Fluorescence in situ hybridization (FISH) in the diagnosis of bladder and upper tract urothelial carcinoma: the largest single-institution experience to date

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Introduction: *We evaluated the UroVysion (Abbott Molecular, IL, USA) fluorescence in situ hybridization (FISH) assay for the diagnosis of urothelial cancer in patients diagnosed with or suspected to have bladder, upper tract urothelial carcinoma (UTUC), and combined upper and lower tract urothelial carcinoma (BC).*

Materials and methods: *A single institution retrospective analysis comparing sensitivity, specificity, positive predictive value, and negative predictive values for FISH and urinary cytology. FISH within 6 months of endoscopic evaluation were obtained from outpatient voided urine samples. Our institutional pathology department confirmed pathologic disease from specimens obtained during endoscopic evaluations for lower tract disease. For upper tract disease, disease was confirmed by retrograde ureteroscopy, biopsies of visual lesions, and site-specific upper tract cytology.*

Results: *A total of 415 patients submitted FISH specimens. Overall, FISH was more sensitive than cytology 54.9% in comparison with cytology 42.2% ($p = 0.01$), specificity favored cytology 92.9% compared to 73.5% with FISH ($p < 0.01$). For BC only patients, the same significant finding of increased sensitivity and decreased specificity was identified, but for UTUC alone and combined UTUC and BC, there was no significant difference. Cytology had improved positive predictive value (PPV) over FISH, 76.9% in comparison to 64.6% ($p = 0.02$). Negative predictive value (NPV) also favored cytology 74.2% versus 64.9% ($p = 0.02$). When analyzing individual cohorts, cytology had improved PPV for BC alone patients. UTUC showed no difference for PPV and NPV. For both UTUC and BC, NPV was slightly favored for FISH over cytology 93.2% versus 91.2% ($p = 0.03$).*

Conclusions: *Voided urine FISH testing does offer a higher detection of urothelial carcinoma for BC compared to voided cytology; however, specificity was worse. FISH does not appear to improve detection of urothelial carcinoma in patients with either UTUC only or both BC and UTUC.*

Key Words: urothelial carcinoma, bladder cancer, FISH, urinary cytology

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Introduction

Bladder cancer (BC) is the second most common urological cancer and fifth most prevalent overall with an estimated 74,000 new cases per year and 16,000 deaths in the United States, upper tract urothelial carcinoma (UTUC) is only 5% of renal and urothelial tumors with approximately only 7000 new cases per year.[1,2] Due to various anatomic and behavioral differences, BC and

UTUC have different management pathways, but both require numerous office and/or operative visits, and result in significant patient discomfort and financial strains. They impose the highest cost per patient of all malignancies.[1]

Urinary markers are currently adjunct to direct vision diagnoses and interventions. Over 18 markers have been tested for bladder cancer surveillance. However, of all, urine cytology is the only widespread used marker.[2] However, cytology has its limitations with low sensitivity, wide interpreter variability, and lack of cellular sloughing from well-differentiated urothelial tumors. These restrictions have in turn stimulated investigations into developing better urinary markers such as fluorescence in situ hybridization (FISH) provided by UroVysion (Abbott Molecular, IL, USA) utilizes a multi target assay with microscopy targeting aneuploidy of chromosomes 3, 7, 17, and loss of 9p21 and is FDA approved for surveillance of bladder cancer. Its priority in test algorithms still has not been determined and it is unclear if it belongs as a substitution to the gold standard currently held by cytology.[3,4] FISH also adds significant cost burden in surveillance for BC and UTUC.[5] Several retrospective studies with limited numbers in single-institutions have examined sensitivities and specificities in comparison to cytology, and there is evidence suggesting that FISH is more sensitive but less specific test in comparison to cytology. We aim to review the largest single-institution prospectively acquired institutional database of patients with de novo and recurrent BC, UTUC, and both BC and UTUC and determine whether it improves detection of urothelial carcinoma in comparison to urine cytology.

Materials and methods

With our institutional review board's approval, we conducted a retrospective review of consecutive patients undergoing urothelial carcinoma diagnostic and therapeutic evaluations. A 4 year institutional experience from January 2006 to September 2010 with 3 year follow up identifying voided urine specimens for FISH analysis were compared to voided or intraoperative catheterized cytology specimens from the bladder. When clinically indicated, patients underwent endoscopic evaluation in the operating room. Biopsies from the lower urinary tract were viewed by our institution's pathologist and staged according to the World Health Organization 2004 classifications. UTUC was diagnosed based on methods previously described with retrograde ureteroscopy, biopsies of visualized lesions, and obtainment of selective upper tract cytology with preparation of a cell block whenever possible.[6] When a positive biopsy specimen was not retrieved, UTUC was diagnosed by the presence of visible tumor and positive cytology and/or highly suspicious cytology.

Only FISH specimens within 6 months of endoscopic evaluation were considered valid for this study. Cytologic specimens were obtained by catheter on the day of operative endoscopic evaluation or by voided specimen in the office. FISH study was considered positive by interpretation of our pathology department with utilization of the Urovysion kit. A positive FISH result consisted of a gain in 2 or more chromosomes (3, 7, and 17) in the same cell of four or more separate cells or loss of locus 9p21 in 12 or more cells. Sensitivity, specificity, positive predictive value, and negative predictive values were calculated using chi square analysis and Microsoft Excel programming.

Results

We identified 415 patients with voided FISH specimens submitted. Ages of subjects ranged from 27 to 100 years of age, with a mean of 71.2 years. The male to female ratio was 3:1. The number of patients that underwent endoscopic evaluation was 350 with a total of 1203 endoscopic procedures recorded. The total number of patients that presented with recurrence was 175/273 (64%).

TABLE 1. **Disease stratification**

Diagnosis	**n**	**%**
No evidence of UCa	77	22.0%
BCa	176	50.3%
UTUCa	65	18.6%
BCa and UTUCa	32	9.1%

UCa = urothelial carcinoma; BCa = bladder urothelial carcinoma; UTUCa = upper tract urothelial carcinoma

TABLE 2. **Pathology breakdown for bladder cancer**

Bladder pathology	**n**	**%**
Ta LG	110	52.9%
Ta HG	22	10.6%
CIS	23	11.1%
T1 LG	5	2.4%
T1 HG	39	18.8%
T2	9	4.3%

LG = low grade; HG = high grade; CIS = carcinoma in situ

TABLE 3. **Sensitivity and specificity**

	Cytology		FISH		p value
Overall					
Sensitivity	180/427	42.2%	73/133	54.9%	0.01
Specificity	711/765	92.9%	111/151	73.5%	< 0.01
BCa only					
Sensitivity	66/200	33.0%	25/50	50.0%	0.03
Specificity	628/673	93.3%	85/123	69.9%	< 0.01
UTUCa only					
Sensitivity	62/147	42.2%	27/52	51.9%	ns
Specificity	83/92	90.2%	25/28	89.3%	ns
BCa and UTUCa					
Sensitivity	52/80	65.0%	21/31	67.7%	ns
Specificity	711/766	92.9%	111/151	73.5%	< 0.01

UCa = urothelial carcinoma; BCa = bladder urothelial carcinoma; UTUCa = upper tract urothelial carcinoma

Disease stratification is depicted in Table 1. Seventy-seven patients after evaluation for urothelial carcinoma had no evidence of malignancy, 176 had only BC, 65 had UTUC only and 32 had both BC and UTUC. The pathology breakdown for BC is listed in Table 2. The majority of bladder tumors, 110 patients, were superficial, low grade including the BC only and BC with synchronous UTUC. Twenty-two patients had HG Ta, 23 had CIS, 5 had LG T1, 39 had HG T1, and 9 had T2 disease.

Table 3 highlights the sensitivity and specificity, with overall FISH being more sensitive in comparison to cytology, 54.9% and 42.2% (p = 0.01) respectively. However, specificity was in favor of cytology 92.9% compared to 73.5% (p < 0.01). For patients with BC only, the same finding of increased sensitivity at the sacrifice of specificity was once again found. In UTUC patients, although FISH had a trend toward relatively increased sensitivity, the findings did not yield significance. For patients that had both upper and lower tract disease there was no significant difference between sensitivities. The specificity in this category is the same as the study group overall.

Positive predictive value (PPV) results were calculated for cytology and FISH and are highlighted in Table 4. Cytology yielded better positive predictive values with 76.9% in comparison to 64.6% (p = 0.02) for FISH, as well as an improved negative predictive

TABLE 4. **Positive and negative predictive values**

	Cytology		FISH		p value
Overall					
PPV	180/234	76.9%	73/133	64.6%	0.02
NPV	711/958	74.2%	111/171	64.9%	0.02
BCa only					
PPV	66/111	59.5%	25/62	40.3%	0.02
NPV	628/762	82.4%	85/111	77.4%	ns
UTUCa only					
PPV	62/71	87.3%	27/30	90.0%	ns
NPV	83/168	49.4%	25/50	50.0%	ns
BCa and UTUCa					
PPV	52/106	49.1%	21/61	34.4%	ns
NPV	711/739	96.2%	111/121	91.2%	0.03

PPV = positive predictive value; NPV = negative predictive value; UCa = urothelial carcinoma; BCa = bladder urothelial carcinoma; UTUCa = upper tract urothelial carcinoma

TABLE 5. **High grade cytology versus FISH**

	Cytology		FISH		p value
Sensitivity	34/80	42.5%	11/15	73.3%	0.03
Specificity	628/673	93.3%	86/123	70.0%	< 0.01

value (NPV) 74.2% versus 64.9% (p = 0.02). Cytology also had an improved positive predictive value when investigating the cohort of BC alone with 59.5% versus 40.3% (p = 0.02). UTUC showed no difference between FISH and cytology. For patients with both BC and UTUC, there was no significant difference between cytology and FISH for PPV, but negative predictive value favored cytology over FISH, 96.3% versus 91.2% (p = 0.03), however, both were high in excluding synchronous upper and lower tract disease with a negative test result.

Subset analysis of patients with high grade bladder lesions that included HG Ta, HG T1, and HG T2 lesions was performed as well and can be found in Table 5. While FISH had improved sensitivity 73.3% versus 42.5% (p = 0.03) with cytology, specificity for this group was the same with bladder lesions overall, with 93.3% favoring cytology over 69.9% for FISH. Positive predictive value and negative predictive value could not be recorded for this subset population because the negative disease cohort was included with the bladder tumor only subset population.

Discussion

It is important for a test to be continuously scrutinized in comparison to a gold standard prior to acceptance as a vital cancer marker for detection and surveillance. Hajdinjak et al performed a meta-analysis of 14 urothelial carcinoma studies with a total of 2477 FISH tests. Unfortunately, their analysis did not specify location of urothelial carcinoma and each study included ranged in number of FISH tests from 19-473.[7,8] Meta-analysis revealed an overall 72% sensitivity with a 95% confidence interval of 69%-75% and an overall specificity of 83% (82%-89% confidence interval).[9] The pooled data is more benevolent to FISH with our study finding sensitivity and specificity results lower than the pooled analysis, with 55% sensitivity and a specificity of 73.5%. Although our findings were lower than the pooled analysis, our large test may be different simply as a result of our data resulting from a single tertiary referral population combined with our institution's strict FISH criteria for positivity.[7] In the included Sarosdy study with 473 patients, mean sensitivity was 69% (54%-81%) and specificity was 79% (75%-83%), this single institutional study more closely aligns to our own data. Another large study included by Caraway et al reported 1006 urinary specimens from 600 patients found overall sensitivity of 58% and specificity of 66%. Positive and negative predictive values of FISH were 42% and 79%. Our data is similar to that presented in their study, however our positive predictive value was higher with a slightly lower negative predictive value.[10]

Hajdinjak analysis of pooled cytologies found 42% sensitivity (38%-45% CI) and 96% specificity (95%-97% CI). Our cytology data is consistent with those findings of lowered sensitivity and higher specificity with 42.2% sensitivity and specificity of 92.9%. However, the reproducibility of cytology is limited as there is a significant inter-pathologist difference in diagnosis. Our high volume tertiary referral institution has dedicated urologic oncology pathologists reviewing large numbers of urine cytologies, thus explaining why our sensitivity and specificity might be higher than in the community setting. Additionally, we optimize the number of cells sent for cytopathology analysis by performing bladder washing via catheterization when collected in the operating room as opposed to simply voided specimens. This has been reported to be better than voided urine for detection of BC.[11] Nonetheless, in comparison to FISH, our institution's interpretation of cytology has a higher specificity than FISH. This lowered specificity for FISH indicates that detecting de novo urothelial carcinoma or recurrence will still require the need for direct vision by means of endoscopic evaluation. According to our data FISH as an individual test does not have the accuracy to replace cytology in the clinical decision pathways for management of urothelial cancers.

Additionally, when considering urothelial carcinoma, degree of invasion correlates with worse survival. Similarly, high grade disease is far more concerning than low grade with reports of progression to invasive disease at 2% for low grade and up to 45% for high grade disease.[12] In the analysis of our database, FISH did have an increased sensitivity in comparison to cytology for the detection of high grade bladder cancer, 73.3% compared to 42.5% p = 0.03. However, specificity once

again favored cytology, 93.3% (628/673) versus 70.0% (86/123). Increased detection of higher grade cancers may justify FISH utilization in cohorts of patients with higher risk of progression and recurrence; however, the lowered specificity in comparison to cytology does not change the need for endoscopic visualization to confirm the presence or absence of disease. This is especially significant as a study by Lotan et al of 664 patients with prior diagnosis of BC underwent FISH and cytology, and although many were initially negative for identification of an immediate recurrence, both tests' positivity foretold future recurrences. Positive FISH patients had a positive recurrence on average at 12.6 months compared to 17.9 months if FISH negative ($p < 0.05$). Furthermore, they found that on multivariate analysis only initial T-stage and FISH positivity were independent risk factors for progression to T2+ disease. While this study only included bladder cancer, it illustrates the important principle that FISH positivity may herald a positive diagnosis of recurrence or cancer even if initially negative as well as the potential for cancer progression.[13]

Comparison of noninvasive voided urine test, such as urine cytology and FISH, in reducing the number of operative interventions for primary detection and surveillance of UTUC would be very valuable.[14] Given the far lower incidence of UTUC in comparison to BC, most studies have limited numbers. When interpreting these small, often single, institutional studies, it is important to consider the population of UTUC patients being examined. Voided FISH sensitivities for upper tract disease will likely be higher in those UTUC series in which extirpative surgeries are the predominant treatment modality of choice since these patients are more likely to have high grade or bulkier high volume disease. This is in stark contrast to predominantly endoscopic treated UTUC series in which the majority of patients will have low grade and low volume disease. For example, Marin-Aguilera et al presented a series of 30 patients evaluated, 21 of which underwent extirpative surgery in the form of nephroureterectomy or segmental ureterectomy. They reported 76.7% sensitivity for FISH in comparison to 36% for cytology; specificity was reported at 94.7% in comparison to 100% for cytology. This study also reported positive and negative predictive values with FISH having 95.8% PPV and 72% NPV; in comparison, cytology had 100% PPV and 54% NPV.[15]

The prior largest upper tract study to date comparing FISH and cytology comes from Xu et al from China examining voided specimens from 85 patients suspected to have UTUC and reported a 78.9% sensitivity for FISH and 45.1% for cytology, but the combination of the two was 85.9% which was not significantly better than just FISH alone. The specificity of FISH and cytology both neared 100% and were not significantly different alone or combined.[16] Importantly, 70 of 85 of these patients were treated with extirpative surgery, thus explaining the relatively impressive voided FISH sensitivities compared to our study in which the vast majority of our UTUC patients were treated conservatively with endoscopy. Not surprisingly, when compared to voided samples, site-specific specimens directly from the upper tract will yield improved numbers. Mian et al analyzed 55 patients with a total of 68 specimens analyzed from upper tract washings. Their results showed outstanding sensitivities in 100% for FISH compared to 20.8% in cytology. Specificity, however, favored cytology at 97.4% in comparison to FISH at 89.5%.[17] Given these improved results over voided FISH specimen, utilization of ureteral washings may have some benefit although requiring invasive means of obtaining samples.[18]

Economically, FISH testing is costlier compared to cytology. A hypothetical analysis was proposed by Lotan et al describing a bladder tumor marker with a potential sensitivity of 50% and specificity of 70%, with the possibility of replacing cystoscopy and cytology for which they set a combined sensitivity and specificity of 100%. In their analysis, cost savings per patient would only apply in replacing cystoscopy if the bladder tumor marker was less than $302. The cost analysis resulted in utility only at a lower price as recurrence rates increased and progression increased, at which they used 80% recurrence and 40% progression rates. The hypothetical bladder tumor marker would thus only be cost effective if it was less than $75.[19,20] The cost of FISH testing is the highest among available urothelial tumor markers and certainly exceeds this value. However, in a subsequent analysis of atypical cytologies, the utility of FISH provided significant cost savings of $216 per patient for office biopsies, and $1740 per patient for operating room biopsies.[20]

While not a perfect test, in certain circumstances where results of cytology are unclear, FISH may provide additional vital information. For example, if cytology is atypical, equivocal or suspicious, an option that has been described is reflex FISH testing. Ferra et al identified 161 urine specimens with 68.3% sensitivity and 39.7% specificity utilizing criteria suggested by the manufacturer. However, when utilizing the test in cases where cytology is atypical or suspicious, FISH had improved sensitivity but not surprisingly again lowered specificity with 82.9% and 21.7% respectively.[21] Schlomer et al evaluated 29 patients with equivocal

cytologic findings. They separated these patients into one group with a history of urothelial cancer and no history of urothelial cancer. Those with a history (14 patients) had 100% sensitivity and 60% specificity. Those with no history (15 patients) had 100% sensitivity and 100% specificity. These numbers are smaller than the previous study mentioned with promising results.[22] In our study FISH testing in a reflex scenario yielded 76 patients who had at least one atypical cytology. Forty-four of these patients had a history of urothelial carcinoma. We found sensitivity for reflex testing in patients with a history of BC to be 56.8% (25/44 patients) and a specificity of 62.5% (20/32 patients), PPV and NPV were 67.6% (25/37 patients) and 51.3% (20/39 patients) respectively. However, in comparison to prior studies, our data shows a lowered sensitivity and higher specificity in comparison to the study reported by Ferra et al.[21] But, in comparison to sensitivities and specificities overall in our database, the values are relatively similar and we did not find FISH to have a considerable advantage in detecting urothelial carcinoma in patients with atypical voided cytology. Thus any additional benefit in utilizing FISH as a reflexive test for abnormal cytology still remains in question according to our findings.

Our study provides an extensive patient population with a series of endoscopic evaluations from both upper and lower tract disease. Our study does have its limitations. First, it is a retrospective analysis. Second, urine cytology was retrieved at the same time as the pathologic tissue diagnosis. However, voided FISH specimens in the office were obtained between the time of examination and up to 6 months prior. This could very well be in the time frame of what is described as anticipatory positive, where a positive finding may reflect recurrence or presentation of urothelial carcinoma in the future.[23] This describes another limitation, which is inherent in the analysis of all long term follow ups of patients. In addition to anticipatory factor measuring recurrences, clinical outcomes could have been analyzed to identify if FISH made a difference in clinical decision-making.

Conclusions

Voided urine FISH testing does offer a significantly higher detection of urothelial carcinoma specifically for BC compared to voided cytology; however, the specificity was significantly worse. In a secondary analysis, FISH does not appear to improve detection of urothelial carcinoma in patients with either UTUC only or both BC and UTUC. It is always important to consider that when lower tract disease is clearly not evident and cytology/FISH remain positive, the likelihood of upper tract disease may be higher. In the utilization of FISH as a replacement for cytology, the improvements in sensitivity at the sacrifice of poorer specificity will yield more operative endoscopic evaluations for disease confirmation. Thus, as a stand-alone test the gold standard of cytology should not be abandoned. FISH analysis and its role as a reflex study may have potential benefit and needs to be further elucidated.

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