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Prospective analysis of sensitivity and specificity of urinary cytology and other urinary biomarkers for bladder cancer

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Abstract

Introduction: Although urinary cytology (C) is the most widely used noninvasive test for the detection and surveillance of bladder cancer (BC), it has poor sensitivity especially for low-grade tumors. We prospectively tested the performance of urine markers on patients with BC using C and 4 commercially available urinary marker tests: Hemoglobin Dipstick (H), BTA *Stat* (B), NMP22 BladderChek (N), and ImmunoCyt (I).

Methods: Urinary samples from 109 consecutive patients with BC were prospectively collected. All samples were tested using conventional C and available biomarkers. Prior and subsequent surgical specimen reports were examined, and sensitivity and specificity were calculated for each. Collected variables included patient demographics, date of urinary collection, type of specimen (voided, washing, or catheterized), surgical pathology, recurrence, and follow-up.

Results: Sensitivity values for each marker were as follows: C, 48% (16% for low-grade tumors and 84% for high-grade [HG] tumors); B, 61% (36% and 91%); H, 51% (38% and 66%); N, 58% (25% and 92%); and I, 62% (47% and 83%). Specificity results for each marker were as follows: C, 86%; B, 78%; H, 58%; N, 85%; and I, 79%. On multivariate analysis, higher stage (C and N) and HG disease (C, H, B, N, and I) were independent prognostic factors for improved test performance. When urinary markers were combined with C, sensitivity/specificity values for HG disease were as follows: C + H, 85%/57%; C + B, 91%/78%; C + N, 94%/84%; and C + I, 90%/78%.

Conclusions: Based on these data, C seems to yield the highest specificity and N the highest sensitivity for HG tumors. The combination "C + N" seems to be the better approach to improve the sensitivity for HG tumors compared with single markers and other combinations.

Keywords: Urinary cytology; Urinary markers; Surveillance; Bladder cancer; Sensitivity; Specificity

1. Introduction

Urothelial carcinoma of the bladder (BC) is the most common malignancy in the urinary tract and the eighth most common cancer in men in North America [1]. The overwhelming majority of these tumors are non–muscle-invasive ones and are confined to the mucosa (stage Ta and carcinoma in situ) or submucosa (stage T1) at the time of diagnosis [2]. Although these lesions are not immediately life-threatening, they do have a high predilection for recurrence (up to 70%) and progression to more aggressive disease (up to 30%) even after transurethral resection with or without adjuvant intravesical therapy [3–5].

The current gold standard in the detection and surveillance of these tumors is a combination of cystoscopy and urinary cytology (C). Ever since its inception in 1878, cystoscopy has evolved to include improved flexibility and visual definition. It, however, continues to be a subjective and invasive tool that can often miss discrete flat lesions [2]. C is an easy, readily available noninvasive test with historically high specificity for BC (in excess of 95%) [2]. However, recently, some contemporary data have suggested that these initially reported high specificity rates may not be

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reproducible [6]. More clinically relevant is its notoriously poor sensitivity especially for low-grade (LG) tumors [2]. Furthermore, it is operator dependant, and in certain settings, such as inflammation and infection, its interpretation can be arduous [7].

As such, much research has been geared to identify the best possible novel noninvasive urine-based bioassays for the detection, surveillance, prediction of recurrence, and progression of urothelial cancers. We present a prospective analysis of performance of urine assays on patients with BC using C and 4 Food and Drug Administration–approved commercially available urinary marker tests: Hemoglobin Dipstick (H), BTA *Stat* (B), NMP22 BladderChek (N), and ImmunoCyt (I).

2. Materials and methods

Between July 2007 and January 2009, urinary samples from 109 consecutive patients with a known history of bladder cancer or suspicion of bladder tumor based on workup of hematuria were prospectively collected within a clinical trial from a single institution following institutional review board approval. All urinary samples were tested for urinary C as well as 4 other urinary biomarkers: H, B, N, and I. Collected variables included various patient demographics, date of urinary collection, type of specimen (voided, washing, or catheterized), surgical pathology, recurrence, and follow-up.

All voided urine C samples were prepared as ThinPrep slides whereas other samples (washings or catheterized) were prepared as Cytospin or as a smear preparation after centrifugation. All were subsequently stained with the Papanicolaou stain. All were reviewed by 1 of 4 academic pathologists with training in cytopathology. As previously reported, only carcinoma or those lesions that were suspicious for carcinoma were considered clinically positive [8].

On the same day before cystoscopy, all biomarkers were prepared according to the instructions provided with the commercially available kits. Subsequent surgical specimen reports were examined, and sensitivity and specificity were calculated for each. Histologic specimens were graded according to the 2004 World Health Organization grading system [9].

Sensitivities, specificities, negative predictive values, positive predictive values, and efficacy of C and the other biomarkers were calculated for single tests and were further stratified according to LG and high grade (HG). Subsequently, receiver operating curve analyses were performed to determine the area under the curve. Calculations were performed after correlation with urinary cystoscopy and biopsy (histology specimens). Analyses were assessed for each test separately then as part of combinations including urinary C (C + H; C + B; C + N; and C + I). Similar to clinical practice, histology obtained from cystoscopy with biopsies was considered the reference standard for confirmation of diagnosis. Finally, to avoid the correlation of C or biomarkers or both with histology specimens, which may have included de novo tumors not previously present, we allowed a maximal arbitrary period of 1 year between the urine marker test and histologic specimen if there were any further histologic evaluations performed after the initial evaluation within that time period. This time interval has been previously used and reported in the literature as well as in prior reports from our institution [7,8,10].

We then proceeded to assess the performance of C and biomarkers, which was defined as having a positive test in the presence of a positive histology. To account for differences in baseline clinical variables and follow-up time and to adjust for the effects of potential confounders on the performance of the various markers, we conducted univariable and then multivariable analyses by using Cox proportional hazards models. All models were adjusted for age, smoking, reason for collection, pathological stage, and grade. Follow-up started at the initial time of urinary marker testing, and the criteria to end a patient's follow-up were time of histology or death or the aforementioned arbitrary cutoff period of 12 months, whichever came first. Accordingly, each marker was modeled as a time-fixed binary variable. $P < 0.05$ was considered statistically significant. All analyses were performed using the SAS version 9.1.3 Service Pack 4 statistical (window platform).

3. Results

3.1. Clinical characteristics

The median age was 69 (33–96) years, 81% of patients were white, and 83% were males. Specimens were collected in the morning in 84 patients (77%) and in the afternoon in 25 (23%). Methods of collection included voided urine in 82 patients (75%) with the remaining specimens obtained by washings, catheterization, or cystoscopy (Table 1). Histology result was positive in 83 patients (76%) and showed HG tumor in 23 (27%).

3.2. Single test sensitivity and specificity

Sensitivity values for each marker were as follows: C, 48% (16% for LG tumors and 84% for HG tumors); B, 61% (36% and 91%); H, 51% (38% and 66%); N, 58% (25% and 92%); and I, 62% (47% and 83%). Specificity results for each marker were as follows: C, 86%; B, 78%; H, 58%; N, 85%; and I, 79% (Table 2).

3.3. Combination test sensitivity and specificity

When urinary markers were combined with C, sensitivity values for HG tumors were as follows: C + H, 85%; C + B, 91%; C + N, 94%; and C + I, 90%. Specificity results for each combination were as follows: C + H, 57%; C + B, 78%; C + N, 84%; and C + I, 78% (Table 3). None of the triple combination permutations (all including C) yielded improved results compared with C + N for both