Urothelial carcinoma (UC) of the bladder is the fourth most frequent malignant disease in men and the fifth most common cancer overall. Most patients present with early-stage disease confined to either the urothelium (Ta) or the lamina propria (T1). The standard treatment of such patients has been transurethral resection (TUR) of all visible tumor. However, even under ideal conditions, the efficiency of TUR has been lower than expected. The percentage of positive histologic findings at the second TUR has been reported to be 6%-78%. Therefore, some investigators have suggested repeat TUR routinely in all cases of non-muscle-invasive bladder cancer. However, the role of repeat TUR is still controversial because of the treatment costs, morbidity, and delay in adjuvant topical therapy.

Early studies had demonstrated that multitarget fluorescence in situ hybridization (FISH) analysis for aneuploidy of chromosomes 3, 7, and 17 and loss of the 9p21 locus provided high sensitivity and specificity in the detection of bladder UC. However, to our knowledge, no study has evaluated the role of multitarget FISH in the detection of residual tumor after TUR of nonmuscle-invasive bladder cancer. The aim of our present study was to assess the utility of FISH as a predictor of the residual tumor load after TUR of bladder UC.

MATERIAL AND METHODS

From August 2007 to July 2009, 125 consecutive patients with a suspicion of bladder UC were evaluated prospectively at our institutions. TUR was performed in all 125 patients, who had presented with gross hematuria and an ultrasound diagnosis of a bladder lesion. All patients underwent upper urinary tract imaging studies, including renal ultrasonography, intravenous urography, and computed tomography. No patient had a posi-
tive result for an upper urinary tract tumor. All TURs were performed or supervised by a single experienced surgeon (L.Y.Y.). The Institutional Ethical Committee approved the study, and all patients provided written informed consent before entry into the study.

The first TURs were fully standardized, with the bladder carefully reviewed using both 12° and 70° lenses. Next, a macroscopically complete resection was performed. Intravesical therapy was not administered after the first TUR, regardless of the pathologic results from this TUR. The 34 patients in whom no tumor was identified after TUR and with other pathologic findings, including chronic cystitis and urothelial hyperplasia, were excluded from the present study. The remaining 91 patients had UC of the bladder. The tumor staging was in accordance with the 2002 TNM classification and grading was done using the World Health Organization 2004 classification. The patients with UC underwent a second procedure 4-6 weeks after the initial TUR. The intervention included a second TUR or cystectomy, depending on the histopathologic findings of the first TUR. The patients with Stage Ta or T1 tumors underwent a second TUR, and those with Stage T2 tumors underwent radical cystectomy. Two patients with UC who did not undergo a second procedure were excluded from the present study. The second TUR began with an accurate inspection of the bladder using the white light. The scar of the first procedure was resected, and any macroscopic overt or suspicious lesion found was excised. Using the pathologic findings of the second procedure, the patients with bladder tumors were divided into 2 groups, those with (n = 38) and without (n = 51) residual tumor.

Voided urine samples (50-300 mL) were collected just before the first TUR and 4-6 weeks after the first TUR but before the second procedure. The cells from the voided urine samples were centrifuged at 600g for 10 minutes, and the cell pellets were harvested using prewarmed (37°C) potassium chloride hypotonic solution for 20 minutes, with subsequent fixation with Carnoy solution (3:1 vol/vol methanol/glacial acetic acid) for 10 minutes (×3), centrifuged at 300g for 8 minutes. Next, 20 µL of the final cell pellet suspension was placed on a glass slide. The slides had been pretreated using a FISH pretreatment kit (Abbott Molecular/Vysis, Des Plaines, IL) according to the manufacturer’s instructions. Subsequently, they were hybridized with the multitarget, multicolor FISH probes. The probe mixture consisted of 4 directly labeled probes to the pericentromeric regions of chromosomes 3 (SpectrumRed), 7 (SpectrumGreen), 17 (SpectrumAqua) and the locus 9p21 (SpectrumGold). This mixture was applied to slides under cover slips. The slides were sealed with rubber cement and denatured at 73°C for 5 minutes and hybridized overnight (16-22 hours) at 42°C in a moist chamber. The posthybridization washes were performed in 50% formamide/2× standard saline citrate (pH 7.0) at 42°C for 8 minutes (×3), in 2× standard saline citrate (pH 7.0) for 10 minutes, and NP-40 buffer (2× standard saline citrate/0.1% NP-40), for 10 minutes, and then dehydrated by gradient alcohol. Counterstaining was performed with 10 µL of 4,6-diamidino-2-phenylindole.

The evaluation of the samples was performed by 2 independent observers who were unaware of the clinical findings. A sample was considered FISH positive if ≥1 of the following criteria were met: (a) identification of ≥10 nuclei with the same polysomy in 1 chromosome (3, 7, or 17); (b) identification of ≥5 nuclei with gains in ≥2 different chromosomes (3, 7, or 17); and/or (c) observation of homozygous deletion of 9p21 in >20% of the nuclei counted. The counting process was stopped when 1 of these criteria were met. If none of the criteria for a FISH-positive result was met, all15

The differences between the 2 groups were assessed using the chi-square test or Fisher’s exact test, and 2-sided tests of significance were performed. Results with P < .05 were considered significant. All analysis was done using the Statistical Package for Social Sciences, version 15.0, software (SPSS, Chicago, IL).

RESULTS

Table 1 lists the characteristics of the 91 patients who were histopathologically confirmed to have bladder UC after the first TUR. After the initial TUR, 38 (42.7%) had residual disease in the histopathologic specimens obtained from the second procedure. The stage and grade are also listed in Table 1. The pathologic data from the patients with and without residual tumor are listed in Table 2. A significant difference was found in the stage distribution between the groups with and without residual tumor.

Of the 89 patients with bladder UC who had undergone a second procedure (repeat TUR or cystectomy), 66 had FISH-positive findings before the initial TUR. We compared the positive percentages of urinary FISH results in the patients with and without residual tumor before and after the initial TUR (Table 3). Before the first TUR,
Routine second TUR for all cases of nonmuscle-invasive bladder cancer is controversial.4,5 Thus, several investigators have recommended a routine second TUR for all cases of nonmuscle-invasive bladder UC. Therefore, it is unlikely that the findings of residual tumor could have resulted from incomplete endoscopy or the lower experience of the operating surgeon performing TUR.

In our series, we found a residual tumor rate of 42.7% after the initial TUR. Although less than that reported in published studies, the rate of residual carcinoma found after the second TUR was still significant. The presence of residual disease after the initial TUR increases the risk of early tumor recurrence and progression. Those patients without residual disease or recurrence had a better prognosis.4,5 Thus, several investigators have recommended a routine second TUR for all cases of nonmuscle-invasive bladder cancer.7 However, the role of repeated TUR is still controversial. The treatment costs, morbidity, and delay in adjuvant topical therapy are among the most frequently cited reasons for not performing a second TUR.8 Therefore, a tumor marker that would early show which patients will require another intervention would not only decrease the number of unnecessary procedures but might also reveal those with high-risk disease.

The Food and Drug Administration-approved UroVysion FISH assay is a multitarget test that can be performed on urine cells and will detect ≤4 chromosomal aberrations frequently associated with bladder cancer.18 These alterations, consisting of aneuploidy of chromosomes 3, 7, and 17 and the loss of the 9p21 band, have demonstrated increased sensitivity compared with cytology for detecting UC in urine specimens.19-21 In addition, it has been suggested that this test might be useful for the detection of tumor recurrence before it is visible by cystoscopy.22 In the present study, we assessed the use of the multiprobe FISH assay to predict the residual tumor load after TUR of bladder UC. Our preliminary results have suggested that urinary FISH assay, in addition to being a good diagnostic marker of bladder UC, could be used as a predictor of residual disease after TUR. After the initial TUR, the FISH-positive percentage in those with residual tumor was significantly greater than those without residual tumor. Furthermore, the percentage of conversion from FISH-positive to FISH-negative status in those with residual tumor was significantly lower than

### Table 2. Histopathologic findings of patients with and without residual tumor after initial transurethral resection

<table>
<thead>
<tr>
<th>Variable</th>
<th>No</th>
<th>Yes</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients* (n)</td>
<td>51</td>
<td>38</td>
<td>.007</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ta</td>
<td>46 (90.2)</td>
<td>16 (42.1)</td>
<td>.001</td>
</tr>
<tr>
<td>T1</td>
<td>5 (9.8)</td>
<td>15 (39.5)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>0</td>
<td>7 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-grade (G1)</td>
<td>16 (31.4)</td>
<td>15 (39.5)</td>
<td>.484</td>
</tr>
<tr>
<td>High-grade (G3)</td>
<td>35 (68.6)</td>
<td>23 (60.5)</td>
<td></td>
</tr>
<tr>
<td>Associated carcinoma in situ</td>
<td>5 (9.8)</td>
<td>3 (7.9)</td>
<td>.339</td>
</tr>
</tbody>
</table>

Data in parentheses are percentages.

* Two patients with tumor who did not undergo second procedure were excluded.

### Table 3. Comparison of urinary fluorescence in situ hybridization results in patients* with and without residual tumor

<table>
<thead>
<tr>
<th>FISH Result</th>
<th>No (n = 51)</th>
<th>Yes (n = 38)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before initial TUR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>39 (76.5)</td>
<td>27 (71.1)</td>
<td>.436</td>
</tr>
<tr>
<td>Negative</td>
<td>12 (23.5)</td>
<td>11 (28.9)</td>
<td></td>
</tr>
<tr>
<td>After initial TUR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>9 (17.6)</td>
<td>16 (42.2)</td>
<td>.003</td>
</tr>
<tr>
<td>Negative</td>
<td>42 (82.4)</td>
<td>22 (57.8)</td>
<td></td>
</tr>
<tr>
<td>Before/after initial TUR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive/negative</td>
<td>30 (58.9)</td>
<td>11 (28.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Positive/positive</td>
<td>9 (17.6)</td>
<td>16 (42.2)</td>
<td></td>
</tr>
<tr>
<td>Negative/negative</td>
<td>12 (23.5)</td>
<td>11 (28.9)</td>
<td></td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization; TUR, transurethral resection.

Data in parentheses are percentages.

* Two patients with tumor who did not undergo second procedure were excluded.

no significant differences were seen in the FISH-positive percentage between those with and without residual tumor (71.1% vs 76.5%, P = .436). After the first TUR, the FISH-positive percentage in those with residual tumor was significantly greater than in those without residual tumor (42.2% vs 17.6%, P = .003). Moreover, before and after the initial TUR, the percentage of conversion from FISH-positive to FISH-negative status in those with residual tumor was significantly lower than that in those without residual tumor (28.9% vs 58.9%, P < .001). No patients were observed with conversion of the FISH results from negative to positive in those with and without residual tumor after initial TUR. The positive and negative predictive value of FISH for residual tumor was 64.0% and 65.6%, respectively.

**COMMENT**

The standard treatment of nonmuscle-invasive (Stage Ta, T1) UC of the bladder is TUR of all visible tumor, possibly followed by adjuvant intravesical chemotherapy or bacille Calmette-Guérin therapy.16 However, endoscopic resection is limited by visual identification of the tumor, especially in patients with greater tumor stages (T1). The rate of residual tumor after TUR has been 6%-78%.2,6 This rate increased with the extent of tumor infiltration noted on the initial TUR. It was 33%-78% after the resection of Stage T1 tumors and was only 6% after resection of Stage Ta tumors. Additionally, Schwabold et al4 reported that only 14% of the malignant tissue was at other locations outside the initial tumor resection area. Zurkirchen et al17 found no statistically significant difference in the residual tumor rates between the experienced urologist and trainee groups in their series of 214 patients who had undergone repeat TUR for Stage Ta or T1 bladder UC. Therefore, it is unlikely that the findings of residual tumor could have resulted from incomplete endoscopy or the lower experience of the operating surgeon performing TUR.
that in those without residual tumor before and after the initial TUR.

Multiple urine-based markers have been evaluated to determine whether they could assist in detecting residual tumor after TUR. However, patients with bladder cancer can have factors, such as hematuria, infection, instrumentation, or intravesical therapy, that can cause inaccurate readings in both bladder tumor markers and conventional cytology. In contrast, the DNA changes detected by FISH will not be affected by infection, hematuria, or instrumentation. Therefore, conversion from FISH-positive to FISH-negative status after TUR will indicate a tumor response to therapy and not simple manipulation of the karyotypic findings. This potentially supports the use of FISH for monitoring the success of TUR. Our results have suggested that the FISH assay could be valuable for the prediction of the residual tumor load after the initial TUR. The residual tumor, which could be the source of recurrence or progression, could potentially be identified and resected earlier, thus increasing the effectiveness of any adjuvant intravesical therapy. When the post-TUR FISH assay finding is positive after the initial TUR, it might be helpful to select those patients who require immediate re-evaluation. In contrast, if the post-TUR FISH assay finding is negative, the relative risk of missing residual tumor should be evaluated if the patient is not going to undergo a second procedure, a situation for which close follow-up is recommended. All patients in our cohort have received close follow-up for tumor recurrence and/or progression after surgery. For 9 patients with a positive FISH result but no residual tumor, 4 patients (44.4%) developed bladder tumor recurrence at a median follow-up of 19.4 months (range 16-29), in accordance with the published data.

Our series included 8 patients (8.8%) with Stage T2 bladder UC, for whom cystectomy was indicated, and 14 patients (15.4%) with Stage Ta, low-grade UC according to the pathohistologic results of the first TUR. For patients with Stage T2 UC, searching for residual tumor seems to be of less value if the patient will be undergoing cystectomy. However, it has been reported that a bladder with Stage pT0 at cystectomy would imply a lower tumor burden than a bladder with residual tumor. Those patients with muscle-invasive cancer who had no evidence of residual tumor in the cystectomy specimen had better survival. Additionally, we acknowledge that the indication for a second TUR for those with low-grade Stage Ta cancer is debatable. Some investigators have suggested a second TUR could provide valuable information to distinguish between residual tumor and true early recurrence at 3 months, considered a poor prognostic factor.

The positive and negative predictive value of FISH for residual tumor was 64.0% and 65.6%, respectively. These results have demonstrated that a positive FISH result can define the presence of residual tumor in 64.0% of patients. In contrast, a negative FISH result correctly predicted the absence of residual tumor in 65.6% of cases. However, 22 patients with residual tumor would have been missed if the FISH assay were used and 9 patients with a positive FISH result had no residual tumor at the second procedure. Thus, some limitations are still present in the use of FISH in predicting residual bladder UC after TUR. For example, considering that more than one half of the patients with residual tumor had had a negative FISH result after the initial TUR, omitting a second TUR was not an option. A positive FISH result would not change the current standard procedures for bladder UC treatment (ie, repeat TUR) because UC, especially nonmuscle-invasive bladder cancer, has a wide variation in clinical behavior. We agree that FISH should not be incorporated into rigid schemes of clinical decision-making for predicting residual tumor after TUR, and everyone should be aware of its restrictions. However, we would emphasize that the present preliminary study is the first in a series of our investigations. Additional prospective and multicenter trials with larger sample sizes are needed to confirm our results.

CONCLUSIONS

Our preliminary data have suggested that urinary FISH assay, in addition to providing high sensitivity in the detection of bladder UC, could also be used to predict the presence of residual tumor after TUR of bladder UC. Patients with a positive post-TUR FISH result were more likely to have residual tumor after surgery. Negative urinary FISH results after initial TUR could indicate the possibility of avoiding a second procedure. Additional larger, prospective, and multicenter trials are needed to confirm our results.

References


EDITORIAL COMMENT

Restaging resection after initial transurethral resection of bladder tumor (TURBT) for bladder cancer has gained favor in selected high-risk patients (in particular, high-grade and/or Stage T1) owing to the recognized high rate of incomplete resection of the initial tumor and the high rate of upstaging. Both of these facts have important implications for definitive treatment choices, such as the type of intravesical therapy or even the decision to proceed with radical cystectomy. Whether or not restaging TURBT should be routine remains controversial.

The commercial urine fluorescence in situ hybridization (FISH) assay, UroVysion (Abbott Molecular/Vysis, Des Plaines, IL), is more sensitive than urine cytology in tumor detection. Against this background, the authors of the present study examined the utility of the urine FISH results in predicting the presence of residual tumor after initial TURBT as a method to assess the need for restaging TURBT among 89 patients who underwent restaging TURBT or cystectomy within 4-6 weeks after their initial resection. Several comments about study design and methods are indicated before commenting on the results regarding the study hypothesis. First, 15.7% of the patients (14 of 89) had only low-grade, Stage Ta tumors. Most investigators would not recommend restaging TURBT for this patient group. Second, 8.9% of the patients (8 of 89) had Stage T2 tumors and neither the urine FISH result nor the results from restaging TURBT would likely change the decision to proceed with cystectomy in a medically suitable patient. Thus, the need for a costly follow-up urine FISH assay or the need for restaging TURBT with its attendant costs and risks could be questioned for 24.2% of the entire study group. Most importantly, among the 38 patients with residual tumor, the urine FISH assay results were positive for only 42.2% and were falsely negative in 57.8% of the cases. Furthermore, of the 51 patients who did not have residual tumor found on the restaging TURBT, the urine FISH assay was falsely positive in 17% of the cases.

The goal to optimize and selectively use restaging TURBT for patients in the greatest need is good. However, the results from the present study do not support the use of the urine FISH assay results after the initial TURBT to determine the need for restaging TURBT.

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doi:10.1016/j.urology.2010.10.047

REPLY

We appreciate the reviewer's comments. Our present study was mainly designed to assess the utility of fluorescence in situ hybridization (FISH) as a predictor of the residual tumor load after transurethral resection (TUR). Depending on the histo-