 male) met the search criteria and had valid results of both cytology and UroVysion from the same urine sample. The source of urine included 1112 void, 558 bladder washing, 98 catheterized, and 67 ileal conduit urine. Seven hundred ninety cases from 652 patients were tested for hematuria/symptoms of urinary tract obstruction, and 1,045 cases from 305 patients were for surveillance of recurrent UCC. A total of 299 cases were determined to be Clinically Positive for UCC, and among them, 281 were confirmed by surgical biopsy performed concurrently and/or within 4 months of the tests; 10 had a bladder mass(es) seen in the concurrent cystoscopy; and 8 were diagnosed in outside institutions and referred to our institution for treatment. There were 1,536 Clinically Negative cases: 245 had negative biopsy results within 6 months; 375 had negative concurrent cystoscopy and no evidence of UCC within one year; and 916 had no evidence of UCC within 3 years based on the information from electronic medical records.

The results of UroVysion tests and cytologic diagnoses of 1,835 cases were listed in Table 1. Among 299 Clinically Positive cases, UroVysion was positive in 185 (61.9%)and cytology was positive in 87 (29.1%) cases; in 1,536 Clinically Negative cases, UroVysion and cytology were negative in 1,378 (89.7%) and 1287 (83.7%) cases, respectively. The false positive and false negative cases were 158 (10.3%) and 114 (38.1%), respectively for UroVysion, and 48 (3.1%) and 117 (39.1%), respectively for cytology. Two hundred ninety-six (16.1%) cases were cytologically diagnosed as AUC, 95 of them (32.1%)were Clinically Positive for UCC, including 52 cases of high grade UCC and 43 cases of low grade UCC. UroVysion was positive in 107 of 296 AUC cases: 61 (57.0%) cases were clinically positive

for UCC, including 48 cases of high grade UCC(Table 2). The sensitivity, specificity, PPV and NPV of UroVysion and cytology in detecting UCC were shown in Table 3. Comparing UroVysion to cytology, UroVysion had higher sensitivity (61.9% vs. 29.1%, p<0.0001) and NPV (92.4% vs. 87.5%, p<0.0001), and cytology had higher specificity (96.9% vs. 89.7%, p<0.0001) and PPV (64.4% vs. 53.9%, p=0.008). When stratified by the reasons for the tests, PPV of both UroVysion and cytology was higher in the surveillance group than in patients with hematuria, and NPV of both tests was higher in the hematuria group than in the surveillance group. For both tests, the sensitivity and specificity values were comparable between the two groups (Table 4). Both tests demonstrated higher sensitivity in detecting high grade UCC when stratified by the tumor grade based on the patients' history and/or follow up histological diagnosis(Table 5).

Discussion

Urine cytology is a noninvasive, inexpensive, and simple way to detect UCC. It is a valuable tool for detecting high grade UCC, especially those flat lesions that are difficult to detect on cystoscopy[22, 23]. However, it suffers from generally low sensitivity with a range of 29% to 84%, which varies according to tumor grade and stage, with the lowest sensitivity seen in non muscle invasive low grade tumors[10, 24, 25]. Accurate statistical analysis on urine cytology is very difficult owing to many factors, including very high recurrent rate and the existence of the cases with cytologic diagnosis of AUC which, by definition, is an indeterminate and belongs to neither positive nor negative for UCC. Our data showed that the overall sensitivity and specificity of cytology in detecting UCC was 29.1% and 96.9%, respectively with AUC cases counted as negative in statistical analysis, and 60.9% and 83.8%, respectively with AUC counted as positive for UCC. While the statistical analyses with AUC as positive and negative are listed in the tables, for convenience, the discussion below will mainly be based on those with AUC counted as negative, consistent with most of previous studies.

In the present study, urine cytology had similar sensitivity and specificity for UCC detection in the patients with surveillance compared to those with hematuria and/or urinary obstruction symptom. As expected, PPV for UCC detection was higher and NPV was lower in surveillance group compared with hematuria group, mostly likely because of the difference in the prevalence of UCC between these two groups. When stratified by tumor grade, the sensitivity was 14.3% for low grade, and 36.8 % for high grade UCC.

The cytologic diagnosis of the low grade UCC remains a challenge because of the lack of significant cytologic atypia and clear diagnostic criteria[26, 27]. Most urine samples with low grade UCC show either no special cytologic features or only architectural abnormalities, i.e., the presence of significant number of clusters of benign appearing and/or minimally atypical urothelial cells, which can also be seen in many other benign conditions, especially urinary tract lithiasis and catheterization. Other challenges in urine cytology include cellular atypia caused by reactive changes, such as those caused by human polyoma viral infection or prior therapy, including chemo-radiation and Bacillus Calmette–Guérin treatment related changes, which can mimic high grade UCC. In addition, the experience and preference of a pathologist in urine cytology would certainly play a role in the outcome of urine cytology[10]. For these reasons, up to one-third of cases are placed in AUC category in routine practice[27, 28]. Our data showed that the malignant rate was 70.4%, 32.1%, and 8.3% in cytological diagnostic category of Positive for UCC, AUC, and Negative for UCC, respectively. The higher malignant rate in AUC category may partially explain the lower sensitivity of urine cytology in this study. Fortunately most low grade UCC have a papillary growth pattern that can be easily detected by cystoscopic examination. Molecular tests, on

the other hand, would be important ancillary tests for detection of high grade UCC, especially the flat lesions that could be missed on cystoscopy.

UroVysion is a multiprobe FISH test performed on urinary cytology preparations, that identifies polysomies of chromosomes 3,7,17 and locus specific 9p21 deletions that are commonly seen in UCC. The test has been frequently studied and widely used in detection of UCC. Most of the studies comparing UroVysion and cytology concluded that UroVysion was superior to cytology in detection of UCC in almost all categories[10–16], although a few showed the opposite results [17, 29]. In a meta-analysis of assay characteristics of UroVysion in comparison to cytology, Hajdinjak reported a pooled sensitivity and specificity of UroVysion were 72 and 83%, as compared with 42 and 96% for cytology[30]. UroVysion, like cytology, has been shown to have a lower sensitivity for the detection of low grade, as compared to high grade UCC. False positive UroVysion results have been attributed to the presence of abundant umbrella cells(could be uniform tetrapoloid cells)and human polyoma viral infection, while false negative results could result from a low grade UCC, paucity of cancer cells, therapy effects, obscuring inflammation, lubricant, and heavy squamous contamination of vaginal origin[31, 32, 33]. The data from our study showed the sensitivity and specificity of UroVysion was 75.6% and 84.8%, respectively for high grade UCC, 40.8 % and 87.8%, respectively for low grade UCC, and 61.9% and 89.7%, respectively for all UCC, which were similar to recently published studies[11, 13]. The variation in sensitivity and specificity of UroVysion in detecting UCC could be at least partially explained by the difference in study population, the sample size, and perhaps significant changes in demography of the patients. In earlier studies, the UroVysion assay was performed mainly on patients with a history of UCC with recurrent disease, including different proportions of invasive, high grade, and low grade UCC, therefore there was higher sensitivity and specificity. With extended utility to screen patients with hematuria and other symptoms, such as urinary tract obstruction, the sensitivity, specificity, PPV and NPV of the assay have reportedly been decreasing[16, 26]. Our data showed PPV was higher when UroVysion was applied in surveillance of recurrent UCC (59.2%)as compared to screening the patients with hematuria (35.5%), while NPV was higher in screening the patients with hematuria (97.5%), as compared to surveillance (87.7%). However there was no obvious difference observed in the sensitivity and specificity of the assay between these two groups in this study. As expected, the sample size of the studies had also been a source of biases. The studies with the most extreme results were those that included only a small number of cases confounding the typical selection bias. Interestingly, a recent study demonstrated that the demography of patients tested had changed significantly in recent year with increased female patients with fewer true positive results, which could also have impact on the effectiveness of UroVysion test[34]. The present study represents one of the largest series on the utility of UroVysion, including 1,835 cases with histological and/or detailed clinical follow up information. About two-third of the patients (652/957) in the study were tested for hematuria and urinary tract symptoms, a population with lower prevalence of UCC, which might partially explain the lower sensitivity and PPV in comparison with those in the previous studies.

Other potential applications of UroVysion include monitoring effectiveness of the treatments, predicting recurrence of UCC, and assisting with clarification of cytologically atypical cases via the use of “reflex test”. Studies showed that patients with positive UroVysion test post BCG treatment was associated with failure of the treatment compared with those with negative result, and the patients with superficial bladder cancer who had positive UroVysion at the end of BCG treatment were at a higher risk for progression to muscle invasive disease[35, 36]. Thus, the patients with positive UroVysion test but negative cytology and cystoscopic findings were more likely to suffer from future recurrence and should be more closely monitored[37, 38]. Yoder et al observed that over a period of 29 month, 65% of the cases with positive UroVysion but no visible lesions developed recurrent tumor

on follow-up[38]. Our data showed that 46% (158/343) of cases with positive UroVysion tests did not develop UCC during up to the 3 year follow up. Since currently no special management is available to these patients, the use of UroVysion in predicting recurrence may cause significant anxiety in both the patients and clinicians, limiting the clinical utility of the assay. In fact, the relatively high rate of false positive and/or the ability of UroVysion to detect potential UCC years before the morphological changes could be detected have limited and may have partially contributed to the downward trend of clinician ordering UroVysion test in our institution. Use of UroVysion as a reflex test, i.e., testing only on the cases with equivocal cytologic diagnoses, such as those with suspicious for malignant cells and AUC, has been studied[16, 39–41]. It has been found that UroVysion analysis was useful in the clarification of equivocal or atypical cytology and a sensitivity of 100%, 89%, and 60% were obtained in patients with suspicious, atypical, and negative cytology, respectively, with the overall specificity was 97%[16]. A negative FISH test result under these situations likely correlated with benign cytological changes, although a low-grade UCC could not be excluded, since these tumors can also be negative with the UroVysion assay. In the present study, UroVysion test was positive in 48 of 52 high grade UCC cases in AUC category, indicated that a clinical management algorism including UroVysion as a reflex test following equivocal cytology might be beneficial in detecting UCC for our patient population.

Interestingly, the study showed that the results of UroVysion for UCC detection were similar to those of cytology when AUC counted for positive, which could just be a coincidence.

In summary, the present study evaluated the assay characteristics of UroVysion in detecting UCC in comparison to cytology in the same urine sample. In accordance with most of the previous studies, our data showed that UroVysion test had higher sensitivity and lower specificity compared with cytology, although the sensitivity of UroVysion was slightly lower than that in earlier studies. These results suggest that UroVysion should be reserved for cases with the highest risk; in particular, those with equivocal urine cytology.

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Table 1

Correlation of UroVysion and Cytologic Diagnosis with The Clinical Findings

	No. of Cases	UroVysion +	UroVysion −	Cytology +	AUC*	Cytology −
Clinical Positive	299	185 (61.9%)	114 (38.1%)	87 (29.1%)	95 (31.7%)	117 (39.1%)
Clinical Negative	1536	158 (10.3%)	1378 (89.7%)	48 (3.1%)	201(13.1%)	1287 (83.8%)
Total	1835	343	1492	135	296	1404

• AUC: Atypical Urothelial Cells

Table 2

Correlation of Cytologic diagnosis of Atypical Urothelial Cells (AUC) AND UroVysion with The Clinical Findings

	AUC	UroVysion +	UroVysion −
Clinical Positive	95	61	34
Clinical Negative	201	46	155
Total	296	107	189

AUC: Atypical Urothelial Cells

Table 3

The Effectiveness of UroVysion and Cytology for Bladder Cancer Detection

	Sensitivity	Specificity	PPV	NPV
UroVysion	61.9%	89.7%	46.153.9%	92.4%
Cytology				
With AUC as −	29.1%**	96.9%**	64.4%*	87.5%***
With AUC as +	60.9%	83.8%**	42.2%**	91.7%

AUC: Atypical Urothelial Cells; PPV: Positive Predict Value; NPV: Negative Predict Value

* p<0.01 when compared to the same diagnostic test characteristic for UroVysion;

** p<0.0001

Table 4

The Effectiveness of UroVysion and Cytology for Bladder Cancer Detection in Patients with Hematuria and History of Urothelial Cell Carcinoma

	Sensitivity	Specificity	PPV	NPV
Hematuria				
UroVysion	60.0%	88.193.4%	35.5%	97.5%
Cytology				
With AUC as −	33.3%**	98.5%†	57.7%*	96.1%**
With AUC as +	57.8%	88.6%***	23.4%**	97.2%
UCC				
UroVysion	62.2%	86.2%	59.2%	87.7%
Cytology				
With AUC as −	28.3%†	95.3%†	66.1%	80.56%†
With AUC as +	61.4%	79.3%†	48.8%***	86.5%

UCC: Urothelial Cell Carcinoma; AUC: Atypical Urothelial Cells; PPV: Positive Predict Value; NPV: Negative Predict Value

* p<0.05 when compared to the same diagnostic test characteristic for UroVysion;

** p<0.01;

*** p<0.001;

† p<0.0001

Table 5

The Effectiveness of UroVysion and Cytology for Bladder Cancer Detection in Patients with History of Low Grade and High Grade Urothelial Cell Carcinoma

	Sensitivity	Specificity	PPV	NPV
Low Grade UCC (n=468)				
UroVysion	40.8%	87.8%	4247.1%	84.9%
Cytology				
With AUC as −	14.3%†	96.8%†	53.8%	81.0%***
With AUC as +	45.9%	80.2%**	38.1%*	84.9%
High Grade UCC (n=576)				
UroVysion	75.65%	84.8%	64.86%	90.4%
Cytology				
With AUC as −	37.236.8%†	94.1%†	70.069.5%	80.2%†
With AUC as +	71.2%	78.3%**	55.054.7%**	88.0%

UCC: Urothelial Cell Carcinoma; AUC: Atypical Urothelial Cells; PPV: Positive Predict Value; NPV: Negative Predict Value

* p<0.05 when compared to the same diagnostic test characteristic for UroVysion;

** p<0.01;

*** p<0.001;

† p<0.0001