RESEARCH ARTICLE

Fluorescence-in-situ-hybridization in the Surveillance of Urothelial Cancers: Can Use of Cystoscopy or Ureteroscopy be Deferred?

Christopher Chee Kong Ho[1]*, Wei Phin Tan[2], Rajadurai Pathmanathan[3], Wei Keith Tan[4], Hui Meng Tan[5]

Abstract

Background: Fluorescence in situ hybridization (FISH) testing may be useful to screen for bladder carcinoma or dysplasia by detecting aneuploidy chromosomes 3, 7, 17 and deletion of the chromosome 9p21 locus in urine specimens. This study aimed to assess the sensitivity, specificity, positive and negative predictive value of FISH in a multi-ethnic population in Asia. Materials and Methods: Patients with haematuria and/or past history of urothelial cancer on follow-up had their voided urine tested with FISH. Patients then underwent cystoscopy/ureteroscopy and any lesions seen were biopsied. The histopathological reports of the bladder or ureteroscopic mucosal biopsies were then compared with the FISH test results. Results: Two hundred sixty patients were recruited. The sensitivity and specificity of the FISH test was 89.2% and 83.4% respectively. The positive (PPV) and negative predictive values (NPV) were 47.1% and 97.9%. By excluding patients who had positive deletion of chromosome 9, the overall results of the screening test improved: sensitivity 84.6%; specificity 96.4%; PPV 75.9% and NPV 97.9%. Conclusions: UroVysion FISH has a high specificity of detecting urothelial cancer or dysplasia when deletion of chromosome 9 is excluded. Negative UroVysion FISH-tests may allow us to conserve health resources and minimize trauma by deferring cystoscopic or ureteroscopic examination.

Keywords: Fluorescence-in-situ-hybridization - urothelial - cancer - UroVysion - Asia

Asian Pac J Cancer Prev, **14 (7)**, 4057-4059

Introduction

Bladder cancer (BC) is the second most common malignancy of the urinary tract (Hentschel et al., 2011). On the other hand, 5% of all urothelial tumours are found in the upper urinary tract (Steffens and Nagel, 1988). Diagnosis of urothelial cancer would require cystoscopy/ureteroscope and biopsy. Unfortunately, cystoscopy/ureteroscopy is invasive and is not acceptable for a considerable number of patients. Thus, the need for a reliable non-invasive diagnostic tool for urothelial cancer. Urine cytology is currently the gold standard for non-invasive methods of diagnosing urothelial cancer. Other modalities developed include molecular tumour markers. Fluorescence-in-situ-hybridization (FISH) is a multi-targeted technique designed to detect chromosomal aberrations associated with urothelial cancer, including aneuploidies of chromosomes 3, 7, and 17 and loss of 9p21. Studies done in the West have demonstrated that FISH has a higher sensitivity than cytology but a similar specificity, where the sensitivity was 76% (65-84%) and specificity was 85% (78-92%) (Mowatt et al., 2010).

The aim of this study was to assess the sensitivity, specificity, positive and negative predictive value of the FISH test in a multi-ethnic population in Asia and to determine whether this test can be a substitute for cystoscopic or ureteroscopic examination in patients at risk of urothelial cancer or dysplasia in the bladder.

Materials and Methods

Between January 2004 and December 2011, 627 consecutive urine samples were tested with UroVysion which is a FISH test and were reviewed retrospectively. The hospital's ethics committee approved this study. Informed consent was obtained from all patients. Indications for UroVysion tests were patients with haematuria and/or past history of urothelial cancer on follow-up. Exclusion criteria were patients who refused to consent and those below the age of 18 years.

The UroVysion bladder cancer kit (Abbott Laboratories, Abbott Park, IL) was used in all cases. Cytospin preparations were created from fixed spontaneously voided urine samples. These samples were denaturized

*[1]Urology Unit, Department of Surgery, Universiti Kebangsaan Malaysia Medical Centre, [5]Department of Primary Care, Faculty of Medicine, University of Malaya, Kuala Lumpur, [3]Department of Pathology, Sime Darby Medical Centre Subang Jaya, Selangor, Malaysia, [2]Thomas Jefferson University, Philadelphia, United States of America, [4]University of Bristol, Senate House, Tyndall Avenue, Bristol, United Kingdom *For correspondence: chriscksho2002@yahoo.com*

according to the manufacturer's protocol and hybridized using the FISHR multicolor probe blend (centromere samples CEP3, CEP7, and CEP17, as well as the DNA sample LSI 9p21). Uncombined samples were removed through wash steps and the nuclei were counterstained with 4',6-diamidino-2-phenylin- dole (DAPI).

After arrival at the laboratory, the urine samples were stored in a cool and dry place and worked up within 24-48 hours. The evaluation was performed microscopically in 25-50 morphologically "abnormal" cells from a urine sample as described previously (Papanikolaou and Marshall, 1945; Rathert and Roth; 1991). A positive result was given if ≥2 copy numbers at defined loci of the chromosomes (3, 7, and/or a 17) were observed in at least 4 cells or if at least 12 cells did not show any detectable signal for 9p21 (Vysis, 2010).

Patients then underwent cystoscopy/ureteroscopy and any lesion seen is biopsied. The results of the histopathological report of the bladder or ureteroscopic mucosal biopsy were evaluated with their respective UroVysion FISH test done prior to the endoscopic biopsy.

Data analysis was done using the Statistical Package for the Social Sciences (SPSS Inc., Chicago IL, USA) version 16. For the analysis of sensitivity, specificity, and positive/negative predictive values of UroVysion, a chi-square test was used, and a 95% confidence interval was considered.

Results

A total of 260 patients met the inclusion and exclusion criteria and underwent the UroVysion test followed by cystoscopic or ureteroscopic biopsies. The median age was 56.3 years (range 23-84). 185 (71.9%) were men.

Seventy UroVysion tests were positive (Table 1). True positive was 33, of which 13 were found to have transitional cell carcinoma (TCC) of the bladder, seven upper tracts TCC and 13 dysplasia of the bladder mucosa. There were 37 false positive and 4 false negative UroVysion results.

The sensitivity and specificity of the UroVysion test was 89.2% and 83.4% respectively. The positive (PPV) and negative predictive value (NPV) was 47.1% and 97.9%. Excluding patients who had positive deletion of chromosome 9, improved the overall results of the screening test: sensitivity 84.6%; specificity 96.4%; PPV 75.9% and NPV 97.9% (Table 2).

Table 1. Patients with Haematuria/Past History of Urothelial Cancer on Follow-Up

		Cystoscopy/Ureteroscopy	
		Positive (+)	Negative (-)
UroVysion	Positive (+)	33	37
	Negative (-)	4	186

Table 2. Patients Without Deletion of Chromosome 9

		Cystoscopy/Ureteroscopy	
		Positive (+)	Negative (-)
UroVysion	Positive (+)	22	7
	Negative (-)	4	186

Discussion

The gold standard for diagnosis of bladder or urothelial cancer is via cystoscope or ureteroscope with biopsy. However, this is invasive and may not be preferred by many patients. The non-invasive diagnostic stool frequently used is urine cytology. While the specificity of urine cytology has been reported to be >90%, the same cannot be said about the sensitivity of the test. The quoted range has been far less encouraging, at 27-80% (Lotan and Roehrborn, 2003). Besides that, cytology accuracy is also highly dependent upon the skill of the cytopathologist (Sherman et al., 1984; Brown, 2000). Cytology is useful in detecting high-grade tumours and carcinoma in situ, but not low-grade tumours (Sherman et al., 1984; Van der Poel, 1998). To increase yield of cytology, at least 3 urine samples should be analyzed. Another shortfall of cytology is the high false positive result following BCG instillation therapy which may complicate cystoscopic surveillance.

FISH allows a detailed view of chromosomal aberrations and, thus, of the number of copies of the chromosomes or specific genes of cancer cells within an otherwise heterogeneous tissue. A few studies have shown promising results for FISH (Schwarz et al., 2008; Volpe et al., 2008).

When FISH was compared with other diagnostic markers such as bladder tumour antigen (BTA), hemoglobin strips, and telomerase, FISH yielded the highest sensitivity of 81% and specificity of 96% (Halling et al., 2002). In another study on recurrent transitional cell carcinoma of the urinary bladder, the sensitivity was higher for FISH (71%) compared with the BTA Stat test (50%) and cytology (26%), whereas specificity was 94.5% (Sarosdy et al., 2002).

BTA test is generally not useful because of its high false-positive rate and low sensitivity for low-grade tumour (Babjuk et al., 2008; Raitanen, 2008). Similarly, nucleus matrix protein 22 (NMP-22) suffers from high false positive rate but has good negative predictive rate. ImmunoCyt test appears to be the best to detect low grade tumour as it has the highest sensitivity (76-85%) compared to all other tests. However, the positive predictive value of 60% is still inadequate to replace cystoscopy (Schmitz-Drager et al., 2007; Mowatt et al., 2010).

In a recent study on bladder cancer, sensitivity of FISH test was found to be 45% and PPV (positive predictive value) was 16.4% in all and 53.85% and 13.21% in high-grade tumours. Specificity and negative predictive value (NPV) were 96.97% and 99.26% in all bladder tumours (Banek et al., 2012). In another study by Dimashkieh et al. (2013) comparing cytology and urovision, the overall sensitivity, specificity, positive predictive value, and negative predictive value in detecting UCC were 61.9%, 89.7%, 53.9%, and 92.4%, respectively, for FISH and 29.1%, 96.9%, 64.4%, and 87.5%, respectively, for cytology.

Similarly, our study showed a high specificity and negative predictive value (NPV) of 83.4% and 97.9% respectively. The positive predictive value (PPV) was

also similarly low at 47.1% but the sensitivity in contrast was higher at 89.2%. When patients who had positive deletion of chromosome 9 were excluded, the overall results of the screening test improved. Sensitivity was 84.6%; specificity 96.4%; PPV 75.9% and NPV 97.9%.

The current urinary molecular marker tests are not good enough to replace cystoscopy yet UroVysion like cytology may not be good enough for the surveillance of low-grade tumours. However in clinical practice, it is more important that we pick up high-grade tumours early. UroVysion has good sensitivity for high-grade tumours and certainly can be used as an adjunct in the detection or surveillance of urothelial tumour. Currently, UroVysion, ImmunoCyt and NMP-22 appear to be the most useful markers, at least as an adjunct in the detection and surveillance of urothelial cancer (Grossman et al., 2005; 2006; Hajdinjak, 2008; Lotan et al., 2008; Vrooman and Witjes, 2008; Van Rhijn et al., 2009; Mowatt et al., 2010). Bubendorf and Piaton (2012) in their review, concluded that multi-target UroVysion(®) FISH remains an excellent tool to improve diagnosis in urinary cytopathology, provided that FISH results are interpreted in the light of the clinical situation. However, in cases of clearly positive, high-grade cytology, FISH adds no diagnostic value.

In a nutshell, our study has shown that FISH has a very high negative predictive value and the exclusion of positive deletion of chromosome 9, increased the specificity of the test. In other words, those who have negative FISH test results, may not need to undergo immediate cystoscopic or ureteroscopic examination and endoscopic surveillance can be deferred to a longer interval. This will be beneficial in terms of cost and reduce the need for patients to undergo too many invasive procedures.

In conclusion, UroVysion FISH shows a high specificity of detecting urothelial cancer or dysplasia when deletion of chromosome 9 is excluded. A negative test may allow a patient to avoid the trauma of having to undergo cystoscope or ureteroscope which is invasive.

References

Babjuk M, Soukup V, Pesl M, et al (2008). Urinary cytology and quantitative BTA and UBC tests in surveillance of patients with pTapT1 bladder urothelial carcinoma. *Urology*, **71**, 718-22.

Banek S, Schwentner C, Tager D, et al (2012). Prospective evaluation of fluorescence-in situ-hybridization to detect bladder cancer: results from the UroScreen-Study. *Urol Oncol*, [Epub ahead of print].

Brown FM (2000). Urine cytology. Is it still the gold standard for screening? *Urol Clin North Am*, **27**, 25-37

Bubendorf L, Piaton E (2012). UroVysion® multiprobe FISH in the triage of equivocal urinary cytology cases. *Ann Pathol*, **32**, 438-43.

Dimashkieh H, Wolff DJ, Smith TM, et al (2013). Evaluation of urovysion and cytology for bladder cancer detection: a study of 1835 paired urine samples with clinical and histologic correlation. *Cancer Cytopathol*, [Epub ahead of print].

Grossman HB, Messing E, Soloway M, et al (2005). Detection of bladder cancer using a point-of-care proteomic assay. *JAMA*, **293**, 810-6.

Grossman HB, Soloway M, Messing E, et al (2006). Surveillance for recurrent bladder cancer using a point-of-care proteomic assay. *JAMA*, **295**, 299-305.

Hajdinjak T (2008). UroVysion FISH test for detecting urothelial cancers: meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. *Urol Oncol*, **26**, 645-51

Halling KC, King W, Sokolova IA, et al (2002). A comparison of BTA stat, hemoglobin dipstick, telomerase and Vysis FISH assays for the detection of urothelial carcinoma in urine. *J Urol*, **167**, 2001-6.

Hentschel S, Pritzkuleit R, Schmid-Höpfner S, et al (2011). Epidemiologis- che Krebsregistrierung in Deutschland. *Onkologie*, **17**, 97-106

Lotan Y, Roehrborn CG (2003). Sensitivity and specificity of commonly available bladder tumor markers versus cytology: results of a comprehensive literature review and metaanalyses. *Urology*, **61**, 109-18.

Lotan Y, Svatek RS, Malats N (2008). Screening for bladder cancer : a perspective. *World J Urol*, **26**, 13-8.

Mowatt G, Zhu S, Kilonzo M, et al (2010). Systematic review of the clinical effectiveness and cost-effectiveness of photodynamic diagnosis and urine biomarkers (FISH, ImmunoCyt, NMP22) and cytology for the detection and follow-up of bladder cancer. *Health Technol Assess*, **14**, 1-331.

Papanikolaou GN, Marshall VF (1945). Urine sediment smears as a diagnostic procedure in cancers of the urinary tract. *Science*, **101**, 519-20.

Raitanen MP (2008). The role of BTA Test in follow-up of patients with bladder cancer: results from FinnBladder studies. *World J Urol*, **26**, 45-50

Rathert P, Roth S (1991). Urinzytologie. In "Praxis und Atlas". Springer, Berlin pp 1-515.

Sarosdy MF, Schellhammer P, Bokinsky G, et al (2002). Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. *J Urol*, **168**, 1950-4.

Schmitz-Drager BJ, Beiche B, Tirsar LA, et al (2007). Immunocytology in the assessment of patients with asymptomatic microhaematuria. *Eur Urol*, **51**, 1582-8.

Schwarz S, Rechenmacher M, Filbeck T, et al (2008). Value of multicolor fluorescence in situ hybridization (FISH) in the differential diagnosis of flat urothelial lesions. *J Clin Pathol*, **61**, 272-7.

Sherman AB, Koss LG, Adams SE (1984). Interobserver and intraobserver differences in the diagnosis of urothelial cells. *Anal Quant Cytol*, **6**, 112-20.

Steffens J, Nagel R (1988). Tumours of the renal pelvis and ureter. Observations in 170 patients. *Bri J Urol*, **61**, 277-83.

Van der Poel HG, Van Balken MR, Schamhart DH, et al (1998). Bladder wash cytology, quantitative cytology, and the qualitative BTA test in patients with superficial bladder cancer. *Urology*, **51**, 44–50.

Van Rhijn BWG, van der Poel HG, van der Kwast HG (2009). Cytology and urinary markers for the diagnosis of bladder cancer. *Eur Urol*, **8**, 536-41.

Volpe A, Racioppi M, D'Agostino D, et al (2008). Bladder tumor markers: a review of the literature. *Int J Biol Markers*, **23**, 249-61.

Vrooman OPJ, Witjes JA (2008). Urinary markers in bladder cancer. *Eur Urol*, **53**, 909-16.

Vysis (2010). Produktinformation® Vysis: FISH Bladder Cancer Recurrence Kit. Vysis Inc., Abbott Park, IL.