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Diagnostic values of urine CYFRA21-1, NMP22, UBC, and FDP for the detection of bladder cancer

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ABSTRACT

Background: We compared the diagnostic utilities of CYFRA 21-1, nuclear matrix protein-22 (NMP22), urinary bladder cancer antigen (UBC), and fibrin/fibrinogen degradation products (FDP) for detecting urinary bladder cancer.

Methods: We assayed CYFRA 21-1, NMP22, UBC and FDP from urine samples for 250 subjects. Among them, 54 were diagnosed as bladder cancer, and the remaining 196, which consisted of healthy individuals and patients with hematuria, inflammation/infection, or benign prostate hyperplasia, were assigned to the control group.

Results: Urinary levels of all 4 markers were higher in the bladder cancer group than the control group. The areas under the receiver operating characteristic curves (ROC-AUCs) of CYFRA 21-1, NMP22, UBC and FDP, corrected with urine creatinine concentrations, were 0.90, 0.89, 0.80 and 0.77, respectively, for discriminating bladder cancer from controls. The ROC-AUCs for the combinations of the markers were not significantly higher than those with CYFRA 21-1 or NMP22. NMP22 was the only independent variable for predicting bladder cancer among the four markers in the multivariate analysis.

Conclusions: All 4 tumor biomarkers exhibited diagnostic utility for predicting bladder cancer. Among them, CYFRA 21-1 and NMP22 were the most effective at predicting bladder cancer.

1. Introduction

Prompt diagnosis and treatment are essential to reduce the morbidity and mortality associated with urinary bladder cancer. In the year 2011, about 69,250 new bladder cancer patients were diagnosed, and the estimated deaths from bladder cancer was 14,990 in the United States [1]. About 75% of patients with the disease had superficial tumors, which were restricted to the mucosa or the lamina propria [2]. Early detected superficial tumors can be eradicated effectively by transurethral resection and intravesical therapy without the need for more aggressive surgical procedures. Cystoscopy has been the clinical standard for the identification of bladder cancer. However, this procedure is expensive and uncomfortable despite flexible cystoscopy improving patients' tolerability. Furthermore, flat tumors may be difficult to detect, and errors made by the operator may be neglected [3]. Cytology has been recognized as one of the diagnostic modalities to replace cystoscopy, but its sensitivity is too low to detect low grade neoplasms [4]. Considerable effort has been made to find noninvasive biomarkers for bladder cancer with sufficient diagnostic ability.

Among the discovered biomarkers for the diagnosis of bladder cancers, some have been reported to be useful. CYFRA 21-1 and urinary bladder cancer antigen (UBC) assays are tests based on the measurement of cytokeratins (CKs), those are intermediate filament proteins found in epithelial cells. In malignant conditions, such as urothelial cell carcinoma (UCC), particular chain-specific CKs have been found to be overexpressed. CYFRA 21-1 assays usually detect fragments of CK19 with the help of two monoclonal antibodies, and UBC kit measures CK8 and CK18 in urine samples [5,6]. Meanwhile, nuclear matrix proten-22 (NMP22) is a nuclear protein which controls chromatid regulation and cell separation during replication. As the measurement of this protein had been reported to be useful for the evaluation of bladder cancer patients, NMP22 assay was approved by the United States Food and Drug Administration (FDA) [7]. In addition, fibrin/fibrinogen degradation products (FDP) are generated by the activation of the fibrinolytic system. Clotting factors, which can be released by the bladder tumor cells, rapidly convert fibrinogen into fibrin deposit. Plasmin can then break down the fibrin clot into FDP [8].

In this study, we evaluated the diagnostic performances of CYFRA 21-1, NMP22, UBC and FDP, which have been widely used as biomarkers for bladder tumors over the past decade. We also investigated which of these markers are the most useful for detecting bladder cancer.

Abbreviations: AUC, area under the curve; BPH, benign prostate hyperplasia; CI, confidence interval; CK, cytokeratin; FDP, fibrin/fibrinogen degradation products; NMP22, nuclear matrix protein-22; ROC, receiver operating characteristic; UBC, urinary bladder cancer antigen; UCC, urothelial cell carcinoma.

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2. Materials and methods

2.1. Study design

A total of 250 urine samples from patients who visited Severance Hospital for NMP22 testing and were not suffering from malignant diseases other than bladder cancers were collected during December 2010 and February 2011. Voided mid-stream random urine samples were collected, and the residual specimens after measuring the levels of NMP22 were frozen at −20 °C until assayed for other markers. The specimens were thawed at room temperature and centrifuged for 15 min at 3500 RPM. The supernatant was divided into aliquots and the CYFRA 21-1, UBC and FDP levels were measured with the aliquots.

The subjects were classified into five groups: bladder cancer (n = 54), healthy subjects (n = 47), patients with hematuria (n = 59), urinary inflammation/infection (n = 22) and benign prostate hyperplasia (BPH) (n = 68). All of the bladder cancer patients were diagnosed based on histological examination, and they were staged as superficial (pTa, pT1) or muscle invasive (pT2, pT3, pT4) cancer according to TNM criteria [9]. Histological grade of any tumors was assessed following the WHO tumor grading system [10].

The median interval between sample collection and diagnosis of bladder cancers was 7.0 days (n = 53, 1st to 3rd quartiles = 3.0 to 14.3). The diagnosis of bladder cancer was based on the pathologic findings of cystoscopy-guided biopsy or transurethral resection of bladder tumor (TURB).

2.2. CYFRA 21-1 assay

CYFRA 21-1 levels were measured using a commercially available electrochemiluminescence immunoassay (Elecsys CYFRA 21-1 Immunoassay) and cobas e 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). The assay used monoclonal cytokeratin 19-specific antibodies which were either biotinylated or labeled with a ruthenium complex. Urine samples and antibody complexes were bound to the solid phase through the interaction of biotins and the added streptavidin-coated microparticles. The bound microparticles were magnetically captured onto the surface of the electrode. After applying voltage to the electrode, the induced chemiluminescent emission was measured by a photomultiplier and automatically converted to the concentration of CYFRA 21-1 (μg/L) using the calibration curve automatically drawn by the analyzer. Measured serum CYFRA 21-1 levels were suggested to be linear in the range between 0.10 and 500.0 ng/mL by the manufacturer, and the linearity was also validated in our laboratory to be from 1.18 to 460.7 ng/mL of serum CYFRA 21-1 concentrations. The lower limit of detection (LOD) suggested by the manufacturer is 0.10 ng/mL. However, assay characteristics for the determination of CYFRA 21-1 levels in the urine samples were not suggested or validated by the manufacturer.

2.3. NMP22 assay

NMP22 concentrations were determined using Alere NMP22 Test reagent kits (Alere Inc., Waltham, MA, USA). The kit was based on the enzyme-linked immunosorbent assay (ELISA), and employed two monoclonal antibodies raised against nuclear mitotic apparatus protein. Patient's urine samples reacted with antibodies coated onto wells of microplates. After one wash, the captured NMP22 antigen reacted with a second antibody labeled with digoxigenin. Then, the second antibody combined with an anti-digoxigenin antibody, which had been conjugated with horseradish peroxidase. Using *O*-phenylenediamine as a substrate, reaction developing color occurred and was terminated by the addition of sulfuric acid. The level of NMP22 was proportional to the intensity of the developed color and the actual concentration (U/mL) was calculated from a standard curve. Linear regression analysis was used to generate the calibration curves for the respective assay plates, for example, the equation of a standard curve for an assay plate was $y = 243.83x - 20.193$ ($R^2 = 0.9943$), where x means the optical density (OD) and y indicates the NMP22 concentration. This assay is suggested to be linear in the range between 2.1 and 116.0 U/mL of urine NMP22 by the manufacturer.

2.4. UBC assay

UBC concentrations were determined using a UBC II ELISA assay (IDL Biotech AB, Bromma, Sweden). Urine samples were incubated with monoclonal antibodies (6D7 and 3F3), which detect epitopes on cytokeratins 8 and 18, and the detector antibody that had been conjugated with horseradish peroxidase. After washing, a substrate solution was added and the absorbance was read to calculate the concentration of UBC (μ/L) in the sample. The OD from a blank sample provided by the manufacturer (background OD) was subtracted from the respective samples' OD, and then the values were used to calculate the concentrations of the respective samples. Standard curves for the respective assay plates were generated by tertiary polynomial regression fitting, for instance, the equation of the standard curve for an assay was $y = -0.5357x^3 + 1.8813x^2 + 5.5359x - 0.1064$, where x means the OD and y indicates the concentration. R^2 values for the standard curves used in our results were between 0.9967 and 0.9999 throughout the study. This assay is suggested to be linear in a range from 0.1 to 500.0 μg/L of urine UBC by the manufacturer.

2.5. FDP assay

FDP were measured using a NANOPIA P-FDP assay (Sekisui Chemical Co., Ltd., Osaka, Japan) based on the latex immunoturbidimetric method. FDP in the patient's urine specimen reacted with latex coated anti-FDP mouse monoclonal antibody. A spectrophotometry analyzer (STA COMPACT®) (Diagnostica Stago S.A.S., Asnières sur Seine, France) detected the degree of turbidity by agglutination reaction, and automatically reported the concentration of FDP (μg/mL). The analyzer generated the calibration curve by a tertiary polynomial regression fitting ($R^2 = 1.0000$). Linearity was validated in the range from 2.5 to 120 μg/mL of plasma FDP concentration in our laboratory.

2.6. Urinary creatinine, RBCs and WBCs

Creatinine was measured in urine samples by alkaline picrate method (Jaffe's reaction) with the Hitachi 7600 automated analyzer (Hitachi, Ltd., Tokyo, Japan) and Daichi CRE reagents (Daichi, Tokyo, Japan). CYFRA 21-1, NMP22, UBC and FDP levels were corrected by dividing each concentration of the biomarkers by those of urinary creatinine. Five patients' results (1 from bladder cancer group, 2 from healthy control, 1 from hematuria group and 1 from BPH group) could not be corrected because their creatinine levels were not measurable due to the small quantities of specimens. The number of RBCs and WBCs was counted per high power field in patients with hematuria or inflammation/infection using a flow cytometric method with Sysmex UF-1000i (Sysmex Co., Kobe, Japan). Among the remaining 245 corrected urine samples, which were composed of 53 bladder cancers and 192 total controls, urine RBCs or WBCs were not tested in 14 samples from the bladder cancer group and 2 from the total control group. A total of 229 samples with the results for both urine RBCs and WBCs were analyzed for the correlations between CYFRA 21-1, NMP22, UBC, FDP, urine RBCs and urine WBCs.

2.7. Statistical analysis

Statistical analyses were performed using the Analyse-it Method Evaluation Edition, ver 2.26 software (Analyse-it Software Ltd.,