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RESEARCH ARTICLE

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An ensemble approach of urine sediment image analysis and NMP22 test for detection of bladder cancer cells

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Abstract

Background: Bladder cancer is the eighth most common cancer and the second most common urological cancer in Korean males. Current diagnostic tools for bladder cancer include cystoscopy (an upper tract study), urine cytology, and nuclear matrix protein 22 (NMP22) test. In this study, we evaluated the detection rate of atypical/malignant urothelial cells in urinary sediment images when flagged for positive NMP22 test.

Methods: NMP22 was measured by NMP22 BladderChek Test (Abbott Laboratories) and urine chemical and sediment analysis were performed by fully automated cobas 6500 urine analyzer (Roche Diagnostics). Specimens that met the manual microscopic examination (MME) criteria were then subjected to an on-screen review of images. We subsequently reviewed sediment images and examined under the microscopy for the flagged cases.

Results: Of the 1217 patients, 345 (28.3%) had positive NMP22 results, whereas 872 (71.7%) had negative results. Out of the positive results, 154 (12.7%) were positive and 191 (15.7%) weakly positive for NMP22. Screened review of flagged specimens (ie, positive NMP22 result) with sediment imaging analysis revealed that suspicious urothelial carcinoma cells were detected in only two cases (0.8%). In the NMP22 negative flagged cases, the suspicious neoplastic cells were not found.

Conclusions: Our findings suggest that the NMP22 test should be added to the flagging criteria for MME to improve diagnostic accuracy. The combination of urine sediment imaging analysis and NMP22 test can significantly assist technicians in the review of specimens.

KEYWORDS

bladder cancer, manual microscopic examination, NMP22, transitional cell carcinoma, urine analyzer

1 | INTRODUCTION

Bladder cancer is the sixth most common cancer in men, as well as the ninth leading cause death among men worldwide.[1] It also is the eighth most commonly diagnosed cancer in Korean males (~2.8%).[2] Transitional cell carcinoma (TCC), also known as urothelial carcinoma, is the most common histological subtype of bladder cancer. It accounts for over 90% of all bladder cancer cases. Additional categories of bladder cancer include squamous cell carcinoma, adenocarcinoma, small cell carcinoma, and several less common subtypes.[3,4] Cystoscopy and urine cytology are the current recommended tools for diagnostic assessment of bladder cancer. Initial diagnosis of bladder cancer is confirmed by cystoscopic examination and transurethral resection of bladder tumor (TURBT).[5-7] Urinalysis, urine cytology, and urinary biomarker tests are commonly accepted screening strategies for bladder cancer. These methods have a restricted role as initial diagnostic tests due to reduced sensitivity and utility for low-grade tumors. However, the value of these diagnostic methods is attributed to characteristics, such as noninvasiveness, cost effectiveness, reproducibility, and ease of handling.[8-11]

Multiple clinical practice guidelines exist for bladder cancer screening, which differ with the respect to the recommended tests and target populations of low versus high risk. The 2016 American Urological Association/Society of Urologic Oncology guidelines recommend the use of biomarkers to assess response to intravesical Bacillus Calmette-Guerin and adjudicate equivocal cytology.[6] The National Comprehensive Cancer Network recommends an optional urinary biomarker test during follow-up, whereas the National Institute for Health and Care Excellence guideline recommends that urinary biomarker test be used for patients with suspected bladder cancer.[5,11] Chromosomal alterations, carcinoembryonic antigen, two bladder tumor cell-associated mucins, nuclear matrix protein 22 (NMP22), and bladder tumor-associated antigen (BTA) are all current Food and Drug Administration-approved assays for bladder cancer.[12,13] The qualitative point-of-care test (POCT) measuring urinary NMP22 has used for detecting bladder cancer universally in Korea. Additionally, manual screening procedures have been replaced by more efficient automated microscopy image-based urine sediment instruments. The purpose of this study was to evaluate the detection rate of atypical/malignant urothelial cells in urinary sediment images in samples flagged with positive results from NMP22 POCT and urinalysis.

2 | MATERIALS AND METHODS

2.1 | Study subjects

Urine samples were collected from the patients that underwent a NMP22 POCT and urinalysis from January to December 2017. All tests were performed on the same day. Exclusion criteria included a positive test for NMP22 with a history of bladder cancer or other urinary tract cancers. The urine specimens were tested for NMP22 prior to urine analysis in the laboratory.

2.2 | NMP22 assay

Urinary NMP22 concentration was measured with the NMP22 BladderChek Test (Abbott Laboratories), which is based on the lateral flow immunochromatographic qualitative assay. The cutoff value for the assay was 10.0 U/mL.

2.3 | Urine analysis

Urine chemical and sediment analysis were performed using the cobas 6500 urine analyzer (Roche Diagnostics International). Specimens meeting criteria for manual microscopic examination (MME) were subjected to on-screen review of images using a review program developed for analyzer. The MME were carried out for samples when an on-screen image review was not possible. The criteria for MME used in this study were as follows: (a) discrepant results of at least one grade in red blood cell or white blood cell count between the strip testing and sediment findings; (b) specimens flagged for MME due to the presence of crystals, yeasts, small round cells, bacteria, or pathologic casts; and (c) weakly positive or positive results in NMP22 test. Recently, a third criterion was added to the review of existing standard. We linked the NMP22 results and laboratory information system inspection (LIS) first, and then, the LIS results and on-screen review program for the cobas 6500 urine analyzer were connected bidirectionally. Ethical Committee approval was obtained from the Institutional Review Board of The Catholic University of Korea (UC19ZESE0070).

3 | RESULTS

Of 1217 patients, 345 (28.3%) had positive results for NMP22, whereas 872 (71.7%) had negative results. Of those patients with positive test, 154 (12.7%) were classified as positive and 191 (15.7%) as weakly positive NMP22. There were 108 (31.3%) patients diagnosed with a positive NMP22 results but were excluded based on a history of urinary tract cancer (Table 1). After the urine sediment analysis of remaining patients (n = 237), there were only two cases that exhibited atypical transitional cells. We did not identify the malignant cells for the suspicious category in the patient groups showed negative NMP22 results and flagging urinary results. These two cases were subsequently confirmed to have urothelial carcinoma of the bladder. The first diagnosed case was a 67-year-old male presented to the department of urology in July 2017. His chief complaint was painless gross hematuria. The patient had a medical history of myocardial infarction and prediabetes. He was currently taking aspirin and statin medication. The patient tested positive for NMP22, and urinalysis demonstrated high-grade hematuria (50-99

TABLE 1 NMP22 test results of the patients with and without cancer history

NMP22 results	With cancer history (%)	Without cancer history (%)
Positive	56 (4.6)	98 (8.1)
Weakly positive	52 (4.3)	139 (11.4)
Negative	115 (9.4)	757 (62.2)

RBC/HPF). Clusters of atypical transitional cells were observed in the on-screen image review. These abnormal cells had marked variation in sizes and shape with a high nuclear/cytoplasmic ratio. This observation demonstrated malignant cells, which was consistent with transitional cell carcinoma with manual microscopy. Urinary cytology concurrently from the same urine sample revealed the positive finding for a high-grade urothelial carcinoma. The patient underwent dynamic contrast-enhanced computerized tomography and cystoscopy. The former showed a 5.3 cm fungating mass at the left posterolateral wall of the bladder with perivesical extension, whereas the cystoscopy revealed a large papillary bladder tumor. The tumor was resected incompletely via TURBT as the patient refused radical cystectomy.

The second diagnosed case was a 61-year-old male that presented with macroscopic hematuria in October 2017. His medical history included a benign prostatic hyperplasia. The patient tested positive for NMP22 and urinalysis revealed high-grade hematuria (50-99 RBC/HPF) along with the presence of bacteria. Multiple atypical transitional cells were observed in the on-screen image review. Urine cytology showed numerous clusters of malignant urothelial cells consistent with high-grade cellular atypia. TURBT was performed and tissue was sent for histopathologic examination, which was diagnosed as urothelial carcinoma. The results of urinalysis and sediment image for two cases by the cobas 6500 urine analyzer are shown in Table 2 and Figure 1.

TABLE 2 Urinalysis results with the cobas 6500 urine analyzer in Case 1 and 2

	Case 1	Case 2
Urine strip testing		
Glucose	2+	Normal
Color	Amber	Amber
Leukocyte	Neg	Neg
Bilirubin	Neg	Neg
Ketone	Neg	Neg
Specific gravity	1.022	1.023
Occult blood	4+	4+
PH	6.0	6.0
Protein	1+	1+
Urobilinogen	Normal	Normal
Nitrite	Neg	Neg
Urine sediment examination (image analysis)		
WBC	5-9/HPF	1-4/HPF
RBC	50-99/HPF	50-99/HPF
Bacteria	Neg	Few/HPF
Squamous epithelial cell	0-1/HPF	0-1/HPF
Transitional epithelial cell	50-99/HPF	30-49/HPF

4 | DISCUSSION

Microscopic examination of urine sediment is a widely used tool for screening, diagnosing, and managing conditions affecting the urinary tract. The main advantage of the test is its speed, convenience, noninvasiveness, and low cost.[14] Fully automated platforms based on the imaging analysis have also improved diagnosis over traditional manual procedures. The automation of the diagnostic process has contributed to lower labor costs and inter-observer variability.[15] The NMP22 test was introduced for detection of a nuclear mitotic apparatus protein elevated in bladder cancer.[16,17] In newly diagnosed or recurrent TCC, the sensitivity and specificity of assay ranged from 33%-98% and 40%-92%, respectively.[18-20] Despite the high variability in sensitivity and specificity, several clinical studies have suggested that it still can be an adjunctive tool to enhance diagnostic accuracy of cystoscopy.[21,22] However, additional work has reported that false-positive results of the NMP22 test are common in patients with impaired renal function, urinary tract infections, mechanical manipulations, and gross hematuria.[23,24] High-grade microscopic hematuria is particularly suggested to increase the rates if false-positive results, whereas false-negative rates are less frequent in patients with hematuria. Hematuria is present in 85%-90% of patients with bladder cancer. As such, a combination of biomarkers along with urinalysis may help increase specificity and clinical usefulness of the NMP22 test as a diagnostic tool.[23,25,26]

Clinical laboratories use of criteria for MME is often based on the types or performance of the automated analyzer, as well as the specific target population.[27,28] Corresponding with the introduction of the cobas 6500 urine analyzer, increased hospital visits, and positive NMP22 test results, we reestablished the manual review criteria. Because the NMP22 test was performed manually using POCT, we directly inputted the data into the laboratory information system (LIS). Our results showed that positive NMP22 results flagged in the on-screen image review program as a middleware connected with LIS. We then reviewed the urine particles identified as epithelial cells and classified transitional epithelial cells from either squamous epithelial cells or renal tubular epithelial cells.

In this study, the 28.3% (ie, 345) patients with positive and 19.6% (ie, 239) patients with negative NMP22 generated flags. We subsequently reviewed sediment images and examined under the microscopy for 48.0% (ie, 584) patients. From the NMP22 positive flagged cases without cancer history, suspicious urothelial carcinoma cells

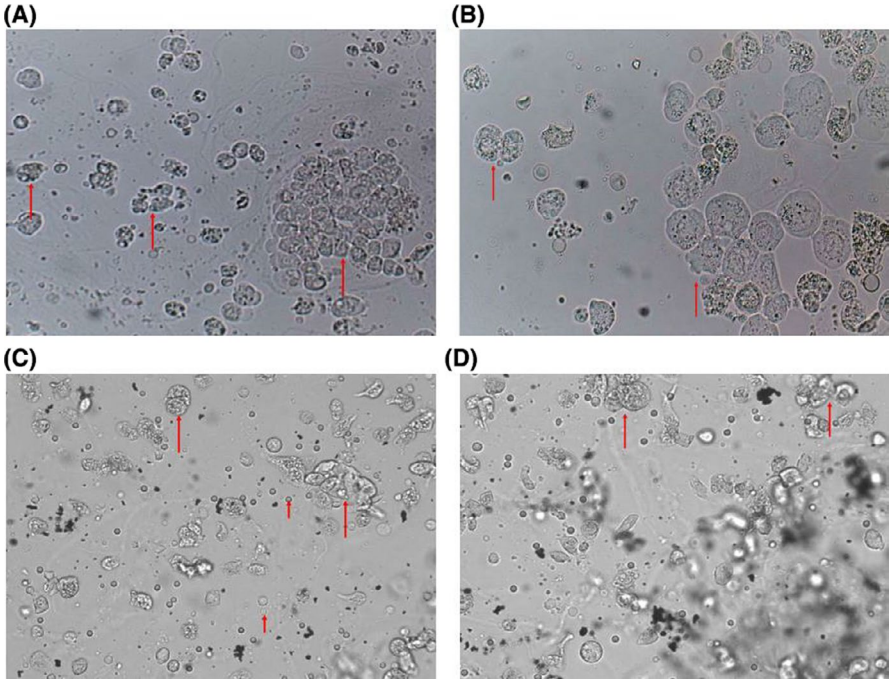

FIGURE 1 The sediment image of unstained urine wet mount provided by the cobas 6500 urine analyzer. In the following cases, atypical transitional epithelial cells or large clusters of epithelial cells were identified by on-screen review of images. The erythrocytes (approximately 5-7 μm in diameter, short arrows) and transitional epithelial cells (more than 20-30 μm in diameter, long arrow) were presented. Case 1: epithelial cells in cluster demonstrated mild to moderate variability in cell size and nuclear-to-cytoplasmic ratio (A, B). Case 2: the majority of the cells, presumably epithelial cells and erythrocytes. The small aggregates of the atypical transitional epithelial cells were present (C, D)

were detected in only two cases based on the sediment image analysis of voided urine. These newly diagnosed cases were pathologically confirmed as transitional cell carcinoma. In the NMP22 negative flagged cases, the suspicious neoplastic cells were not found by MME and urine cytology. The main cause of the NMP22 negative samples flagged was associated with hematuria, pyuria, and microorganisms; these samples give rise to discrepant results between urine strip and sediment testing or low-quality images which are not recognized by the software of the cobas 6500 analyzer.

Our study had some limitations, we did not review all the cases that underwent the NMP22 test. In other words, manual microscopic examination of NMP22 negative cases without abnormal flags did not be performed. As such, our ability to properly assess the detection rate of transitional cells and diagnostic properties of NMP22 was limited. We could not determine the false-negative rate in the MME of NMP22 negative cases. The six patients (2.5%) among 239 patients with negative NMP22 generated flags had previous history of cured bladder cancer, and the urine cytology results of these were negative. Atypical cells were not observed in 239 cases, those of transitional epithelial origin.

The false-negative assay results among the patients with cancer were generally low, and the NMP22 test was false-negative in 1 (5.0%) out of 20 bladder cancers.[29,30] The urinary retention time or presence of urinary cellularity was noted for frequent false-negative results occasionally. False-positive results are more common than false-negative results.[31,32] Various studies have reported that a low specificity of NMP22 is frequently associated with interference from components in the urine sample or by confounding factors (eg, accompanying diseases or symptoms).[23,33,34] Despite this disparity, the NMP22 test has an important role as an adjunct to urine cytology and cystoscopy.[18,20,35] Under the Korean health insurance system, a urine cytology costs almost 13 times as much as a urinalysis.[36] A urinalysis cannot replace urine cytology, but the concomitant testing of NMP22 and urinalysis in the most patients is routine. This study revealed that urine sediment findings combined with NMP22 results are the practical and economic strategy for improving urinalysis result interpretation. However, NMP22 tests were not yet universally applicable in other countries and there may be some additional cost related to the tests.

To our knowledge, this is the first clinically significant findings to recommend the NMP22 test be added to the flagging criteria of MME. The combination of urine sediment imaging analysis with the cobas 6500 urine analyzer platform and NMP22 testing can significantly assist technicians in review of flagged bladder cancer specimens. Additionally, our approach for flagging criteria of MME can also enhance the diagnostic accuracy for clinical laboratories.

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