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The role of FISH and cytology in upper urinary tract surveillance after radical cystectomy for bladder cancer★

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Abstract

Objectives—Cytology and fluorescence in situ hybridization (FISH) (Urovysion) assay are often used during upper urinary tract surveillance in patients following radical cystectomy with urinary diversion, without much available data regarding efficacy in this population. Here, we evaluate the value of FISH and cytology in detecting upper tract recurrence in the face of a urinary diversion.

Materials and methods—A review of our cystectomy database revealed 270 patients who had at least one FISH and/or cytology assay performed during surveillance after radical cystectomy. Workup included upper tract imaging in all patients and upper tract endoscopy as indicated. A total of 163 FISH assays and 474 urinary cytology examinations were included in the analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of FISH and cytology were assessed.

Results—Ten patients (3.4%) developed upper tract recurrence after a median follow-up time of 31 months (2–202). All but 1 patient presented either with gross hematuria or positive finding on imaging; 6 had positive FISH and cytology, and 2 had positive cytology only (no FISH done). For detection of upper tract recurrence, sensitivity, specificity, PPV, and NPV of cytology were 80.0%, 85.6%, 10.7%, and 99.5%, respectively; and that for FISH were 85.7%, 86.5%, 23.1%, and 99.2%, respectively.

Conclusions—The FISH assay and urinary cytology both demonstrate high rates of false positivity and are useful mainly for their negative predictive ability in patients with a urinary diversion. Unless prospective trials show otherwise, both— or at least the more expensive test— can be omitted from surveillance strategies.

Keywords

Bladder cancer; Urinary diversion; FISH; Cytology; Surveillance; Urothelial cancer

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1. Introduction

Primary upper tract urothelial carcinoma (UTUC) is an infrequent urological malignancy, accounting for approximately 5% of all urothelial tumours [1,2]. Upper tract recurrence is also uncommon after radical cystectomy for bladder cancer, ranging from 2% to 7%, and occurring generally about two years after surgery [3]. Prognosis of upper tract recurrence has been reported to be worse than for primary UTUC, with previous series showing node-positive rates up to 83% and locally-advanced tumors in up to 75% of cases [3,4]. Hence, patients often undergo rigorous surveillance of their upper tracts after radical cystectomy, despite evidence suggesting that detection prior to symptomatic presentation may not improve survival. Current upper tract surveillance regimens are variable and usually include cross-sectional imaging and, most frequently, urinary cytology. A positive cytology after radical cystectomy has been reported to be an independent risk factor for urothelial recurrence in this setting [5]. However, overall sensitivity of this test when used for diagnosis of UTUC is poor (36%) and highly depends on tumor grade and stage [6]. UroVysion test is a multi-target fluorescence in situ hybridization (FISH) assay for detection of aneuploidy in chromosomes 3, 7, and 17 as well as loss of the 9p21 locus in exfoliated cells from voided urine. Several studies have reported a high sensitivity and specificity in the detection of bladder cancer, especially in cytologic equivocal urine samples [7,8]. Moreover, it has shown a greater sensitivity than cytology for detecting of primary UTUC whilst maintaining a similar specificity [6]. Thus, despite lack of data, many have started adopting this test in their surveillance protocols, adding to the rising cost to which bladder cancer patients are subjected. The aim of our study was to assess the value of FISH (and compare with cytology) in detecting UTUC in patients with previous radical cystectomy and urinary diversion.

2. Materials and methods

We performed an institutional review board approved analysis of our radical cystectomy database and identified 270 patients who had undergone a radical cystectomy with urinary diversion for bladder cancer in our institution and had at least one FISH and/or cytology assay performed during follow-up after surgery. Medical records were retrospectively reviewed and a total of 163 FISH assays and 474 cytology examinations were included in the analysis. All patients had complete follow-up data available and were therefore considered for the analysis.

Surveillance workup included history and physical examination, routine serum chemistry studies, urinary cytology, FISH, chest radiography, and upper tract imaging by abdominal/pelvic computerized tomography (CT) or intravenous pyelogram (IVP). Patients were usually evaluated every 3 months for the first year, semi-annually from the second through the fifth year, and annually thereafter. Upper tract endoscopy was performed when clinically indicated according to surveillance workup findings. Indications for ureteroscopy included a positive cytology and/or FISH even in the absence of radiological findings in the upper tract. All recurrences were confirmed by histologic analyses. Urinary cytology was obtained from urinary diversions and assessed by experienced pathologists in conjunction with the clinical history. Results were reported as negative (normal and inflammatory), atypical cells, suspicious for malignancy, positive for malignant cells or nondiagnostic. The UroVysion test (Abbott Laboratories Inc., Des Plaines, IL) was performed by trained cytotechnologists according to the manufacturer's instructions and reported by cytopathologists according to the Food and Drug Administration (FDA) guidelines as follows: positive specimens were defined as containing ≥ 4 abnormal or aneusomic cells, or homozygous deletion of 9p21 for ≥ 12 cells or tetrasomy in ≥ 10 cells.

For the analysis of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each test alone and in combination, a χ^2 test was used, and a 95% confidence interval was considered.

3. Results

The pT stage and tumor grade of all cystectomy specimens are summarized in Table 1. Nearly 70% of cases were either high grade or carcinoma in situ (CIS) tumors. Two patients (0.7%) had synchronous UTUC at cystectomy and are excluded. Ten (3.7%) patients developed upper urothelial tract recurrence within a median follow-up of 31 [2–202 (range)] months. Median time to recurrence was 23 months (range 2–38). Table 2 summarizes the histologic diagnoses, imaging, cytology, and FISH results of all patients with upper tract recurrence. All but one of these patients had positive imaging findings (CT scan or IVP) and/or symptoms (gross hematuria).

A total of 474 urinary samples were analyzed for cytology. Of those, 75 (15.8%) were positive, 399 (84.1%) were negative, and only 1 was nondiagnostic. FISH was performed for 163 samples. Overall, 27 (16.0%) were positive, 129 (79.1%) were negative, and 8 (4.9%) were inadequate for analysis due to an insufficient amount of cells. No differences regarding the type of urinary diversion were noted. Test performances are listed on Table 3. Overall, sensitivity and specificity showed values over 80% for each test. Sensitivity and PPV for detection of upper tract urothelial recurrence were not significantly better for FISH than for cytology. A combined analysis of both tests was also done for cases having assays performed on concurrent dates. The only significant difference between individual performances and those of combined evaluation was an improved PPV for the latter compared with cytology (42.9% vs. 10.7%; $P = 0.008$).

4. Discussion

Our study found an equivalent performance of urinary cytology compared with FISH in the detection of UTUC in patients after radical cystectomy and urinary diversion; however, both have low positive predictive ability and are useful more to exclude tumors. Notably, all but 1 patient with upper tract recurrence presented either with gross hematuria or positive finding on imaging.

The multi-target FISH assay was approved by the Food and Drug Administration for bladder cancer diagnosis and surveillance. Initial studies reported overall sensitivity rates of 81%–84% with specificity of 81%–96% for diagnosis [9,10]. In a large multicentric study, overall sensitivity was 71% compared with 26% for urinary cytology. Most relevant, the sensitivity for high grade tumors and CIS was 94% and 100%, respectively [11]. These results have been confirmed by further studies, concluding that FISH improves sensitivity for detection of primary and recurrent bladder cancer compared with urinary cytology in all tumor grades and stages, whilst offering a similar specificity [7,8,12]. Similar results have also been observed with specimens obtained from bladder washings [13]. The FISH test has also been shown to predict urothelial recurrence. Skacel et al. reported that patients with positive tests and concurrent negative cytology and cystoscopy subsequently developed bladder cancer after 12 months [8]. This phenomenon might exist in the UTUC surveillance population as well. While our report is not designed to evaluate this particular issue, in our series, we did not observe this to be the case. However, we will be prospectively collecting data on existing patients to study whether a positive FISH mandates more frequent imaging.

Few earlier reports have analyzed the role of FISH for diagnosis and detection of primary UTUC. Similar to bladder cancer, the FISH test has shown better sensitivity and equal specificity compared with cytology [6,14,15]. Results of FISH analysis obtained by upper

tract washings have shown to be consistent with those obtained from voided samples [14]. However, the role of FISH for upper urothelial tract recurrence after radical cystectomy has not been assessed to date.

Urinary cytology and imaging (CT scan and IVP) are the standard methods considered in upper urinary tract surveillance protocols following bladder cancer treatment. However, most upper urinary tract urothelial recurrences after radical cystectomy (40%–80%) are detected only after progression to tumor-related symptoms [3,16]. In our series, half of recurrences presented with gross hematuria. This fact highlights the poor performance of current protocols, as recurrences are not detected in earlier stages. In contrast to previous reports [3], we found an overall sensitivity of 80.0% for cytology in our series. A possible explanation for this difference might be the high proportion of high grade and carcinoma in situ tumors (69.3%) observed in our study. In fact, all patients with upper tract recurrence had either high grade or CIS tumors at the time of radical cystectomy.

The observed sensitivity and specificity rates of FISH are comparable to those of a previous study analyzing its role in detection of primary UTUC [6]. However, when compared with cytology, no statistically significant improvements in terms of sensitivity (85.7% vs. 80.0%; $P = 1$) and PPV (23.1% vs. 10.7%; $P = 0.18$) were found. This is not consistent with the mentioned study by Marín-Aguilera [6], and the explanation may be the expertise of cytopathologists in a high-volume cancer center, resulting in improved sensitivity of cytology observed in our study.

Combined analysis of the 2 assays only showed a significantly higher PPV rate than those of cytology (42.9% vs. 10.7%; $P = 0.008$); no significant difference was noted with any value of the FISH assay. Consequently, no clinical benefit in terms of upper tract recurrence detection was noted after addition of FISH to the surveillance protocol, as sensitivity and specificity are the most relevant indicators in this setting. Similar conclusions have been obtained by 2 recent studies evaluating performance of FISH in the surveillance of non-muscle-invasive bladder cancer, showing no improvement over cytology [17,18].

As mentioned, both tests showed a good performance in terms of sensitivity and specificity. However, false positive rates were high (57.1%–89.3%), raising doubts about the role of these non-invasive methods in upper tract surveillance protocols, and imaging and direct visualization remain the gold standard for recurrence detection. In other words, up to 90% of imaging studies and/or endoscopic workup following positive FISH and/or urinary cytology tests would result in negative findings. On the other hand, only 1 recurrence in our series was detected exclusively by FISH and cytology. In patients felt to be at increased risk for upper tract recurrence, e.g., positive ureteral margins, cytology and FISH may be helpful. However, because of the modest PPV and the known fact that a positive FISH may precede clinically detectable recurrence for a considerable period of time, invasive investigation does not appear to be indicated without radiologic confirmation of an abnormality.

We do acknowledge certain limitations to our study. Our results were analyzed retrospectively, and surveillance protocols were not uniform across the period studied. While urinary cytology and FISH are both operator-dependent and the analysis is, therefore, exposed to inter observer variations, this is a limitation inherent to the tests themselves and applicable to all who use them in daily practice.

5. Conclusions

The FISH assay and urinary cytology provided equally good sensitivity and specificity values for upper urinary tract recurrence detection in patients after radical cystectomy for bladder cancer in our series. However, the high rates of false positives for both tests coupled

with the fact that most recurrences are detected with imaging and/or symptoms, argue against their routine use in surveillance strategies. Unless prospective trials show otherwise, both—or at least the more expensive test, i.e., FISH—can be omitted from surveillance strategies.

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Table 1

Histologic features at the time of cystectomy

	<i>n</i>	%
pT Stage		
pT0	70	25.9
pTis	58	21.5
pTa	11	4.1
pT1	47	17.4
pT2	34	12.6
pT3	36	13.3
pT4	7	2.6
Unknown	3	1.1
Tumor grade		
1	2	0.7
2	7	2.6
3	129	47.8
CIS	58	21.5
(Unknown e.g., pT0)	76	28.1

Table 2

Patients with upper tract urothelial recurrence during follow-up

Histology at cystectomy	Type of diversion	Time to upper tract recurrence (months)	Gross hematuria	Imaging	FISH	Cytology
pTaG3	Indiana	38	Negative	Positive	Positive	Positive
pT4G3	Indiana	30	Positive	Negative	N/A	Negative
pT2G3	Neobladder	36	Positive	Positive	Positive	Positive
pT1G3	Neobladder	21	Positive	Positive	Positive	Positive
pT1G3	Conduit	12	Negative	Positive	Positive	Positive
pTis	Conduit	26	Negative	Negative	Positive	Positive
pTis	Conduit	28	Positive	Positive	N/A	Positive
pTaG3	Conduit	16	Negative	Positive	Positive	Positive
pTaG3	Conduit	2	Negative	Positive	N/A	Positive
pT1G3	Neobladder	11	Positive	Positive	Negative	Negative

Table 3

Diagnostic Tests Results: Urinary Cytology, FISH and both as Simultaneous tests

<u>Upper urinary tract recurrence</u>		<u>%</u>							
	<u>+</u>	<u>-</u>	<u>Sensitivity</u>	<u>Specificity</u>	<u>PPV</u>	<u>NPV</u>	<u>False positivity</u>	<u>False negativity</u>	
Urinary cytology									
	<u>+</u>	8	67	80.0	85.6	10.7	99.5	89.3	0.5
	<u>-</u>	2	397						
FISH									
	<u>+</u>	6	20	85.7	86.5	23.1	99.2	76.9	0.8
	<u>-</u>	1	128						
Urinary cytology + FISH									
	<u>+</u>	6	8	85.7	88.9	42.9	98.5	57.1	1.5