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Original article

Evaluation of a new quantitative point-of-care test platform for urine-based detection of bladder cancer

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Abstract

Objective: Several commercial point-of-care (POC) tests are available for urine-based detection of bladder cancer (BC). However, these tests are restricted to dichotomized results (positive or negative), which limits their diagnostic value. Quantitative protein-based tests offer improved risk stratification but require complex methods restricted to specialized centers. Recently, the first quantitative POC system based on the detection of cytokeratin fragments became available. The aim of the study was to evaluate the diagnostic accuracy of this quantitative POC test.

Patients and methods: A total of 198 patients having symptoms suspicious for BC were included. All patients received urethrocystoscopy and upper-tract imaging. Urine samples were analyzed by the urine BC antigen (UBC) rapid POC system and evaluated both visually and quantitatively using the concile Omega 100 POC reader. For visual evaluation, different thresholds of band intensity for considering a test positive were applied. Moreover, the UBC enzyme-linked immunosorbent assay (ELISA), urine cytology, and the nuclear matrix protein 22 BladderChek were performed. Sensitivities and specifities were calculated by contingency analyses. Optimal cutoffs of quantitative tests were determined by receiver operating characteristic curves.

Results: A total of 61 patients (30.8%) were diagnosed with BC. Visual evaluation of the UBC revealed sensitivities of 38.1% to 71.4% with corresponding specificities of 54.1% to 89.1%, dependent on the threshold of band intensity applied. The quantitative UBC rapid showed a sensitivity of 60.7% and a specificity of 70.1% at optimal cutoff (area under the curve = 0.68). A constant increase of both the probability of BC and high-risk BC with increasing UBC rapid values was observed. UBC concentrations determined by the reader significantly correlated with the UBC ELISA ($P < 0.001$). The UBC ELISA, the nuclear matrix protein22 BladderChek and cytology showed sensitivities of 48.3%, 16.4%, and 51.7% with specificities of 71.3%, 95.3%, and 78.1%, respectively.

Conclusion: The UBC rapid in combination with a quantitative POC-reader system for the first time enables quantitative determination of a BC marker under POC conditions. Diagnostic accuracy is at least equivalent to elaborate ELISA-based measurement. The quantitative use of the UBC rapid test facilitates risk prediction compared with conventional nonquantitative dichotomized POC testing.

Keywords: bladder cancer; urine markers; UBC rapid; point of care; NMP22; cytology; surveillance

1. Introduction

Cystoscopy is still considered as the gold standard in the diagnosis of bladder cancer (BC). In current guidelines, cytology is recommended as an adjunct to cystoscopy. Cytology is the most widely adopted noninvasive urine test [1] although it has low sensitivity especially for low-grade tumors [2,3]. Several urinary marker tests have been investigated in the last few years regarding their diagnostic accuracy and possibility of complementing cystoscopy in the diagnosis of BC. Although several tests and combinations of markers [4] have shown promising performance, their diagnostic accuracy cannot be considered sufficient to replace cystoscopy. One limitation of some of the broadly

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available urine tests, such as fluorescence in situ hybridization (FISH), is that they are relatively complex to perform and they are expensive [5,6]. At the present time, various point-of-care (POC) test systems are available on the market allowing fast and simple urine marker determination. The nuclear matrix protein 22 (NMP22) BladderChek detects the NMP regulating critical aspects of mitosis [7]. Urinary BC antigen (UBC) rapid is a test that detects fragments of cytokeratins 8 and 18 in urine samples. These cytokeratins are frequently overexpressed in tumor cells [8]. The disadvantage of all these tests is their limitation to a simple positive or negative result. Owing to a lack of studies assessing this issue, no recommendations regarding the semiquantitative use of POC tests for BC exist. Moreover, semiquantitative test bands anticipate reproducibility of test results.

Test systems, such as enzyme-linked immunosorbent assays (ELISA), can provide quantitative results for BC tumor markers. However, they are relatively expensive, complex, and time consuming compared with POC tests.

The concile Omega 100 reader is a photometric POC system for the use with several rapid tests, such as troponin and C-reactive protein. Recently, the system was adopted to the UBC rapid lateral flow test cassette. This platform has been developed to provide quantitative results of the UBC test with the advantage of an easy and fast application.

The aim of this study was to investigate the feasibility and diagnostic accuracy of this new system. Moreover, the performance of the system was compared with conventional visual evaluation of the POC test, the established ELISA method for UBC, the NMP22 BladderChek, and urine cytology.

2. Materials and methods

2.1. Patients

For this prospective study, 198 patients having symptoms suspicious for primary BC (such as hematuria or irritative voiding syndromes) or undergoing surveillance for BC were included between April and December 2012 in the Department of Urology, University of Tübingen, Germany. The study was approved by the local institutional review board (No. 032/2013BO2). All patients underwent cystoscopy, bladder ultrasound, upper-tract imaging (ultrasound, intravenous urography, retrograde pyelography, or computed tomography), and transurethral resection of bladder tumor in case of abnormal findings. Exclusion criteria were any kind of mechanical manipulation (cystoscopy, transrectal ultrasound, and catheterization) within 10 days before urine sampling, as well as existing gross hematuria.

2.2. Procedure

Midstream urine was collected in a sterile plastic container and processed subsequently. Urine samples were analyzed by the UBC rapid test (IDL, Bromma, Sweden), the NMP22 BladderChek test (Alere, Waltham, USA), the UBC ELISA (IDL, Bromma, Sweden), and urine cytology. All tests were done as advised by the manufacturer's instructions. First, results of the UBC rapid test were evaluated visually. The occurrence of a test band after 10 minutes of incubation was subdivided into 4 categories (no band, weak band intensity, intermediate, and strong band intensity). After visual evaluation, the test cartridges were analyzed by the photometric POC system concile Omega 100 reader (concile GmbH, Freiburg, Germany) for quantitative analysis. The Omega 100 reader illuminates the test field with a complementary color light to reduce interference in the analysis. The built-in charge-coupled device–matrix sensor takes a photograph of the light reflection, which is analyzed by the device.

For performing the NMP22 BladderChek, 4 drops of fresh urine sample were given on the test field of the cassette, as advised by the manufacturer, and results were obtained after 30 minutes. For cytology, urine was processed according to Papanicolaou [9] and microscopically reviewed by a urologist experienced in cytopathology. Accepted characteristic features of malignancy were considered [10].

2.3. Statistical analysis

Test performances were evaluated by contingency analysis. Statistical calculations were done with JMP (SAS Institute Inc, Cary, USA). $P < 0.05$ was considered significant. Receiver operating characteristic curves were performed to determine the area under the curve (AUC) and the optimal cutoff for the UBC ELISA and quantitative values of the reader.

3. Results

Overall, 198 patients were included in the study, 51 of these patients were under surveillance of non–muscle invasive BC (NMIBC) and 147 patients had no history of BC. Reasons for performing workup for BC in patients without history of BC included gross hematuria (32.7% of patients), microscopic hematuria (26.5%), irritative voiding syndromes (20.4%), suspicious bladder ultrasound (8.8%), and other reasons (11.9%; e.g., hydronephrosis, recurrent urinary tract infection). During workup in our hospital, 32 patients (16%) had suspicious findings in ultrasound (of whom 26 were diagnosed with BC). Median age of the study population was 70 years (range 20–90). Of these patients, 151 (76.3%) were men and 47 (23.7%) were women. Of the 198 patients, 61 (30.8%) had BC. Among the 61 patients, 39 had primary and 22 had recurrent BC; 48 (78.7%) had NMIBC (pTa and pT1 tumors), 4 (6.6%) had stage pT2, 6 had pT3 tumors (9.8%), and 3 had pure carcinoma in situ. A total of 17 (29.3%) patients had G1 tumors, 26 (44.8%) G2, and 15 (25.9%) had G3 tumors.