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UroVysion™ Testing Can Lead to Early Identification of Intravesical Therapy Failure in Patients with High Risk Non-Muscle Invasive Bladder Cancer

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ABSTRACT

Purpose: In this study, we investigated the ability of UroVysion™ to assess response to intravesical therapy in patients with high risk superficial bladder tumors.
Materials and Methods: We performed a retrospective review of patients undergoing intravesical therapy for high risk superficial bladder tumors. Urine specimens were collected for UroVysion™ analysis before and immediately after a course of intravesical therapy. Cytology and cystoscopy were performed six weeks after treatment, using either a positive cytology or visible abnormality on cystoscopy as a prompt for biopsy. The operating characteristics of the UroVysion™ test were then determined.
Results: 41 patients were identified in whom 47 cycles of induction and 41 cycles of maintenance intravesical therapy were given during the study period. This yielded a total of 88 treatment and evaluation cycles. Median follow-up was 9 months per induction (range 1-21 months) and 13 months per patient (range 1-25 months). A total of 133 urine samples were collected for UroVysion™ of which 40 were positive. Based upon standard clinical evaluation, 41 biopsies were performed which detected 20 recurrences. UroVysion™ testing performed immediately upon completion of therapy for the 41 patients undergoing biopsy yielded a sensitivity, specificity, and accuracy of 85%, 61%, and 71%.
Conclusions: The use of UroVysion™ following intravesical therapy for high-risk superficial bladder tumors helps to identify patients at high risk of refractory or recurrent disease who should undergo immediate biopsy under anesthesia.

Key words: *bladder neoplasms; superficial; BCG; interferons; chemotherapy; follow-up*
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INTRODUCTION

In the United States, there are approximately 68,000 new cases of urothelial carcinoma of the bladder (UC) diagnosed each year, resulting in 14,000 deaths annually (1). A recent population based study found that 32-47% of all bladder cancer deaths may be preventable, and that preventable deaths are more common in patients who initially present with non-muscle invasive disease (2). Currently, the standard of care for high risk superficial bladder tumors (HRSBT) is transurethral resection of bladder tumor (TURBT) and intravesical therapy (IVT). Following a 6 week course of IVT, patients usually undergo a 6 week waiting period prior to cytology and cystoscopy to allow any abnormalities caused by IVT to normalize. However, for patients in whom IVT fails, this is merely a period during which lymph node metastasis could

occur or the bladder disease can progress. Therefore, a test which is able to accurately predict which patients have responded favorably to IVT within a week of completion of IVT could lead to both earlier initiation of second line therapy and potentially significant improvements in survival.

Fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled DNA probes to assess cells for chromosomal alterations. UroVysion™ (Vysis, Downers Grove, IL., USA) is a Food and Drug Administration approved FISH probe set which detects gain in copy number of chromosomes 3, 7, and 17 and homozygous deletion of 9p21. Multiple studies have shown a significantly higher sensitivity than cytology for detecting UC, including even in high-grade cancers, while it maintains the high specificity of cytology (3). Additionally, UroVysion™ is a useful test in cells with atypical or suspicious cytology, as is often observed during IVT, because it relies on DNA alterations rather than morphologic changes (4).

In this study, we assessed the ability of UroVysion™ FISH performed before and at the completion of an induction cycle of IVT to predict the results of biopsy prompted by standard clinical evaluation - cytology and cystoscopy - performed 6 weeks after the last intravesical dose.

MATERIALS AND METHODS

The University of California San Francisco (UCSF) Urologic Oncology Database has Institution Review Board approval to collect clinical, pathologic, and follow-up data on consenting patients who have been seen and treated for genitourinary cancer at UCSF. The database was queried for patients who were treated with IVT for HRSBT between 2006 and 2008, a time which corresponded to the implementation of routine UroVysion™ testing in these patients. This procedure identified a total of 41 patients who comprised the study cohort.

Patients were followed-up according to institutional standard of care. In general, this included voided cytology and UroVysion™ prior to initiation of IVT. Following the induction course of 6 weekly doses, a repeat voided urine specimen was collected for UroVysion™ at or within one week of completion of IVT. Voided/barbotage cytology and cystoscopy were performed 3 months after initiation of IVT. A positive cytology or visible abnormality on cystoscopy was a prompt for biopsy. Documented superficial recurrence on biopsy was an indication for re-induction or radical cystectomy. Maintenance IVT was administered in 3 weekly doses 6 weeks after completion of the induction course and repeated in 3 weekly doses at 6 monthly intervals for the following 18-24 months. Routine surveillance cystoscopy and cytology were performed at 3 monthly intervals for the first 2 years and every 6 months thereafter up to 5 years.

UroVysion™ Analysis

The UroVysion™ test consists of commercially available DNA probes in the pericentromeric regions of chromosomes 3, 7, and 17 as well as to the 9p21 locus. Slides were interpreted by the same molecular cytopathologist (A.B.). They were diagnosed as positive based on ≥ 4 cells showing polysomy of chromosome 3, 7 or 17, or ≥ 12 cells demonstrating hypodiploid 9p21 content. A minimum of 25 cells were considered as a sufficient sample for the test.

Statistical Analysis

The primary objective was to calculate the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of an UroVysion™ test performed immediately after 6 weeks of intravesical therapy. Biopsy results were considered as the reference standard. The secondary objective was to determine whether a change in UroVysion™ over the course of therapy had greater predictive capabilities.

RESULTS

Demographic and clinical data of the 41 patients in this study are shown in Table-1. A total of 47 cycles of induction IVT and 41 cycles of maintenance IVT were given during the study period. Six patients underwent re-induction for biopsy proven superfi-

Table 1 *– Summary of patients and their tumor characteristics.*

Median Patient Age (range)	66 (40-96)
No. Gender (%)	
Male	32 (78)
Female	9 (22)
No. Prior IVT (%)	23 (56)
No. Grade and Stage at Induction (%)	
LGTa	4 (8)
HGTa	7 (15)
HGTa + CIS	3 (6)
HGT1	12 (26)
HGT1 + CIS	7 (15)
CIS	14 (30)
No. Type of Induction IVT (%)	
BCG	31 (66)
BCG + IFN	9 (19)
Chemotherapy	7 (15)

IVT = intravesical therapy; LG = low-grade. HG = high grade; CIS: carcinoma in situ; BCG = bacillus Calmette-Guerin; IFN = interferon.

cial recurrence. This yielded a total of 88 treatment and evaluation cycles. In total, the patients were a relatively high risk group for IVT with 56% already having failed at least one course of prior IVT, and 67% of the patients with a high grade T1 disease and/or carcinoma in situ (CIS). Of the patients who began induction with HG T1 disease, 11 out of 19 had repeat TURBT prior to receiving intravesical therapy. Of the patients who did not undergo repeat TURBT, 4 had focal lamina propria invasion alone, 2 were nonagenarians with multiple medical problems, and 2 were referred after induction had already begun.

Median follow-up was 9 months per induction cycle (range 1-21 months) and 13 months per patient (range 1-25 months). Forty-one biopsies were performed which detected 20 recurrences. Five patients underwent radical cystectomy for disease refractory to multiple courses of intravesical therapy (n = 4), or inability to tolerate induction intravesical therapy (n = 1). One patient had progressed to muscle invasive disease. In addition, 2 patients developed upper tract recurrences with 1 who underwent a nephroureterectomy and 1 who was awaiting surgery.

A total of 133 voided urine samples were collected for UroVysion™. Fifty-two tests were performed prior to IVT (29 before induction, and 23 before maintenance), of which 13 were positive. Eighty-one were performed after IVT (36 after induction and 45 after maintenance), and 27 were positive. The results of testing for the patients who underwent biopsy are illustrated in Figure-1. A total of 34% of patients after IVT had an equivocal cytology. The characteristics of immediate UroVysion™ performed 6 weeks after the last intravesical dose appear in Table-2. Correlation testing before IVT revealed that there was no correlation between cytology and UroVysion™ results ($r = 0.15$ $p = 0.57$). Correlation testing after IVT showed a weak correlation between cytology and UroVysion™ results ($r = 0.27$ $p = 0.06$). We also evaluated the characteristics of the UroVysion™ test with anticipatory positive and upper tract recurrences included as "true positives". These data are shown in Table-2. We defined anticipatory positive as those patients with a positive UroVysion™-negative biopsy at 3 months who later had a positive UroVysion™-positive biopsy within 6 months.

During 44 cycles, the patients had an UroVysion™ test performed both before and after therapy. Of these patients, 19 had a biopsy performed based

Table 2 *– Ability of an immediate UroVysion™ to predict 6 week biopsy findings.*

	Sensitivity	**Specificity**	**PPV**	**NPV**	**Accuracy**
Cytology (n = 33)	56%	88%	82%	68%	73%
UroVysion™ (n = 35)	85%	61%	61%	85%	71%
UroVysion™* (n = 35)	88%	73%	78%	85%	81%

** adjusting for detection of upper tract and anticipatory positive disease. PPV = positive predictive value; NPV = negative predictive value.*

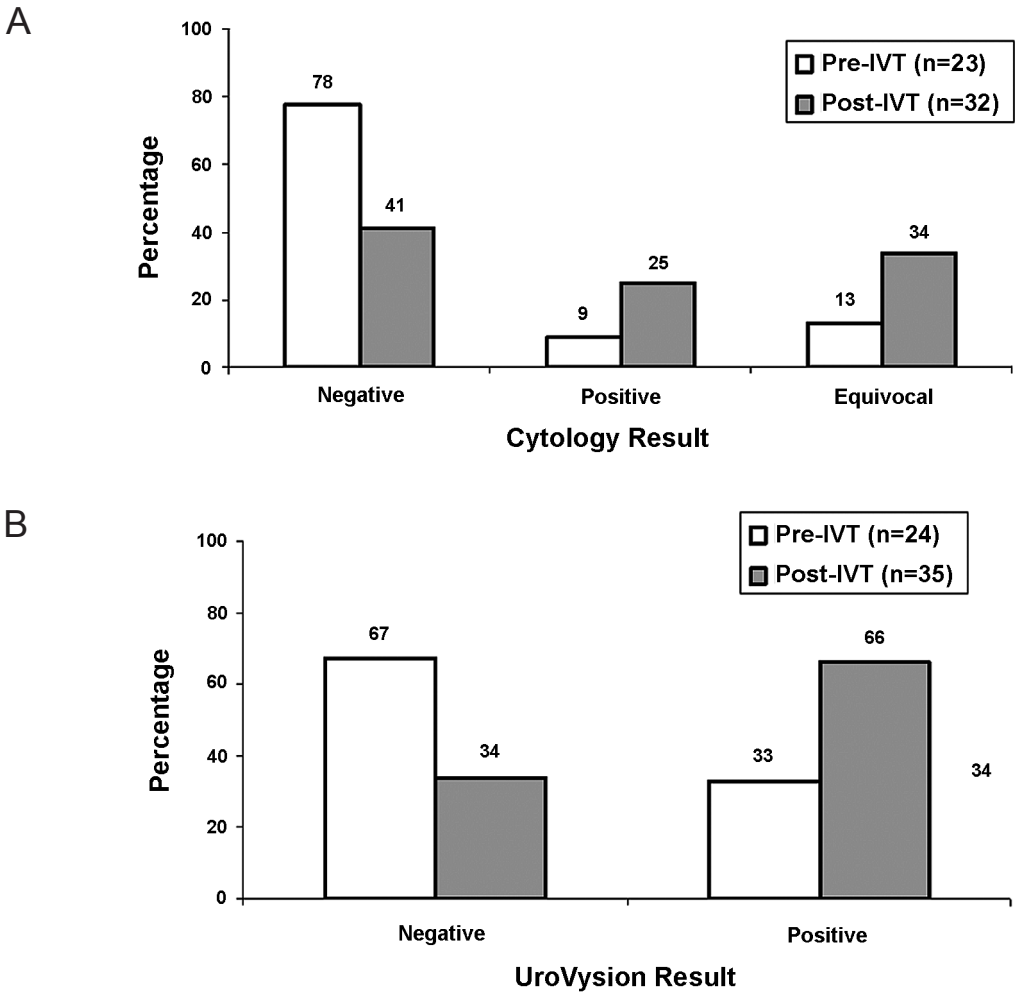

Figure 1 *– Pre- and post-intravesical therapy results. A) Cytology. B) Fluorescence in situ hybridization - FISH. IVT = intravesical therapy.*

upon abnormal standard clinical evaluation. The results are shown in Table-3. The NPV of a change in UroVysion™ from positive to negative compared with a post-IVT negative value alone increased from 85% to 100%. The PPV of a change in UroVysion™ from negative to positive compared with a post-IVT positive value alone increased from 59% to 67%.

In 2 patients, UroVysion™ testing did not predict disease recurrence, which was detected by biopsy (true false negative). In one patient disease recurrence was detected by cystoscopy, while in 1 patient this was missed by clinical tests as well. This patient was 1 of 2 patients included in the study who had a biopsy performed because they had been receiving a novel chemotherapy regimen following immunotherapy failure. In a total of 7 patients, UroVysion™ testing predicted disease recurrence even though the biopsy was negative. Only 3 of these patients had no evidence of recurrence in follow-up (true false positive). Three patients had disease recurrence in the bladder at 3 or 6 month surveillance (anticipatory positive FISH). One patient had an upper tract recurrence 3 months after the positive FISH and negative biopsy. One patient is still awaiting a 3 month evaluation. In a total of 4 patients, a positive UroVysion test was the only predictor of recurrent disease (2 in the bladder and 2

Table 3 *– Results of biopsy in patients with a change in UroVysion™ over course of intravesical therapy.*

UroVysion™ Results	N. Patients	Biopsy Positive	NPV/PPV
Negative/Negative	7	29%	71%
Positive/Negative	1	0%	100%
Negative/Positive	6	67%	67%
Positive/Positive	5	60%	60%

PPV = positive predictive value; NPV = negative predictive value.

in the upper tract), as both cytology and cystoscopy were negative in these patients.

COMMENTS

Despite improvements in surgical technique, refinements in adjuvant intravesical therapy, and small, but real increased disease specific survival with chemotherapy, there has been no real change in the age-adjusted total mortality rate in urothelial carcinoma in the past 20 years (5). Patients with low grade stage Ta disease have as little as a 5% chance of progression and even smaller risk of bladder cancer specific mortality (6). Patients who present with high grade stage ≥ T2 disease may have a somewhat fixed 70% 5 year disease specific survival. In contrast, patients who present with HRSBT have a lethal disease in a potentially curable form.

There are only a few series of patients with HRSBT that have undergone cystectomy at diagnosis; therefore, the 83% 10 year disease specific survival rate reported in one early cystectomy study could have been underestimated (7). In comparison, the 10 year disease specific survival rate of patients with HRSBT in a randomized clinical trial of Bacillus Calmette-Guerin therapy was only 70% (8). A further study has shown that as use of IVT for HRSBT increased, survival of patients who eventually undergo radical cystectomy has dramatically decreased (9). In fact, patients with HRSBT who progress to muscle invasive disease while undergoing IVT have a 10 year disease specific survival of only 27% (7). Reasons for such dramatic differences in survival are multifactorial; however, there is evidence to support the concept that the risk of death from under or untreated high risk disease increases with time.

Three prior studies have evaluated the use of FISH in monitoring response to IVT in patients with HRSBT (10-12). The conclusion of these studies was that a positive post-IVT FISH is useful in predicting eventual relapse, with one study also showing a higher chance of progression. While these studies showed the important prognostic efficacy of FISH, no studies have suggested the use of FISH in order to prompt a change in management. Given the unsatisfactory high mortality in patients who progress during IVT, we believe that changes in the management of this group of UC patients are needed. With the goal of decreasing the time required to detect refractory or recurrent disease, we conducted this study to evaluate the usefulness of UroVysion FISH in patients undergoing IVT.

We found that a voided UroVysion™ performed immediately after completion of an IVT cycle had an accuracy of 71% in predicting findings on biopsy 6 weeks later. This is in agreement with the combined accuracy of cytology and cystoscopy in other reported studies (65-84%), (13-15) but can be achieved without any waiting period. Importantly, cytology was equivocal in one out of every three patients after IVT, potentially limiting its usefulness in this setting. The accuracy of UroVysion™ was lowered mainly by a PPV of 61%. However, in this group of patients with high risk disease, the ideal test may be one with a high NPV. In this study, the NPV of UroVysion™ testing was 85%. Thus, under these conditions, a negative UroVysion™ could be useful to select a group of patients who could be monitored for 6 more weeks prior to standard evaluation with cytology and cystoscopy. In addition, the accuracy of

UroVysion™ in this study was significantly affected by both anticipatory positive FISH tests and by upper tract recurrence. Including these as "true positive" results this would increase the PPV to 78% and the overall accuracy of UroVysion to 81%. Patients who change from positive to negative or negative to positive have even higher NPV and PPV, respectively, although these data are based on a small sample size.

Although there has been a tendency to move away from post-IVT protocol biopsies based on published reports, (15) many randomized trials continue to employ protocol biopsy as the standard method of evaluation rather than cystoscopy alone (16). In our series, biopsy of all patients with a positive UroVysion™ test alone (negative cytology and cystoscopy) would have resulted in 6 extra biopsies. However, biopsy (or upper tract investigation) of all patients with only a positive UroVysion™ would have detected disease in 3 patients missed by routine cystoscopy and cytology. In addition, our data suggest that patients with a negative UroVysion™ can likely be safely monitored until the standard 3 month time point, as the 2 "false negative" cases showed "dysplasia" on biopsy which were treated clinically as a recurrence. Therefore, there were no failures of the UroVysion test to detect biopsy proven recurrence.

Potentially more importantly, we believe there is value in determining which patients need further evaluation with biopsy earlier than the standard schedule. A total of 70% of our patients with a positive UroVysion™ had recurrent HGTa/T1 disease. While CIS has been observed in late responders in 11% of patients, (17) it is unlikely that this would be the case for patients with a frank tumor. Thus, waiting 6 more weeks for these patients would only have led to potential disease progression. Therefore, the positive UroVysion™ would lead to earlier TURBT and potentially earlier re-induction, or possibly earlier cystectomy. It is important to note that the most recent reported guidelines for the management of non-muscle invasive bladder cancer states that cystectomy should be considered for initial therapy of select patients, (18) let alone patients who have likely already failed one induction course of IVT. According to the European Organization of Research and Treatment of Cancer risk tables, this might include (in this setting of already high risk patients) in particular those patients with multifocal disease, large tumors, or HGT1 with CIS (19).

Limitations of our study include its retrospective nature, lack of availability of UroVysion test in all patients, particularly before initial diagnostic TURBT, and that all patients did not undergo a biopsy. We now have a protocol that includes routine pre-TURBT UroVysion testing, followed by tests prior to and after induction IVT as well as before and after maintenance therapy. Another critique might our hipothesis that earlier detection and treatment of refractory disease improves outcome. Given the added cost of UroVysion™, an important next step will be to perform a prospective study to determine whether or not improvement in disease specific and overall survival is observed.

CONCLUSIONS

In patients with HRSBT undergoing IVT, UroVysion testing performed immediately upon completion of therapy can predict 6 weeks post IVT biopsy result with a sensitivity, specificity, and accuracy of 85%, 61%, and 71% respectively.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

This article retrospectively analyses the ability of UroVysion™ FISH test performed immediately after last intravesical therapy instillation to predict outcome of follow-up evaluation after 6 weeks. Aim is to reduce standard 6 weeks waiting while disease may progress.

Unique characteristic of UroVysion™ FISH test lies in its ability to actually demonstrate / identify genetically abnormal - pathological cells. Unfortunately, mere presence of genetically abnormal cells is not synonymous with tumor presence, therefore, test performance, also according to the present study results, is not ideal, but still promising and in agreement with other reports (1).

One should avoid the temptation to act solely on results of this test as in a rather bizarre case I recently observed. Nephroureterectomy was performed solely based on positive Urovysion FISH result from upper urinary tract and only dysplasia, no malignancy was found on the pathology sample.

Approach authors preliminary evaluated - using UroVysion FISH for early identification of non-responders to intravesical therapy, who are at high risk for disease progression and therefore dismal prognosis - is promising, but at the moment far from proven or tested. There are more questions open than answered (for example basic science reasoning, why testing after 6th, not 5th instillation, what is dynamics of treatment response, etc.). However, in the future, role of UroVysion FISH test, sometimes disputed (2), may become established just in such scenarios, which deserve further studies.

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EDITORIAL COMMENT

This study assesses the use of the UroVysion™ FISH test in order to predict whether intravesical Bacillus Calmette-Guerin (BCG) treatment was effective shortly after completion of the therapy course. This allowed identification of patients that are refractory to BCG treatment who should be considered for cystectomy with a sensitivity of 88% and a negative predictive value of 85%. The advantage of this evaluation by FISH is that earlier assessment of refractory disease, i.e. directly after completion of a series of intravesical therapy, may prompt earlier cystectomies. This may then theoretically shorten the time during which metastatic disease may develop and lives may be saved. Although the patient group in this study is small, these results are promising and warrant further extended and prospective studies. Such a study might also include a FISH test on the primary tumor in order to be able to select patients whose tumors do show chromosomal abnormalities in the FISH test.

Another advantage is that FISH and other urine tests can be “anticipatory positive” that is they can detect tumors that were not seen by cystoscopy.

This phenomenon can be explained by the fact that cystoscopy is not 100% sensitive (sensitivity estimates range from 63-85%) (1,2) or that some tumors are yet too small to be seen. Hence, a urine test may identify more patients that have to be followed more stringently. In addition, urine tests are able to detect upper tract recurrences that cannot be seen by cystoscopy as was also the case in this study (3).

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