Comparison of Fluorescence *In Situ* Hybridization –FISH– and Conventional Cytology for Early Detection of Urothelial Carcinoma.

Nadina Erill[1], Anna Colomer[1], Míriam Gorriz[2], Naim Hannaoui[3], Ruth Román[1], Maria Conangla[2], Josep M Banús[3], Carlos Cordón-Cardo[1,2,4], Xavier Puig[1,2].

[1]BIO**PAT**, Grup Assistència, Barcelona, Spain; [2]HISTOPAT Laboratoris, Barcelona, Spain; [3]Institut Català d'Urologia i Nefrologia, ICUN; Barcelona, Spain; and [4]Division of Molecular Pathology, Memorial Sloan-Kettering Cancer Center, New York.

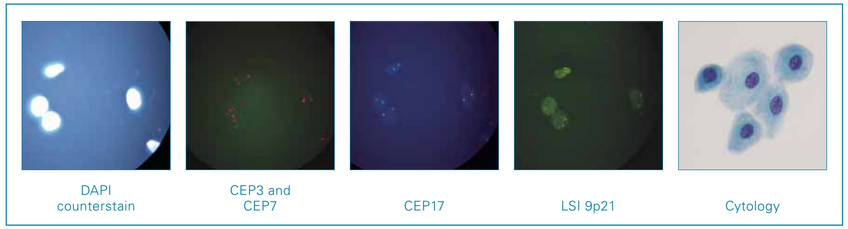

Figure 1

Representative multicolor fluorescence in situ hybridization images (x1000) of normal urothelial cells with 2 copies of each chromosome. Cytology of the same sample is also shown (Papanicolau stain, x400)

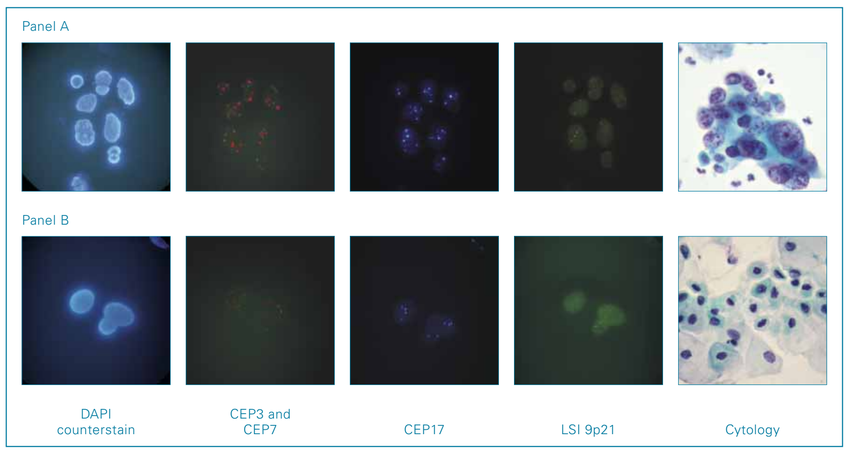

Figure 2

Examples of positive FISH results from patients with biopsy proven urothelial carcinoma (x1000).

Panel A.
Voided urine cell nuclei displaying severe atypical features such as nuclear enlargement, irregular shape, heterogeneous chromatin texture and clustering. Cells display gains of the four chromosomes analyzed. Abnormal cytological features, consistent with urothelial carcinoma, can also be appreciated with Papanicolau stain of the same sample (x400).

Panel B.
A normal urothelial cell with 2 copies of each chromosomes is shown close to two urothelial carcinoma cells with increased copy numbers of chromosomes 3, 7 and 17, and homozygous deletion of 9p21 locus, evidenced as no gold signals. Atypical transitional cells are evidenced in cytology (Papanicolau stain, x400).

REFERENCES

- Mostofi FK, Davis CJ, Sesterhenn IA. Histological typing of urinary bladder tumours. Whorld Helath Organization International Histological classification of tumours. Heidelberg: Springer-Verlag,1999.
- Halling KC, King W, Sokolova IA, Meyer RG, Burkhardt HM, Halling AC et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. *J Urol* 2000;164:1768-75.
- Skacel M, Fahmy M, Brainard JA, Pettay JD, Biscotti CV, Liou LS et al. Multitarget fluorescence in situ hybridization assay detects transitional cell carcinoma in the majority of patients with bladder cancer and atypical or negative urine cytology. *J Urol* 2003;169:2101-5.
- Bubendorf L, Grilli B, Sauter G, Mihatsch MJ, Gasser T, Dalquen P. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. *Am J Clin Pathol* 2001;116:79-86.
- Placer J, Espinet B, Salido M, Solé F, Gelabert-Mas A. Clinical utility of a multiprove FISH assay in voided urine specimens for the detection of bladder cancer and its recurrences, compared with urinary cytology. *Eur Urol* 2002;42:547-52.
- Koss LG, Deitch D, Ramanathan R, Sherman AB. Diagnostic value of cytology of voided urine. *Acta Cytol* 1985;29:810-6.

Bladder cancer is, in most cases, a chronic illness, and patients need continuous surveillance for early detection of recurrence and progression. Noninvasive papillary urotelial tumors (pTa) recur in about 70% of cases, and progress in about 5% to invasive cancer. The tumors with invasion to the lamina propria (stage pT1) stand for the greatest clinical problem, thus 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 and recurrence monitoring of bladder carcinoma. As this is an invasive technique that causes discomfort for the patients and incurs risk, urinary cytology has become the reference standard for the noninvasive diagnosis and follow up of bladder cancer. However, the sensitivity of cytology in urinary specimens is limited, since most of the low grade cancers are missed.

The multicolor, multitarget interphase fluorescence in situ hybridization (FISH) has been recently shown to have high sensitivity and specificity for detecting transitional carcinoma cells in urine specimens. This technology is based on the presence of frequent chromosomal alterations in uroepithelial cancer cells, previously demonstrated by cytogenetic studies, and consists of probes to the centromeres of chromosomes 3, 7 and 17, and to the 9p21 region.

The aim of the present study is to evaluate the use of the UroVysion™ Multicolor FISH assay (Vysis, Downers Gorve, IL) in voided urine and bladder washing specimens for the early detection of bladder cancer and recurrent lesions, and comparing the results with those obtained by conventional urine cytology.

MATERIALS AND METHODS

Patient Population.
A total of 138 voided urine specimens from 92 patients of the area of Barcelona (Spain) were collected from October 2003 to October 2004 for FISH and conventional cytology analysis. Seventy-three out of the 138 samples were consecutive and spontaneous urine specimens, whereas 65 consisted in bladder washings obtained before cystoscopy or transurethral resection of a bladder tumor. The study population comprised 79 men and 13 women (median age 68±9.6 years, range 43 to 96) distributed into two clinical categories: 52 patients having a history of urothelial carcinoma and following conventional controls for recurrence (n= 93 samples), and 40 patients without urothelial cancer antecedents, being evaluated for a variety of genitourinary symptoms and signs, including irritative voiding symptoms or hematuria (n= 45 samples). All patients were visited and treated by the same urology medical team (ICUN). A positive diagnosis was confirmed by biopsy in 38 samples.

Conventional Cytology.
Urine specimens were collected in three consecutive-day samples. Approximately 50 mL used to obtain, after cytospin centrifugation (Shandon, Life Sciences Int., Astmoor, England), resulting in 2 slide preparations per sample that were fixed with 96% ethanol and stained with standard Papanicolau. Slides were evaluated as negative for malignant cells, atypia or suspicious of malignity and positive for malignant cells (consistent with urothelial carcinoma). For sensitivity analysis, suspicious results were scored as positive.

Histology.
Histologic examination was performed in all cases with transurethral resections of bladder tumors. Specimens were processed for routine histopathology by formalin fixation and paraffin embedding. Stage was assigned following the TNM pathological staging system. Tumor stage was assigned as follows: pTa (n=20), pT1 (n=14), and pT2 (n=4). All tumors were also graded following the 1973 WHO classification and according to the Malstroms/Bergkvist system, resulting in grade 1 (n=5), grade 2 (n=9) and grade 3 (n=24).

Fluorescence In Situ Hybridization.
Urine specimens (at least 35 mL) were processed the same day of collection or kept at 4ºC in 2% Carbowax solution for 1-2 days. The laboratory process was performed according to the instructions of the manufacturer. The probe mixture consisted of four fluorescently labeled probes targeted to the peri centromeric regions of chromosomes 3 (CEP3, spectrum red), 7 (CEP7, spectrum green), and 17 (CEP17, spectrum aqua), and to band 9p21 locus (LSI 9p21, spectrum gold). DAPI/Antifade was used as counterstain.

FISH evaluation: The hybridization signals of FISH slides were scored cell-by-cell using a fluorescence microscope equipped with DAPI, aqua, yellow and red-green band-pass filters. Slides were evaluated by scanning at least 60 epithelial nuclei, despite of its normal or athypical morphology. Identification of five or more cells with polysomy, defined as gains of two or more chromosomes in a cell, or the presence of more than 50% of the nuclei with an homozygous loss of 9p21 signals was considered to define positivity for chromosomal abnormalities associated to urothelial cancer cells.

RESULTS

Results obtained from cytology and FISH analysis are summarized in Tables 1 to 3, and representative cases examples of both assays can be appreciated in Figures 1 and 2.

Table 1A summarizes results obtained when analyzing specimens from patients with diagnosed bladder tumors confirmed by biopsy (n=38). The clinical indication of samples analyzed were 12 clinical symptoms of de novo carcinoma and 26 follow up controls (with or without clinical signs of recurrence). The overall frequency of positive results for cytology analysis in this group of samples is almost 66%, whereas for FISH analysis it rises up to 84%. Complementarily, Table 1B summarizes results obtained when analyzing samples not associated to an urothelial carcinoma positive diagnosis. Of these 100 samples analyzed, 67 were follow up controls (with and without clinical signs of recurrence) and 33 corresponded to other genitourinary pathologies including irritative voiding symptoms and hematuria associated to infectious disease. It is important to note that no suspicious FISH results, defined as presence of two or more polysomic chromosomes in less than 5 nuclei, were observed in this series.

Table 1A

UROTHELIAL CARCINOMA			
	FISH		
CYTOLOGY	Negative	Positive	Total
Negative	5	8	13
Positive	0	22	22
Suspicious	1	2	3
Total	6	32	38

Table 1B

NO UROTHELIAL CARCINOMA			
	FISH		
CYTOLOGY	Negative	Positive	Total
Negative	97	0	97
Positive	0	0	0
Suspicious	3	0	3
Total	100	0	100

Table 2
Comparison of cytology and FISH results for bladder cancer detection in relation to the histological grade of its biopsy (n=38).

	CYTOLOGY		FISH	
	Negative	Positive*	Negative	Positive
Grade 1	5	0	4	1
Grade 2	3	6	2	7
Grade 3	5	19	0	24
Total	13	25	6	32

* Cytology suspicious results are scored as positive.

Table 3
Comparison of overall sensitivity, accuracy and predictive values obtained with urine cytology and FISH assays.

	CYTOLOGY	FISH
Sensitivity	68	84
Specificity	97	100
Positive predictive value	89	100
Negative predictive value	89	94
Accuracy	89	96

CONCLUSIONS

Our data corroborates that the UroVysion multicolor FISH markedly improves the sensitivity and specificity of cytology for the detection of bladder cancer cells in voided urine specimens and bladder washings. This highest accuracy makes FISH assay very useful to improve the management of patients with symptoms of primary bladder cancer or during surveillance for detection of recurrence in asymptomatic patients.

The unbeatable positive predictive value (100%) obtained with FISH analysis support data of previous studies on which the presence of FISH abnormalities in urine samples precede a demonstrable bladder tumor, although in some reported cases confirmative biopsy was obtained several months later.

In samples associated with other genitourinary symptoms and signs not directly related to urothelial carcinoma FISH analysis does not seem to improve cytology results and thus it is not the assay of election.

CASE REPORT

Case report of a patient on which FISH analysis evidenced a cytological unnoticed tumor. The CTscan follow up for a previous history of prostate cancer evidenced a new recurrence of a grade 2 pTa tumor. Biopsy H&E of the original tumor, negative follow up cytology and CTscan images are shown.