The role of fluorescence in situ hybridization assay for surveillance of non-muscle invasive bladder cancer

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Objective: *To compare the sensitivity and specificity of UroVysion fluorescence in situ hybridization assay (FISH) with cystoscopy and urine cytology in the surveillance of patients with documented non-muscle invasive bladder cancer (CIS, pTa and pT1).*

Methods: *This retrospective study was done on a consecutive series of patients undergoing surveillance for non-muscle invasive bladder cancer. The results of FISH were analyzed with concurrent cystoscopy and urine cytology.*

Results: *In all, 94 follow up visits from 59 patients were evaluated. The mean follow up was 52 months. FISH detected 30/48 recurrences of bladder cancer, as compared to 20/48 for cytology and 47/48 on cystoscopy. Hence, the sensitivity of FISH was 63% compared to 42% for cytology (p value 0.03) and 98% for cystoscopy (p value 0.0001). However, cytology was significantly more specific (89%) than FISH (65%) or cystoscopy (41%).*

FISH was significantly more sensitive in diagnosing Grade 3 tumors (p = 0.0005) than Grades 1 and 2 tumors, when compared with cytology. There was no significant difference in the sensitivity and specificity between FISH and cytology for Grade 1 and 2 tumors. Sensitivity of urine cytology was similar for Grade 3 versus Grades 1 and 2 tumors (p = 0.56). FISH was able to detect all three CIS recurrences whereas cytology was positive in two and atypical in one sample.

Conclusions: *FISH has a significantly higher sensitivity than cytology in diagnosing patients with Grade 3 bladder tumors. The low specificity of FISH seen in our study and based on the currently available evidence, the test does not satisfy the criteria for replacing cystoscopy or cytology for surveillance of patients with non-muscle invasive bladder cancer.*

Key Words: bladder cancer, cytology, cystoscopy, UroVysion, fluorescence in situ hybridization

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Introduction

The majority of newly diagnosed urinary bladder cancers are non-muscle invasive (carcinoma in situ, pTa and pT1). Early detection of both recurrences and progression of these tumors requires meticulous surveillance. Serial cystoscopy and cytology are the current tests used for surveillance of these non-muscle invasive bladder cancers.[1] Based on the shape and size

of these tumors, cystoscopy is very reliable for detecting papillary tumors, whereas it sometimes fails to identify carcinoma in situ (CIS) lesions. Cytology has a high specificity in the detection of Grade 3 tumors that may not be evident on cystoscopy. However, cytology has been reported to have a low sensitivity for detecting Grade 1 and 2 lesions.[2] As such, cystoscopy and cytology have been used to complement each other. However, despite the advent of flexible video cystoscopes, cystoscopy remains an invasive procedure that entails inherent risks and discomfort for the patient.

Recently, there has been an intense search for urine based markers that could improve or perhaps replace cystoscopy and possibly cytology for bladder cancer surveillance. Ideally, a urine test should be both highly sensitive and specific if it is to replace those methods that are presently in use.

In January 2005, the FDA approved the UroVysion fluorescence in situ hybridization assay (FISH) not only as an aid in diagnosing bladder cancer in patients with hematuria, but also for the surveillance of patients already known to have bladder cancer. The FISH assay comprises a mix of probes to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus. The sensitivity of FISH for detection of bladder cancer has shown to be in the range of 69%%-96%.[1,3-6] However, sensitivity for FISH as low as 30% has also been reported, casting doubt on its application,[7,8] and prompting others to abandon it altogether.[9]

During the past 3 years, we have used the UroVysion FISH test in our clinic and because of the reported conflicting data on the usefulness of this test in the clinical setting; we decided to retrospectively assess its value in the management of non-muscle invasive bladder tumors [CIS (carcinoma in situ), pTa (tumor confined to mucosa) and pT1 (tumor invading submucosa/lamina propria)] in our patients.

Methods

This retrospective study was done on a consecutive series of patients undergoing surveillance for non-muscle invasive bladder cancer. The medical records were reviewed and all follow up visits between September 2005 and August 2008 were included in the study. Along with FISH, concurrent cystoscopy, urine cytology and biopsy were performed. FISH assay and cytology were carried out on the same urine specimen obtained by bladder wash during cystoscopy.

Exclusion criteria from our analysis included reporting of either a non-diagnostic cytology (acellular or hypocellular) or a non-diagnostic FISH assay, a diagnosis of muscle invasive bladder cancer either at initial diagnosis or during a follow up visit, and those patients whose histopathology reports were unavailable for review (n = 1). Assays sent during initial assessment of hematuria were also excluded. The FISH assay was performed by an independent operator who was blinded to the cystoscopy and cytology results. The cytology was performed by one cytopathologist (RV) who was given information about the cystoscopic findings as per existing clinical practice. The Mostofi three-grade system was used for tumor grading.

Cystoscopy was regarded as positive in cases involving visually apparent tumor recurrence and was considered negative if no visual abnormalities were found. However, a suspected or unconfirmed lesion which led to a transurethral biopsy was, for the purpose of this analysis, considered a positive cystoscopy (n = 29). In accordance with our clinical practice, the cytology was categorized as positive for malignancy only if definitive cancer cells were reported by the cytopathologist to be present. The cytology was considered negative for all others including those that were reported as "atypia" or "suggestive of a malignancy." Our analysis, therefore, differs from some other authors where atypical cells suggestive of malignancy have been considered to be positive for malignancy.[4] The histopathology results were compared to the results of the FISH assay, cystoscopy and cytology.

Sensitivity and specificity were calculated relative to the biopsy as denoting the truth of recurrence. The sensitivity was calculated as the percentage of times the test (cytology or FISH) was positive among true positives and false negatives, i.e. when bladder cancer was present according to the biopsy. Specificity was calculated as the percentage of times the test was negative among true negatives and false positives, i.e. when bladder cancer was absent according to the biopsy. The results of FISH and cytology were compared using the McNemar test in order to account for the fact that determinations for these different methods were made on the same samples. P values < 0.05 were considered statistically significant.

TABLE 1. **Comparison of sensitivity and specificity of FISH assay with cytology in the detection of bladder cancer recurrences**

	Cytology	FISH assay	p value
Sensitivity	20/48 (42%)	30/48 (63%)	0.03
Specificity	41/46 (89%)	30/46 (65%)	0.005

TABLE 2. **Comparison of sensitivity and specificity of FISH assay with cytoscopy in the detection of bladder cancer recurrences**

	Cystoscopy	FISH assay	p value
Sensitivity	47/48 (98%)	30/48 (63%)	0.0001
Specificity	19/46 (41%)	30/46 (65%)	0.03

TABLE 3. **Combined sensitivity of cystoscopy with FISH assay and cytology in the detection of bladder cancer recurrences**

	Cystoscopy + Cytology	Cystoscopy + FISH assay
Sensitivity	47/48 (98%)	47/48 (98%)
Specificity	19/46 (41%)	12/46 (26%)

Results

We identified 59 patients during this time period, who were on surveillance for non-muscle invasive bladder cancer. There were 40 men and 19 women (male to female ratio 2:1). The mean age was 58 years for men (range 37-82) and 52 years for women (range 33-78). The mean follow up for all patients was 52 months (range 4-150 months). At initial diagnosis, 16 patients (27%) had Grade 3 tumors (poorly differentiated), 20 (33%) had Grade 2 tumors (moderately differentiated) and 23 (38%) had Grade 1 tumors (well differentiated). Two of the 59 patients (3%) had CIS, 33 (59%) had pTa, 22 (37%) had pT1 and 2 (3%) had both CIS and pT1.

A total of 94 follow up visits from these 59 patients qualified for analysis after excluding five FISH assays and eight cytology samples due to non-diagnostic results. In the study period, there were 48 biopsy proven recurrences and 46 negative biopsies. Among these 46 specimens where the biopsy was reported to be negative, 19 were random bladder biopsies. Of these, 14 were performed due to an abnormal FISH result.

Table 1 compares the sensitivity of FISH assay with cytology. FISH detected 30/48 recurrences of bladder cancer confirmed by biopsy, as compared to 20/48 for cytology. Cytology was therefore negative in 28 samples with recurrence of bladder cancer (these included 17 samples reported to have atypical cells). The increase in sensitivity of FISH came at the cost of a lower specificity of 65% when compared to that of cytology (89%).

Cystoscopy detected all but one recurrence (47/48). Twenty-seven biopsies done for suspect lesions visualized in the bladder on cystoscopy were negative for cancer leading to a low specificity of 41% for cystoscopy, Table 2.

When combining the results of cystoscopy and cytology (detection by either cystoscopy or cytology considered positive, and detection by neither as negative), sensitivity and specificity of 98% and 41% respectively were seen. These figures were identical to that of cystoscopy by itself. It was interesting to note that the same high sensitivity (98%) was seen when a combination of FISH with cystoscopy was used for surveillance, Table 3.

We compared the sensitivity of FISH with cytology for Grade 3 tumors versus Grade 1 and 2 tumors, Table 4. While the sensitivity was significantly higher for FISH when compared with cytology in Grade 1 tumors ($p = 0.0005$), the specificity was significantly higher for cytology ($p = 0.005$). There was no significant difference in the sensitivity and specificity between FISH and cytology for Grade 1 and 2 tumors.

Sensitivity of urine cytology was similar between Grade 3 versus Grades 1 and 2 tumors ($p = 0.56$), detecting 9 and 11 bladder cancers, respectively. FISH was able to detect all three CIS recurrences whereas cytology was positive in two and atypical in one sample.

TABLE 4. **Impact of tumor grade on sensitivity and specificity of FISH assay**

	FISH assay	Cytology	p value FISH versus cytology	Cystoscopy
Grade 3 tumors				
Sensitivity	21/25 (84%)	(9/25) 36%	0.0005	(25/25)100%
Specificity	17/25(68.0%)	(25/25) 100%	0.005	(13/25) 52%
Grade 1 and 2 tumors				
Sensitivity	9/23 (39%)	11/23(48%)	0.53	22/23(96%)
Specificity	13/21(61.9%)	16/21(76%)	0.26	6/21(29%)

Discussion

In January 2005, the US Food and Drug Administration approved UroVysion FISH assay as an aid in diagnosing bladder cancer in patients with hematuria and for surveillance of patients known to have bladder cancer. There have been a number of reports since then promoting FISH over urine cytology for diagnosis and surveillance of bladder cancer.[1,3-6,14,15]

The median sensitivity for FISH assay has been reported as 69%-87% with a specificity of 89%-96%.[3] In contrast to these reports, there have been two recent studies reporting a low sensitivity of FISH in patients with non-muscle invasive bladder cancer. Gudjonsson et al[7] prospectively looked at its value in 175 samples sent on 159 patients and found a sensitivity and specificity of 30% and 95%, respectively. Moonen et al have reported a sensitivity and specificity for FISH as 39.1% and 89.7% respectively.[8]

Most studies in the literature have been based on mixed populations of selected patients with primary and recurrent tumors. In our clinical practice, we looked at the role of FISH in management of non-muscle invasive bladder cancer and found a sensitivity of 63% compared to 42% for cytology (p value 0.03). However, cytology was significantly more specific (89%) than FISH (65%) (p value 0.005).

CIS areas are known to shed urothelial cells easily, which might explain the greater sensitivity of the FISH assay for such lesions. We found that FISH has a high sensitivity for CIS (100%), although our numbers are very small to draw any significant conclusions. We also found a higher sensitivity for FISH in detecting Grade 3 tumors. These findings have been observed in other studies as well.[5,7,17]

In our clinical practice, we often come across patients on surveillance for non-muscle invasive bladder cancer where only the FISH assay was positive. There have been reports of discordance with positive FISH and negative cytology, but rarely vice versa.[1] This leads to more aggressive assessments and a shortened interval between examinations. In our experience, these exams rarely discover a significant lesion and subject the patient to the risks of anesthesia and bladder surgery in addition to being expensive studies. These views are shared by Nieder et al in a recent article.[9]

Bladder wash cytology is considered to be more sensitive than voided urine cytology[12] and provides better material for cytology because of greater number of cells, better cell preservation and fewer contaminants.[13] For these reasons, we have always used bladder wash cytology for routine cytological analysis as well as the FISH assay. This may have been a factor resulting in a higher overall sensitivity in our study when compared with other recent articles which mainly utilized voided specimens.[7,8] In our present study, the sensitivity of urine cytology obtained by bladder wash, was no greater and even appeared to be lower for Grade 3 tumors compared to Grades 1 and 2 tumors, although the difference is due to only two additional detections in the low-grade subgroup and is well within random error (36% versus 48%, p = 0.56). As seen with many previously developed urine based tumor markers, the promising early results indicating the usefulness of the FISH assay have not been reproduced consistently,[7-9] leading to skepticism about the true value of this test.

Our study was somewhat limited by being retrospective. Some of the biopsies were performed due to an abnormal FISH assay or cytology resulting in an element of selection bias. However, our mean follow up was 52 months which is longer than most of the published papers on the value of FISH assay.

Because of the low specificity of FISH seen in our study, the test does not satisfy the criteria for replacing cystoscopy or cytology for surveillance of patients with non-muscle invasive bladder cancer, even though we found that FISH had a significantly higher sensitivity than cytology in diagnosing Grade 3 bladder cancers, a finding previously reported by others.[7] Kipp et al evaluated the value of FISH in monitoring response to treatment in 37 patients receiving intravesical therapy for non-muscle invasive bladder cancer.[16] Patients with a positive FISH assay invariably recurred and the majority (7 of 11) progressed, whereas only 11 of 25 with a negative result recurred and only 2 of them progressed.

The FISH assay has also been reported to be useful in diagnosing bladder cancer where the cytology is either equivocal or atypical.[10,11,15] Gofrit et al suggested that FISH may be a useful tool for predicting tumor recurrence by sparing the need for cystoscopy and widening surveillance intervals in patients with history of low-grade tumors and a normal FISH assay.[17]

Conclusion

UroVysion FISH assay seems to have found its way into clinical practice, although its definitive role has not been fully elucidated. The promising early results indicating the usefulness of the FISH assay have not been reproduced consistently. The false-positive results are problematic and lead to unnecessary investigations. Based on our results and currently available evidence, FISH does not justify as a replacement for cystoscopy or cytology in surveillance of patients with non–muscle

invasive bladder cancer. Further studies involving larger numbers of patients are required to determine the accuracy and widespread applicability of FISH assay in guiding treatment of bladder cancer. □

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