Urinary Survivin mRNA Expression and Urinary Nuclear Matrix Protein 22 BladderChek[®] and Urine Cytology in the Detection of Transitional Cell Carcinoma of the Bladder

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Key Words

Bladder cancer • NMP22 BladderChek[®] • Real-time PCR • Survivin • Urine cytology

Abstract

Objective: To compare the diagnostic performance of urine cytology (UC), survivin mRNA expression, and the NMP22 BladderChek[®] (NMP22BC) test for the detection, grading and staging of transitional cell carcinoma (TCC) of the bladder. **Materials and Methods:** Voided urine samples collected from 25 healthy controls and 80 patients diagnosed with TCC of the bladder were subjected to UC, the NMP22BC test and reverse-transcription real-time PCR for survivin mRNA expression. **Results:** Survivin mRNA expression showed the highest sensitivity (87.5%) followed by the NMP22BC test (61.3%) while UC exhibited the lowest sensitivity (40%). All three urine markers had a similar specificity of 96% (95% CI 80.5–99.3%). Survivin mRNA expression was the only urine marker that showed a significant difference in relation to tumour histological grade (χ^2 8.5, p = 0.015). None of the three urine markers was significantly related to tumour pathological stages. **Conclusion:** The diagnostic sensitivity of urinary survivin mRNA expression was superior to that of UC and the NMP22BC test and correlates with tumour pathological grade but not stage.

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Introduction

With the current limitations of standard urine cytology (UC) and cystoscopy for the detection of bladder cancer, focus has shifted toward the development of non-invasive and sensitive urine markers which can be used alone or in combination with UC. One of these urine markers, which has already received approval from the United States Food and Drug Administration (FDA) for clinical use, is the NMP22 BladderChek[®] (NMP22BC) test. The NMP22BC test has a sensitivity of 55.7% while UC detected only 15.8% of the cancers [1]. The sensitivity and specificity of UC and the NMP22BC test varied largely among studies [2]. In addition, many confounding factors affect the results of the NMP22BC test, leading to the generation of false-positive results. The gene expression of survivin, an inhibitor of apoptosis, is globally dysregulated in cancer. Apoptosis is dysregulated as well, which is the hallmark for progression in carcinogenesis.

The aim of the present study was to assess the performance of urinary survivin mRNA expression and the NMP22BC test in detecting transitional cell carcinoma (TCC) of the bladder in comparison to UC and to see whether the results correlate with tumour grade and stage.

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Table 1. Analysis of survivin mRNA, UC and the NMP22BC test in healthy controls and bladder cancer patients

Category	Samples	Survivin-positive	NMP22BC-positive	UC-positive
Healthy controls	25	1 (96) ¹	1 (96) ¹	1 (96) ¹
TCC	80	70 (87.5) ^{2,3}	49 (61.3) ^{2,3}	32 (40) ^{2,4}
Histological grade				
G1	38	29 (76.3)	21 (55.3)	12 (31.6)
G2	23	22 (95.6)	17 (74)	11 (47.8)
G3	19	19 (100)	11 (58)	10 (52.6)
χ^2 (p value)		8.5 (0.015)	2.2 (0.33)	2.9 (0.23)
Clinical stage				
pTa	6	5 (83.3)	6 (100)	0 (0)
CIS	6	5 (83.3)	2 (33.3)	2 (33.3)
pT1	53	48 (90.6)	31 (58.5)	22 (41.5)
pT2	6	4 (66.7)	3 (50)	1 (16.6)
pT3	5	4 (80)	3 (60)	4 (80)
pT4	4	4 (100)	4 (100)	3 (75)
χ^2 (p value)		3.7 (0.58)	8.8 (0.11)	11 (0.05)

Figures in parentheses are percentages.

¹ Specificity. ² Sensitivity. ³ $p < 0.001$, Mann-Whitney test.

⁴ $p > 0.05$, Mann-Whitney test.

Materials and Methods

Fresh voided urine samples from 25 healthy controls and 80 patients diagnosed with TCC of the bladder were used in this study. All patients underwent cystoscopy and bladder biopsy as the reference standard for bladder cancer detection. Patients with known urinary infections, inflammation or any other type of cancer were excluded from the study. This prospective study was approved by the Ethics Committee of the Health Sciences Center and Ministry of Health. All patients signed informed consent forms. The NMP22 levels were measured using the FDA-approved NMP22BC test (+ or -) (Matritech, Inc., Newton, Mass., USA) according to the manufacturer's instructions. UC was performed as described in an earlier study [3]. Tumours were graded according to the WHO classification – grade 1 = low, grade 2 = intermediate and grade 3 = high [4]. The tumours were staged using the standard tumour, node and metastasis TNM WHO classification [4] using data obtained by cystoscopy, computed tomography and pathological specimens. Total RNA from urinary sediments was purified using TRIzol reagent (Invitrogen, Carlsbad, Calif., USA) according to the manufacturer's instructions. RNA was converted to cDNA using reverse transcription (RT) (ABI, Foster City, Calif., USA).

Real-time polymerase chain reactions (PCR) for samples run in triplicates were performed using the ABI Prism 7000 Sequence Detection System and the TaqMan® Universal PCR Master Mix (ABI). Predesigned and standardized primers and TaqMan fluorescent probe sets for survivin and the internal control gene glyceraldehyde-3-phosphate dehydrogenase were purchased from ABI. The relative expression of survivin

mRNA was calculated using the following equation: ratio = $(E_{\text{survivin}})^{\Delta C_{\text{t}}}_{\text{survivin}}(\text{control} - \text{sample}) / (E_{\text{GAPDH}})^{\Delta C_{\text{t}}}_{\text{GAPDH}}(\text{control} - \text{sample})$, where E_{survivin} and E_{GAPDH} represent the real-time PCR efficiency of survivin and GAPDH gene transcription, respectively. ΔC_{t} is the threshold cycle deviation of control minus sample of the gene transcript. All data analyses were conducted using GraphPad Prism v 5.0 (GraphPad Software Inc., San Diego, Calif., USA). A p value < 0.05 was considered statistically significant.

Results

The healthy controls' and patients' ages (mean age $\pm$ SD) were 55 ± 15 (range: 20–70 years) and 53 ± 19 (range: 16–77 years), respectively. There were 65 male and 15 female patients with TCC of the bladder while in the control arm there were 16 males and 9 females. Of the 80 patients with TCC 43 (53.8%) patients were newly diagnosed while 37 (46.2%) patients had recurrent TCC. Nineteen patients with urinary tract infections or urolithiasis who presented with haematuria were excluded from this study. The diagnostic performance of survivin mRNA expression in comparison to UC and the NMP22BC test in our population cohort is given in table 1. Survivin mRNA expression showed the highest sensitivity (87.5%; 95% CI 78.5–93.1%) followed by the NMP22BC test (61.3%; 95% CI = 50.3–71.2%); UC exhibited the lowest sensitivity, i.e. 40% (95% CI 30–51%). All three urine markers had a similar specificity of 96% (95% CI 80.5–99.3%). Comparison of the three urine markers and the different clinicopathological factors revealed that only survivin mRNA expression differed significantly in relation to tumour histological grade (χ^2 8.5, $p = 0.015$). None of the three urine markers was significantly related to tumour pathological stage.

Discussion

Although UC is the standard non-invasive test and is highly specific for the diagnosis of bladder cancer, it is characterized by low sensitivity (30–50%) depending on tumour stage and differentiation [5]. UC also suffers from limitations due to interobserver variability and the need for expert cytopathologists. Such shortcomings were partly overcome by the use of the FDA-approved NMP22BC test for the diagnosis of bladder cancer and follow-up tests. The sensitivity of the NMP22BC test (77.5%) surpassed that of UC (46.3%) and the specificity of the NMP22BC test was 88.8% compared to 97.9% for UC [6]. The results of our study are in agreement with

reported ranges [1, 6]. Many studies reported the high sensitivity and specificity of urinary survivin mRNA in the early detection of bladder cancer by real-time RT-PCR [7, 8]. This method is objective and sensitive for measuring gene expression. In our study, the relative expression of survivin mRNA correlated significantly with tumour grade but not clinical stage. However, similarly to the other two markers, the positivity tends to increase with tumour grade [8, 9]. Hence, survivin expression may be useful as a primary detection technique in patients with suspected urothelial cancer.

Overall, none of the three investigated markers can be considered as a typical marker for bladder cancer detection and/or surveillance due to their low sensitivity (UC), high cost (survivin mRNA) or interference with the results due to the presence of confounding factors (NMP22BC) [6, 8, 9]. However, NMP22BC has acceptable sensitivity, is relatively cheap and the result is available within 1 h; these features may make the NMP22BC test a

useful initial screening test in a haematuria clinic [6, 9]. Further refinement of tumour grade can then incorporate the study of survivin mRNA expression.

Conclusion

Compared to the NMP22BC test and UC, survivin mRNA expression showed paramount sensitivity in the detection of TCC of the bladder with significant correlation with tumour pathological grade but not stage. Its use for further characterization of urothelial tumours warrants more investigations.

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