Multiprobe FISH for Enhanced Detection of Bladder Cancer in Voided Urine Specimens and Bladder Washings

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Key Words: Bladder cancer; FISH; Fluorescence in situ hybridization; Multiprobe; Voided urine; Bladder washing; Recurrence

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Abstract

The aim of this study was to evaluate the UroVysion (Vysis, Downers Grove, IL) fluorescence in situ hybridization (FISH) test for improved detection of bladder cancer in urinary specimens. Three groups of specimens were examined, including voided urine specimens (1) collected before resection of bladder cancer, (2) from cystoscopically negative bladders of patients with previous bladder cancer, and (3) from patients with benign prostatic hyperplasia (controls). FISH positivity was defined as more than 2 urothelial cells with an abnormal signal copy number of at least 1 of the 4 probes. FISH was positive in 1 of 27 control specimens and in 33 (73%) of 45 pTa, 12 (100%) of 12 pT1, and 13 (100%) of 13 pT2-4 tumors. The results were similar in a series of 68 bladder washings. In addition, FISH of voided urine specimens was positive in 5 of 10 patients with negative follow-up cystoscopy results. Subsequent recurrence was found in 4 of these patients but in none of 5 patients with FISH-negative results. Multiprobe FISH markedly improves the sensitivity and specificity of cytology for the detection of bladder cancer in urine specimens.

Diagnosis of primary and recurrent bladder cancer is one of the most difficult problems in urology and cytology. Bladder cancer is a chronic illness, and the patients need continuous surveillance for early detection of recurrence and progression. Noninvasive papillary urothelial tumors (pTa) recur in about 70% and progress in about 5% to invasive cancer.[1] The tumors with invasion limited to the lamina propria (stage pT1) pose the greatest clinical problem. Local progression to potentially life-threatening muscle-invasive cancer (pT2-4) occurs in 20% to 30% of these tumors after conservative surgical treatment.

Cystoscopy has been the standard method for diagnostic evaluation of patients with symptoms of bladder cancer. Cystoscopies at regular intervals also are necessary to monitor patients for recurrence or progression of previously treated bladder cancer. Standard cytology has been regarded as an additional diagnostic tool to select patients for cystoscopic evaluation. However, the sensitivity of cytology in urinary specimens is limited, since most of the noninvasive cancers (stage pTa) are missed.[2-4] Therefore, cytology alone is too unreliable to serve as a basis for therapy decisions. Several attempts have been made to improve the detection of cancer cells in urinary samples by multiple urinary tumor markers or test assays (reviewed by Ross and Cohen[4]). However, most of these markers are technically complicated and not sufficiently sensitive or specific to become an everyday tool to select patients for individual follow-up schemes or even to replace cystoscopy. The main problems are high false-positive rates owing to benign conditions and the lack of reproducibility of some tests if applied at different institutions.

Fluorescence in situ hybridization (FISH) is a rapid and powerful technique to detect cytogenetic abnormalities in malignant cells independent of their functional status.

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Previous studies by FISH or comparative genomic hybridization have shown a number of typical cytogenetic changes in bladder cancer, including increased copy numbers of virtually all chromosomes and deletions at 9p and 9q.[5-10] Detection of specific cytogenetic abnormalities by FISH in voided urine specimens or bladder washings has been suggested to facilitate diagnosis and detection of recurrence of bladder cancer.[11-17] However, these promising results have not gained access to broad diagnostic application. This may partly be due to the fact that commercial systems for FISH of urinary specimens have not been available.

The aim of the present study was to explore the diagnostic usefulness of a new multitarget, multicolor FISH assay (UroVysion, Vysis, Downers Grove, IL), which is composed of 3 chromosome enumeration probes (CEP17, CEP3, and CEP7) and the single locus-specific indicator probe 9p21. Our results show that this multicolor FISH probe is a rapid, simple, and powerful tool for an improved identification of bladder cancer in bladder washings and in voided urine specimens.

Materials and Methods

Patients and Samples

Three sets of prospectively collected urine specimens were examined: group 1, 74 voided urine specimens and 52 bladder washings that were collected immediately before therapeutic transurethral resection of 80 bladder tumors from 68 patients; group 2, 10 voided urine specimens and 7 bladder washings from 11 patients with a history of bladder cancer but no visible tumor at the time of follow-up cystoscopy; and group 3, 33 voided urine specimens and 16 bladder washings obtained before transurethral resection of the prostate for benign prostatic hyperplasia (BPH) from 38 patients without a history or clinical evidence of bladder cancer.

The bladder washings were obtained by instillation of 100 mL of 0.9% sodium chloride in the bladder before transurethral resection. The whole volume of voided urine specimens or bladder washings was centrifuged at 2,850*g* for 10 minutes (Heraeus centrifuge, Sepatech, Osterode, Germany). The pellet was resuspended in 10 mL of the supernatant and recentrifuged. Then 50 µL of the sediment was centrifuged at 72.26*g* using a Cytospin 3 centrifuge (Shandon, Life Sciences International, Astmoor, England), resulting in 2 to 30 slide preparations. One slide was fixed with SprayFix (Medite, Burgdorf, Germany) and stained with standard Papanicolaou. The other slides were air dried and stored at –70°C for subsequent FISH analysis. A cell density between 100 and 200 cells per visual field using a 20× objective was regarded as optimal for analysis. The cell density was adjusted by dilution with supernatant if it was more than 400 per field.

The cytologic grading was done by one cytopathologist (P.D.) in a blinded fashion according to the following criteria: G0, no atypia; GI, slight atypia (not diagnostic of neoplasia); GII, moderate atypia; and GIII, severe atypia. For statistical analysis, G0 and GI were regarded as negative and GII and GIII as positive by cytology. Cytologic grading was not possible because of poor fixation or inflammatory changes in 11 cases, including 6 voided urine specimens from 6 patients with BPH and 4 voided urine specimens and 1 bladder washing from 5 patients with tumor. Histologic examination was performed in all cases of transurethral resections of bladder tumors and transurethral resections of BPH. The pT stage of the 80 tumors was determined according to the International Union Against Cancer criteria.[18] There were 53 pTa, 13 pT1, and 14 pT2-4 tumors. Histologic grading was done on 63 evaluable specimens according to World Health Organization criteria[19] and showed 23 grade I, 35 grade II, and 20 grade III tumors. Histologic grading was not possible in 2 specimens because of heat artifacts.

FISH Assay

The multitarget, multicolor FISH probe obtained from Vysis includes probes for CEP17, CEP3, CEP7, and 9p21 labeled with different fluorescent dyes (aqua, spectrum red, spectrum green, and gold). FISH was performed according to the recommendations of the manufacturer with minor modifications. Briefly, air-dried slides were incubated in 0.5 mg/mL of pepsin in 0.01N hydrochloric acid at 37°C for 10 minutes and washed for 5 minutes in 1× phosphate-buffered saline at room temperature. The slides then were fixed in 1% formaldehyde for 5 minutes and washed for 5 minutes in 1× phosphate-buffered saline at room temperature. The slides were placed in Carnoy fixative (3:1 methanol and acetic acid) for 3 times for 10 minutes each and placed in 70%, 80%, and 100% ethanol for 1 minute each. The slides then were denatured in 2× standard saline citrate (SSC)/70% formamide at 73°C for 5 minutes and dehydrated in 70%, 80%, and 100% ethanol for 1 minute each. The slides were air-dried, and 3 µL of the probe was applied to the slide. The slide was coverslipped, sealed with rubber cement, and incubated at 37°C overnight in a humidified chamber. Posthybridization washings were made in 0.4× SSC/0.3% 4-nonylphenolpolyethyleneglycol (NP-40, BDH Laboratory Supplies, Poole, England) for 2 minutes at 73°C. The slides were rinsed in 2× SSC/0.1% NP-40 at room temperature for 2 minutes, air dried, and counterstained with 4,6-diamidine, 2-phenylindole dihydrochloride (DAPI) solution.

Enumeration of FISH Signals

The slides were scored for hybridization signals on a cell-by-cell basis using a Zeiss Axioplan fluorescence microscope (Zeiss, Jena, Germany) with a filter set including DAPI single

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bandpass (DAPI counterstain), aqua single bandpass (chromosome 17), yellow single bandpass (9p21 locus), and red/green dual bandpass (chromosomes 3 and 7). Enumeration of the FISH signals was done on target cells that appeared morphologically abnormal, as suggested by the manufacturer. These target cells were selected based on large nuclear size, irregular nuclear shape, "patchy" DAPI staining, and cell clusters. In cases of a low number of morphologically abnormal nuclei, cells with the largest nuclei were chosen.

The chromosome pattern in the selected target cells was recorded, if aqua (CEP17), green (CEP7), or red (CEP3) showed 3 or more signals and/or there were more than 2 or fewer than 2 gold signals (9p21). This was repeated until 25 such target cells had been analyzed or until the entire sample had been screened. If fewer than 25 target cells were found, the remaining cells were marked as disomic (ie, 2-2-2-2). This scanning method has been shown to be more efficient and more sensitive than the traditional counting of signals in at least 100 cells.[20] Overlapping cells and cells with blurry signals were not analyzed. Signals that were located very close to each other were interpreted as split signals and counted as 1 signal.

Defining the Cutoff for a Positive FISH Result

In a first analysis, we applied the criteria for a FISH-positive result suggested by the manufacturer of the UroVysion assay based on a previous study.[20] Accordingly, a specimen was considered FISH positive for bladder cancer if at least 1 of the following criteria were met: (1) 5 or more cells with gain of more than 1 chromosome, (2) 10 or more cells with gain of a single chromosome, or (3) 10 or more cells with homozygous loss of the 9p21 locus (both copies lost).

Then we adjusted the criteria based on our hypothesis that the diagnostic value of rare cells with a tetrasomic pattern was less strong than the diagnostic value of cells with other types of aneusomy. A tetrasomic FISH pattern was defined as the presence of 4 copies of each of the 4 probes in a cell. In this second analysis, the presence of 4 or fewer tetrasomic cells was regarded as normal. A nontetrasomic cell was regarded abnormal by FISH if it showed 3 or more copies of any of the signals for chromosomes 3, 7, and 17 and the 9p21 locus, or if there was heterozygous or homozygous loss of 9p21 (one copy or both copies lost). Since tetrasomic cells were not counted, we could lower the cutoff of the number of aneusomic cells to define a FISH-positive specimen and still retain a high specificity. The best sensitivity with the best specificity was found at a cutoff of more than 1 abnormal cell **Table 1**. To further avoid false-positive results, we set the cutoff for a FISH-positive specimen at more than 2 abnormal cells. This cutoff provided a similarly high sensitivity and the same specificity as a cutoff more than 1.

Table 1
Defining the Optimal Cutoff Values for FISH-Positive Voided Urine Specimens*

FISH-Positive Cells	Sensitivity (%) pTa	Sensitivity (%) pT1-4	Specificity (%)†
>0	83.3	100	81.8
>1	77.1	100	97.0
>2	72.9	100	97.0
>3	68.8	100	97.0
>4	66.7	100	97.0
No. of specimens	48	26	33

FISH, fluorescence in situ hybridization.
* FISH-positive cells were defined as a gain of chromosome 3, 7, or 17 or any copy number change of 9p21 including gain or heterozygous or homozygous loss. Specimens with fewer than 5 tetraploid cells (4 of each signal) but no other abnormality by FISH were considered FISH-negative.
† Based on a series of 33 samples from patients with benign prostatic hyperplasia (negative control group).

Statistics

Contingency table analysis was used to calculate the association between grading, pT stage, and FISH results. For the analysis of sensitivity, specificity, and positive and negative predictive values of multiprobe FISH, only the cases were included in which cytologic grade also was available for comparison. Estimation of recurrence-free survival was performed by a log-rank test.

Results

Detection of Bladder Cancer With FISH Compared With Cytology

The sensitivity of the multiprobe FISH assay for the detection of bladder tumors was markedly higher compared with cytology **Table 2** and **Table 3**. Representative hybridization images of FISH-negative and FISH-positive urothelial cells are shown in **Image 1**.

Using the previously suggested criteria to define FISH-positive specimens,[20] FISH detected almost twice as many pTa and pT1 tumors from patients with histologically confirmed bladder tumors (group 1) compared with cytology (Table 2). Most important, FISH on urine specimens identified 22 (88%) of the 25 invasive tumors (pT1 and pT2-4), whereas 8 (32%) of these tumors were missed by cytology. The sensitivity of FISH could be increased by applying adjusted criteria for the definition of a FISH-positive specimen (Table 3). With the adjusted criteria, FISH detected 3 times more pTa tumors than cytology (33/45 [73%] vs 11/45 [24%]) and was positive in all invasive tumors (pT1-4), in which cytology failed to detect 6 (50%) of 12 pT1 and 2 (15%) of 13 pT2-4 tumors.

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Table 2
Multiprobe FISH and Cytology for the Detection of Bladder Tumors in Voided Urine Specimens and Bladder Washings*

	Voided Urine Specimens				Bladder Washings			
	No. of Specimens	Cytology	FISH†	Combined‡	No. of Specimens	Cytology	FISH†	Combined‡
BPH	27	3 (11)	1 (4)	4 (15)	16	2 (12)	1 (6)	3 (19)
pTa	45	11 (24)	19 (42)	23 (51)	34	14 (41)	24 (71)	29 (85)
pT1	12	6 (50)	10 (83)	10 (83)	8	5 (62)	7 (88)	8 (100)
pT2-4	13	11 (85)	12 (92)	13 (100)	9	8 (89)	8 (89)	9 (100)

BPH, benign prostatic hypertrophy; FISH, fluorescence in situ hybridization.
* Using previously suggested criteria to define FISH-positive specimens. Data are given as number (percentage) of positive specimens. The BPH group was the control group.
† Criteria to define FISH-positive specimens as suggested by the manufacturer (Vysis, Downers Grove, IL): 5 or more cells with a gain of more than 1 chromosome, 10 or more cells with a gain of a single chromosome, or 10 or more cells with homozygous loss of the 9p21 locus.
‡ Positive by FISH or by cytology.

Table 3
Multiprobe FISH and Cytology for the Detection of Bladder Tumors in Voided Urine Specimens and Bladder Washings*

	Voided Urine Specimens				Bladder Washings			
	No. of Specimens	Cytology	FISH†	Combined‡	No. of Specimens	Cytology	FISH†	Combined‡
BPH	27	3 (11)	1 (4)	4 (15)	16	2 (12)	1 (6)	3 (19)
pTa	45	11 (24)	33 (73)	35 (78)	34	14 (41)	31 (91)	32 (94)
pT1	12	6 (50)	12 (100)	12 (100)	8	5 (62)	8 (100)	8 (100)
pT2-4	13	11 (85)	13 (100)	13 (100)	9	8 (89)	9 (100)	9 (100)

BPH, benign prostatic hypertrophy; FISH, fluorescence in situ hybridization.
* Using adjusted criteria to define FISH-positive specimens (>2 cells with gain of chromosome 3, 7, or 17 or any copy number change of 9p21 including gain or heterozygous or homozygous loss; specimens with <5 tetraploid cells [4 of each signal] but no other abnormality by FISH were considered negative). Data are given as number (percentage) of positive specimens. The BPH group was the control group.
† Positive by FISH or by cytology.

The analysis of the 27 voided urine specimens from the control group (group 3) of patients with BPH revealed a false-positive result by cytology in 3 cases and by FISH in 1 case. The positive and negative predictive values of FISH for bladder tumors (pTa-pT4) were 98% and 68%, respectively. The sensitivity of FISH was high (71%-94%) across all histologic grades, whereas cytology missed 86% of the highly differentiated (GI) tumors **Table 4**.

Separate analysis of severe cytologic atypia (GIII) showed that GIII cytology was never present in the control specimens from 27 voided urine specimens or 16 bladder washings. In addition, all 31 cytologic GIII specimens in the present study were positive by FISH. The combination of FISH and cytology resulted in a slightly increased sensitivity for the detection of pTa tumors compared with FISH alone, yet at the cost of decreased specificity (Tables 2 and 3). The results for bladder washings were similar to those for voided urine specimens, although the sensitivity for the detection of noninvasive (pTa) tumors by both cytology and FISH was somewhat higher in bladder washings than in voided urine specimens (Tables 2 and 3). Separate analysis of the 4 individual FISH probes in voided urine specimens showed that the sensitivity of the combined probe tended to be higher than the sensitivity of each individual probe **Table 5**. This was most apparent in the group of pTa tumors, in which the sensitivity could be increased from 52% to 65% to 73%. The sensitivity for the detection of pT1-4 tumors was highest for the CEP17 probe, which identified 100% of these tumors. There was no predilection for any probe in the false-positive cases.

A tetraploid FISH pattern with 4 signals of each of the probes as the only alteration in bladder washings or voided urine specimens was found in 11 (29%) of specimens from 38 patients with BPH but in only 4 (5%) of 79 specimens from patients with pTa tumors and in none of 43 specimens from patients with pT1-4 tumors. This tetraploid-only FISH pattern was always restricted to not more than 4 cells in the 11 BPH samples but was found in a higher number of cells in 2 of the 4 pTa tumors (9 and 14 tetraploid cells, respectively).

In the 10 patients with negative follow-up cystoscopy results, a positive FISH result in voided urine specimens was highly predictive of subsequent recurrence when our adjusted criteria for a FISH-positive specimen were applied. Recurrence was found in 4 of 5 patients with FISH-positive results (mean ± SD follow-up, 7.9 ± 1.6 months) but in none

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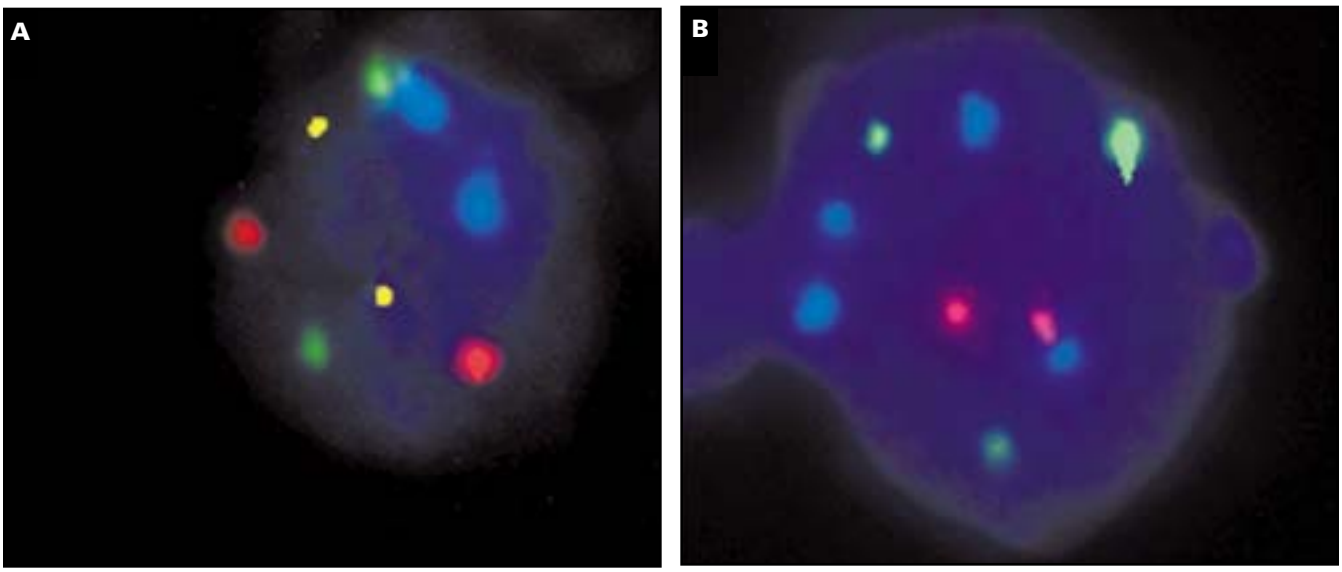

Image 1 Representative multicolor fluorescence in situ hybridization images (×1,000). **A**, Normal urothelial cell with 2 copies of chromosomes 3 (spectrum red), 7 (spectrum green), and 17 (aqua) and the 9p21 locus (gold). **B**, Urothelial carcinoma cell with 2 copies of chromosome 3 (spectrum red), increased copy numbers of chromosome 7 (spectrum green, 3 signals) and 17 (aqua, 4 signals), and loss of both copies of the 9p21 locus (no gold signal).

of 5 patients with FISH-negative results (mean ± SD follow-up, 18 ± 1.6 months; $P = .0034$). There was a high concordance between FISH in voided urine specimens and bladder washings in the 63 patients from whom matched specimens were available **Table 6**. Discrepant results were found in only 9 (14%) of these patients and mostly were restricted to patients with a FISH-positive bladder washing but a FISH-negative voided urine specimen.

Table 4
Multiprobe FISH and Cytology for the Detection of 67 Bladder Tumors Across Histologic Grades I Through III in Voided Urine Specimens*

Histologic Grade	Cytology	FISH†	Combined‡
GI (n = 21)	3 (14)	15 (71)	15 (71)
GII (n = 29)	12 (41)	25 (86)	27 (93)
GIII (n = 17)	13 (76)	16 (94)	16 (94)

FISH, fluorescence in situ hybridization.
* Data are given as number (percentage) of positive specimens.
† Adjusted criteria to define FISH-positive specimens: more than 2 cells with gain of chromosome 3, 7, or 17 or any copy number change of 9p21 including gain or heterozygous or homozygous loss. Specimens with fewer than 5 tetraploid cells (4 of each signal) but no other abnormality by FISH were considered negative.
‡ Positive by FISH or by cytology.

Table 5
Comparison Between Individual FISH Probes and the Combined Probe in 107 Voided Urine Specimens*

	Centromeric Probe				
	3	7	17	9p21†	Combined‡
BPH (n = 33)	2 (6)	2 (6)	2 (6)	2 (6)	1 (3)
pTa (n = 48)	31 (65)	25 (52)	27 (56)	30 (62)	72.9
pT1 (n = 12)	11 (92)	10 (83)	12 (100)	11 (92)	12 (100)
pT2-4 (n = 14)	14 (100)	14 (100)	14 (100)	13 (93)	14 (100)

BPH, benign prostatic hypertrophy; FISH, fluorescence in situ hybridization.
* Data are given as number (percentage). Criteria to define FISH-positive specimens based on individual probes: more than 2 cells with increased copy numbers of the individual chromosomes 3, 7, and 17.
† For 9p21, any copy number change in more than 2 cells was recorded as positive including gain or heterozygous or homozygous deletion.
‡ In the combined analysis, specimens with fewer than 5 tetraploid cells (4 of each signal) but no other abnormality by FISH were considered negative.

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Table 6
Correlation Between Multiprobe FISH in Voided Urine Specimens and Matched Bladder Washings From 63 Patients With Urothelial Bladder Tumors

FISH Result for Urine Specimen/ Bladder Washing	No. (%) of Specimens
+/+	40 (63)
–/–	14 (22)
+/–	1 (2)
–/+	8 (13)
Total	**63 (100)**

FISH, fluorescence in situ hybridization; +, positive; –, negative.

Discussion

The results of the present study strongly suggest that the UroVysion multiprobe FISH is a highly sensitive and specific tool for the detection of bladder cancer in voided urine specimens and bladder washings**.**

Given the limited sensitivity of standard cytology for the detection of bladder cancer in voided urine specimens, regular follow-up cystoscopies are still needed to monitor patients with a history of bladder cancer for recurrence or progression. Ancillary methods with improved sensitivity of urocytologic examination are of high clinical interest, since they could be used to select patients for tailored follow-up schemes based on the individual risk of tumor recurrence or progression. This eventually would minimize cystoscopies in patients at low risk. In addition, such tests could facilitate the diagnosis of urothelial tumors of the upper urogenital tract, which constitute about 5% of all urothelial tumors. Since bladder cancer development and progression are associated with a high number of numeric and structural chromosomal alterations,[5,6,10,21] it is not surprising that their detection in voided urine specimens or bladder washings can be used for noninvasive diagnosis of bladder cancer.

In our study, UroVysion multicolor FISH had a high sensitivity and specificity for the detection of bladder tumors. The 4 probes included in this assay have been suggested previously to show the highest sensitivity for urothelial cancer detection from a series of 10 different probes.[20] By using this multicolor FISH assay, we could detect almost all invasively growing tumors including those in which infiltration was restricted to the lamina propria (pT1). The reliable detection of invasive bladder cancers (pT1-4) is essential, since these tumors are potentially life-threatening and need to be treated. FISH also identified a large fraction of the noninvasive bladder tumors (pTa). In contrast, the sensitivity of standard cytology was markedly lower for both noninvasive and invasive urothelial tumors. The poor performance of standard cytology is not surprising and is in the ranges found in previous studies.[2,3,22,23] The low detection rate of well-differentiated tumors (GI, 14%) reflects the common experience that it is virtually impossible to distinguish these tumors from reactive urothelial changes in urinary samples.[24-26] These difficulties also are emphasized by the fact that slight or moderate urothelial atypia (GI-GII) was diagnosed in 33% of the voided urine specimens of benign BPH control specimens (data not shown). In contrast, the presence of severe cytologic atypia (GIII) is highly specific for bladder cancer. This also is emphasized by the fact that specimens with severe cytologic atypia were always positive by FISH.

In the present study, we initially used the criteria for a FISH-positive specimen suggested by the manufacturer of the UroVysion assay. These criteria had been selected in a preclinical study by Sokolova et al.[20] Our results are in agreement with those of Sokolova et al,[20] which showed a markedly higher sensitivity of FISH for the detection of bladder tumors compared with cytology in 22 pTa and 12 pT1-4 tumors (65% vs 47% and 95% vs 60%). Our data also confirm the previous finding that the combination of the 4 probes has a higher sensitivity than each single probe alone.[20] We could increase the sensitivity and retain the high specificity by using adjusted criteria. We detected 33 (73%) of the 45 noninvasive and 100% of the 25 invasive (pT1-4) bladder tumors with a specificity of 96%. These results are almost identical to the results of a recent report by Halling et al,[22] in which the same multicolor FISH test applied to voided urine specimens detected 65% of 37 pTa and 95% of 19 pT1-4 bladder tumors with a specificity of 96%. In that study, the authors used the manufacturer's criteria for a FISH positivity.[20]

Our results suggest that the optimal criteria to define a FISH-positive result are not absolutely clear. It seems that not all FISH aberrations are equally important. In our nonneoplastic control group, we found a particularly high frequency of tetrasomic cells as the only chromosomal abnormality. Therefore, one should be cautious about considering a tetraploid FISH pattern as FISH positive unless it is found in a large fraction of cells. This fits previous studies using DNA cytometry, in which tetraploidy frequently was found in tumor-negative patients or associated with bladder tumors of low histologic grade and stage.[27,28] To take into account a reduced significance of tetrasomic cells compared with other aneusomies, we defined an alternative scoring system. By using these new criteria, we found highly improved sensitivity and specificity in our cases compared with cytology. Additional studies on larger series are required to fine-tune the definition of FISH-positive specimens.

We also included a small series of patients who had no visible bladder tumor at the time of follow-up cystoscopy. Subsequent recurrence in 4 of 5 cystoscopy-negative, FISH-positive patients (but in none of 5 cystoscopy-negative,

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FISH-negative patients) raises the possibility that FISH also may be useful for early prediction of recurrence. This observation fits previous models that bladder cancer represents a field defect and that numeric chromosomal aberrations already can be found in morphologically normal urothelial cells of affected patients.[29] Our results also suggest that patients with a negative urine FISH result are at a low risk for early recurrence and might be candidates for a reduced frequency of follow-up cystoscopies. Most important, invasive bladder cancer can virtually be excluded in case of a negative FISH result. Hence, one could conclude that only patients with a positive FISH result definitely need to undergo cystoscopy. Regular FISH controls of voided urine to exclude progression to invasive bladder cancer might be an option in the follow-up of patients with low grade pTa tumors. Additional studies with more patients are needed to confirm an application of the UroVysion FISH test as a predictor of an increased risk of subsequent recurrence in patients with bladder cancer with negative cystoscopic results. Currently, the reimbursement for the UroVysion test can be less than $200, including the costs of the assay, labor, and infrastructure. However, one also needs to consider that multicolor FISH may ultimately lead to a decreased frequency of follow-up cystoscopies in many patients with a low recurrence risk in cases of a negative FISH result. Further studies are needed to define the cost/benefit ratio of multicolor FISH for the diagnosis of primary bladder cancer and surveillance for detection of recurrence.

Our data show that UroVysion multicolor FISH is a highly sensitive and specific method for the detection of bladder cancer in voided urine specimens and bladder washings. FISH is easy to perform in routine cytology laboratories and has a high potential to improve the management of patients with symptoms of primary bladder cancer or during surveillance for detection of recurrence.

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References

1. Greene LF, Hanash KA, Farrow GM. Benign papilloma or papillary carcinoma of the bladder? *J Urol*. 1973;110:205-207.
2. Maier U, Simak R, Neuhold N. The clinical value of urinary cytology: 12 years of experience with 615 patients. *J Clin Pathol*. 1995;48:314-317.
3. Mian C, Pycha A, Wiener H, et al. Immunocyt: a new tool for detecting transitional cell cancer of the urinary tract. *J Urol*. 1999;161:1486-1489.
4. Ross JS, Cohen MB. Ancillary methods for the detection of recurrent urothelial neoplasia. *Cancer*. 2000;90:75-86.
5. Richter J, Jiang F, Gorog JP, et al. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. *Cancer Res*. 1997;57:2860-2864.
6. Richter J, Beffa L, Wagner U, et al. Patterns of chromosomal imbalances in advanced urinary bladder cancer detected by comparative genomic hybridization. *Am J Pathol*. 1998; 153:1615-1621.
7. Zhao J, Richter J, Wagner U, et al. Chromosomal imbalances in noninvasive papillary bladder neoplasms (pTa). *Cancer Res*. 1999;59:4658-4661.
8. Reeder JE, Morreale JF, O'Connell MJ, et al. Loss of the CDKN2A/p16 locus detected in bladder irrigation specimens by fluorescence in situ hybridization. *J Urol*. 1997;158:1717-1721.
9. Pycha A, Mian C, Haitel A, et al. Fluorescence in situ hybridization identifies more aggressive types of primarily noninvasive (stage pTa) bladder cancer. *J Urol*. 1997; 157:2116-2119.
10. Sauter G, Gasser TC, Moch H, et al. DNA aberrations in urinary bladder cancer detected by flow cytometry and FISH. *Urol Res*. 1997;25(suppl 1):S37-S43.
11. Meloni AM, Peier AM, Haddad FS, et al. A new approach in the diagnosis and follow-up of bladder cancer: FISH analysis of urine, bladder washings, and tumors. *Cancer Genet Cytogenet*. 1993;71:105-118.
12. Cajulis RS, Haines GK III, Frias-Hidvegi D, et al. Cytology, flow cytometry, image analysis, and interphase cytogenetics by fluorescence in situ hybridization in the diagnosis of transitional cell carcinoma in bladder washes: a comparative study. *Diagn Cytopathol*. 1995;13:214-223.
13. Zhang FF, Arber DA, Wilson TG, et al. Toward the validation of aneusomy detection by fluorescence in situ hybridization in bladder cancer: comparative analysis with cytology, cytogenetics, and clinical features predicts recurrence and defines clinical testing limitations. *Clin Cancer Res*. 1997;3:2317-2328.
14. Jung I, Reeder JE, Cox C, et al. Chromosome 9 monosomy by fluorescence in situ hybridization of bladder irrigation specimens is predictive of tumor recurrence. *J Urol*. 1999; 162:1900-1903.
15. Marano A, Pan Y, Li C, et al. Chromosomal numerical aberrations detected by fluorescence in situ hybridization on bladder washings from patients with bladder cancer. *Eur Urol*. 2000;37:358-365.
16. Junker K, Werner W, Mueller C, et al. Interphase cytogenetic diagnosis of bladder cancer on cells from urine and bladder washing. *Int J Oncol*. 1999;14:309-313.
17. Inoue T, Nasu Y, Tsushima T, et al. Chromosomal numerical aberrations of exfoliated cells in the urine detected by fluorescence in situ hybridization: clinical implication for the detection of bladder cancer. *Urol Res*. 2000;28:57-61.
18. Sobin LH, Wittekind C (International Union Against Cancer [UICC]), eds. *TNM Classification of Malignant Tumours*. 5th ed. Baltimore, MD: Wiley-Liss; 1997.
19. Mostofi SK, Sobin LH, Torloni H. *Histological Typing of Urinary Bladder Tumors*. Geneva, Switzerland: World Health Organization; 1973. *International Histological Classification of Tumors*, vol 10.
20. Sokolova I, Halling KC, Jenkins RB, et al. The development of a multitarget, multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. *J Mol Diagn*. 2000;2:116-123.

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21. Richter J, Wagner U, Schraml P, et al. Chromosomal imbalances are associated with a high risk of progression in early invasive (pT1) urinary bladder cancer. *Cancer Res*. 1999;59:5687-5691.
22. Halling KC, King W, Sokolova IA, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *J Urol*. 2000;164:1768-1775.
23. Casella R, Huber P, Blochlinger A, et al. Urinary level of nuclear matrix protein 22 in the diagnosis of bladder cancer: experience with 130 patients with biopsy confirmed tumor. *J Urol*. 2000;164:1926-1928.
24. Koss LG. Tumors of the bladder. In Koss LG, ed. *Diagnostic Cytology of the Urinary Tract*. Philadelphia, PA: Lippincott-Raven; 1996:71-139.
25. Bastacky S, Ibrahim S, Wilczynski SP, et al. The accuracy of urinary cytology in daily practice. *Cancer*. 1999;87:118-128.
26. Renshaw AA. Subclassifying atypical urinary cytology specimens. *Cancer*. 2000;90:222-229.
27. Kline MJ, Wilkinson EJ, Askeland R, et al. DNA tetraploidy in Feulgen-stained bladder washings assessed by image cytometry. *Anal Quant Cytol Histol*. 1995;17:129-134.
28. Tribukait B, Gustafson H, Esposti PL. The significance of ploidy and proliferation in the clinical and biological evaluation of bladder tumours: a study of 100 untreated cases. *Br J Urol*. 1982;54:130-135.
29. Hartmann A, Moser K, Kriegmair M, et al. Frequent genetic alterations in simple urothelial hyperplasias of the bladder in patients with papillary urothelial carcinoma. *Am J Pathol*. 1999;154:721-727.

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