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Seminar article

Replacing cystoscopy by urine markers in the follow-up of patients with low-risk non–muscle-invasive bladder cancer?—An International Bladder Cancer Network project

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Abstract

Rationale: Numerous molecular urine markers for the diagnosis of bladder cancer have been developed and evaluated mostly in case-control settings through the past decades. However, despite all efforts none of them has been included into clinical decision-making and guideline recommendations until today. The aim of this retrospective longitudinal analysis was to investigate if a molecular marker might be able to replace cystoscopy as a primary examination in diagnosis and follow-up of patients with pTa grade 1-2 bladder cancer.

Materials and methods: Totally 36 patients (32 men) with pTa grade 1-2 bladder cancer underwent 232 follow-up examinations including urine analysis, cytology, immunocytology (uCyt+), and urethrocystoscopy (UC). Mean age at study entry was 63 years. Patients were observed through a median follow-up interval of 3.8 years.

Results: In summary, 47 Transurethral Resection of Bladder Tumors (TURB) procedures were indicated based upon a positive UC (44) or as re-TURB (3) and 33 tumors (plus 1 case of pTa G0) were histopathologically confirmed. Although uCyt+ was positive in 12/13 primary tumors (92.3%), sensitivity dropped to 13/20 (65%) in tumor recurrence presumably because of their smaller size. Urine cytology had a sensitivity and a specificity of 30.3% and 94.9%, respectively, but did not improve the sensitivity of uCyt+ alone. If UC was based upon a positive uCyt+ test, 8/33 tumors (24.2%) would have been overlooked or diagnosed late. In contrast, 173 UCs (74%) would have been saved and 5 presumably unnecessary TURB procedures would not have been indicated.

Conclusions: This longitudinal study suggests a potential of molecular urine tests in replacing cystoscopy in the follow-up of patients with pTa G1-2 bladder cancer. The use of additional markers might further improve sensitivity of urine testing. A prospective randomized study has been initiated to prospectively investigate the performance of a marker panel against UC.

Keywords: Urine markers; Non–muscle-invasive bladder cancer; Immunocytology; Urine cytology; Follow-up; Cystoscopy; TURB; Disease management

Introduction

According to current guidelines follow-up of patients with non–muscle-invasive bladder cancer (NMIBC) stage pTa G1-2 is primarily based upon urethrocystoscopy (UC) [1,2]. For low risk tumors, defined as primary, solitary, Ta LG lesions less than 3 cm of size and without concomitant carcinoma in situ UC is the only examination recommended by the guideline of the European Urological Association

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(EAU). Although follow-up recommendations for low-risk NMIBC are straightforward, for intermediate-risk tumors (defined as either multiple or recurrent and larger Ta G1-2 tumors [>3 cm]) no clear guidance is provided by the EAU guideline leaving the decision how to follow-up these patients to the urologist. It appears noteworthy that despite the high incidence of NMIBC guidelines are largely based upon expert opinion and efficacy of the recommendations in this patient group lacks prospective validation.

This lack of prospective studies and, hence, evidence for follow-up of patients with low/intermediate NMIBC has fueled an ongoing discussion. On the contrary it is a general rule in oncology to diagnose and treat tumor recurrence as early as possible. The rationale behind this concept is to prevent potential tumor progression. However, because of the low risk of tumor progression in patient with pTa G1-2 bladder cancer the relevance of an intense routine follow-up in low/intermediate-risk disease may be questioned. This as UC, the so called "gold standard" in follow-up of NMIBC would cause patient discomfort and is related to side effects such as bleeding and infection.

Theoretically, noninvasive diagnosis using urine cytology or molecular markers might be helpful in this situation as urine has been in direct contact with the tumor lesion and is readily available. Furthermore, cell adherence is known to be decreased in tumor cells resulting in preferential shedding of malignant cells into urine. In general, this concept looks attractive with a potential of sparing at least a significant part of patients from unnecessary invasive diagnosis. However, it remains doubtful if the performance of urine cytology and currently available urine markers is sufficient in this situation with mostly very small tumors exerting low grades of anaplasia [3,4].

The aim of this longitudinal analysis was to investigate if urine cytology and a commercially available immunocytological urine test (uCyt+) might be able to guide a noninvasive follow-up regimen of patients with pTa G1-2 NMIBC.

Material and methods

A retrospective analysis was performed in 36 patients undergoing diagnosis and therapy in a private practice in southern Germany with either primary or recurrent pTa G1-2 NMIBC receiving urine analysis (dip stick and sediment analysis), cytology, uCyt+ tests, and UC for diagnosis or follow-up between 5/2002 and 1/2016. Voided urine specimens were obtained before UC ($n = 194$) or within 32 days after UC ($n = 36$). Follow-up was performed with 4 annual visits for 2 years, biannual examinations for another 3 years followed by annual follow-up visits after 5 years (pending patient compliance). Institutional review board approval (FAU 305_15 B) was granted by the Ethics committee of the Friedrich-Alexander University, Erlangen, Germany, and written informed consent was obtained from all patients.

UCyt+ is a commercially available immunocytological assay based upon microscopic detection of tumor-associated cellular antigens in urothelial cells by immunofluorescence (Scimedx, Denville, NJ). The test was performed according to the manufacturer's protocol as previously described [5,6]. Briefly, voided midstream urine (>30 ml) was prefixed with an equal amount of ethanol (50%) and 0.5 ml fixative solution was added. The samples were then stored at 4°C for up to 7 days until analysis. The samples were filtered through a polycarbonate membrane (pore diameter 8 μm), the cells were then transferred to a slide, fixed with isopropanol (50%), rehydrated with ethanol (80%, 70%, 50%, Aqua dest), and consecutively stained with haematoxylin (4% acetic acid) for 6 seconds. Subsequently, the slides were incubated with blocking solution for 15 minutes, washed and incubated for 1 hour with an antibody cocktail containing fluorescein-labelled monoclonal antibodies M344 and LDQ10 directed against sulfated mucin glycoproteins and Texas-red linked antibody 19A211 against glycosylated forms of high molecular carcinoembryonic antigens. After washing and mounting with Permafluor Medium (ImmunonTM, ThermoShandon, Pittsburgh, PA), the slides were studied for immunofluorescence at 500× magnification. Slides with less than 500 nuclei or less than 1 epithelial cell/HPF (200×) were excluded from the study. Positive and negative controls were performed with each test run. Specimens with ≥1 green or red urothelial cell were considered positive. All analyses were performed by an experienced technician performing >500 tests annually.

Urine cytology was performed using immunocytology specimens as described previously by experienced technicians each performing >1.500 tests annually [5,6]. Briefly, cover slips were removed and counterstaining was performed with hematoxylin (15 s). The slides were mounted again with Entellan Mounting Medium (Merck, Darmstadt, Germany), sealed again with cover slips and examined microscopically at 400× magnification. Test results were provided as normal, atypical (including mild and moderate dysplasia), suspicious (severe dysplasia), and positive.

Urine cytology and uCyt+ were subjected to regular quality control and laboratory staff members were blinded toward because of UC.

Statistical analysis was performed with SAS/STAT and SAS/IML software, version 9.4 (SAS Institute Inc., Cary, NC). To examine the association between tumor weight and recurrence, a frequency table and Fisher exact test were calculated Sensitivities, specificities, positive predictive values (PPVs) and negative PVs (NPVs) with exact Clopper-Pearson 95% CI for uCyt+, urine cytology, and erythrocytes in urine examinations with cystoscopy and tumor resections (TURB) following positive cystoscopy were computed. In addition, the distribution of uCyt+ test results and urinary cytology by time before detection of a bladder tumor because of TURB was analyzed.

Results

The present study is a retrospective longitudinal analysis on 36 patients (32 males and 4 females) with pTa G1-2 NMIBC