The Follow-up of Patients with Non-muscle-invasive Bladder Cancer by Urine Cytology, Abdominal Ultrasound and Urine CYFRA 21-1: A Pilot Study

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Abstract. *Aim: This study aimed to evaluate the accuracy of urine cytology, bladder ultrasound (US), urine cytokeratin 19 fragment assay (CYFRA 21-1) and the combination of these noninvasive modalities in the detection of recurrent bladder cancer. Patients and Methods: In a total of 154 patients that were followed with cystoscopy after endoscopic resection of non-muscle-invasive bladder cancer, we performed and analyzed results of 311 observations that included cytology, CYFRA 21-1, US. The urine concentration of CYFRA 21-1 was measured by an immunoradiometric assay. Results: Cystoscopy and biopsy revealed recurrent bladder tumors in 21 patients. Most of the tumors (77%) were less than 10 mm in their largest diameter. Urine cytology, US and urine CYFRA 21-1 resulted in overall sensitivity of 19.1, 52.4 and 71.4% and specificity of 96.6, 99.7 and 68.6%, respectively. Each of these methods used alone yielded false-negative results in patients harboring tumors at high risk of progression. The combination of all three methods had sensitivity and specificity of 90.5 and 67.2%, respectively. All three tests were negative in 197 of 311 observations (63.3%), missing only 2 low-risk tumors. Conclusion: The combined use of US, urinary CYFRA 21-1 and cytology appears to be an effective, noninvasive approach for the detection of recurrent bladder tumors.*

Bladder cancer is a common malignant neoplasm of the genitourinary tract. At the time of presentation, about 75% of patients have superficial tumors confined to the mucosa (Ta) or the lamina propria (T1). After being treated with transurethral resection (TUR) about 50% of the patients will have tumor recurrence within 2 years. Most recurrent tumors are of low grade and are superficial, but 10-25% may progress to a higher grade and stage (1, 2). This is why a stringent follow-up to detect recurrent tumors is mandatory. The current guidelines recommend cystoscopic surveillance at 3 months initially, and then at increasing intervals (3, 4). Since recurrence may present even many years later, lifelong surveillance is needed. Cystoscopy is an invasive and uncomfortable procedure, therefore many investigators have sought noninvasive tests to replace it. Urine cytology alone has an inadequate sensitivity for the detection of low-grade (LG) tumors but is very accurate in the detection of high-grade (HG) ones, consequently it is still the most common noninvasive test used in the follow-up of patients after TUR (5).

A number of tumor-associated markers have been evaluated and among them, the CYFRA 21-1 assay that measures the concentration of soluble fragments of cytokeratin 19 (6). The results of several studies have shown a significantly higher sensitivity of this assay compared to cytology in detecting LG bladder tumors (7). Recently, however, a combination of CYFRA 21-1 with cytology and/or hemoglobin dipstick was shown not to be a useful enough tool for non-muscle-invasive bladder cancer (NMIBC), since many tumors with high risk of progression were missed (8).

Detection of some bladder tumors can also be achieved by transabdominal ultrasound (US). This method is widely used as a primary screening of bladder problems by primary practitioners and is safe, fast, cost-effective and highly accepted by patients. A close correlation between US and cystoscopy was reported (9). US may visualize bladder papillary growth in the majority of cases, but it has not been recommended for routine follow-up of patients after TUR due to its low sensitivity in the diagnosis of flat tumors and carcinoma *in situ* (CIS) (10).

Earlier, we suggested that an addition of abdominal US to urine CYFRA 21-1 assay with urine cytology may improve the detection of NMIBC (11). Therefore, in this pilot study,

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Table I. *Distribution of primary bladder tumors by grade and stage.*

Grade/stage	N (%)
Grade	
Low	116 (75.3)
High	38 (24.7)
Stage	
pTa	120 (77.9)
pT1	21 (13.6)
Carcinoma *in situ*	13 (8.5)
Total	154 (100)

Table II. *Performance characteristics of urine cytology, abdominal US and CYFRA 21-1.*

	Cytology (95% CI)	US (95% CI)	CYFRA 21-1 (95% CI)
Sensitivity (%)	19.1 (7.7-40.0)	52.4 (32.4-71.7)	71.4 (50.0-86.2)
Specificity (%)	96.6 (93.8-98.1)	99.7 (98.1-99.9)	68.6 (63.1-73.7)
NPV (%)	94.3 (93.0-95.4)	96.7 (94.9-97.8)	97.1 (94.5-98.5)
PPV (%)	28.6 (12.5-52.9)	91.7 (67.9-98.3)	14.2 (10.6-18.6)

CI, Confidence interval; NPV, negative predictive value; PPV, positive predictive value.

we investigated the efficacy of these three noninvasive modalities used alone or in combination for the detection of recurrent bladder tumors.

Patients and Methods

A total of 154 patients, 131 men and 23 women, after TUR of NMIBC were consecutively included in this prospective study approved by the Institutional Ethical Review Board. All participants provided an informed consent. The distribution of the primary bladder tumors by grade and stage is shown in Table I. The median age was 70 years (range, 37-92 years). Twenty-five of the 154 patients (16.2%) had been treated with adjuvant intravesical bacillus Calmette-Guerin (BCG) during the two years preceding this study according to the standard protocol used in our institution: induction course with 6 weekly instillations followed by maintenance instillation every 3 months for 2 years.

Urine samples for cytology and CYFRA 21-1 were obtained and abdominal US and cystoscopy were performed at 3-4 month intervals after TUR. The period for follow-up was 6-12 months. During follow-up, 311 observations were performed in 154 patients (range 1-4, median 2). Patients with positive cystoscopies were treated by TUR. Staging of the recurrent tumors was performed according to the TNM classification and grading by the WHO/ISUP 1998 grading system (12). The cystoscopy was considered as the "gold standard" method for diagnosing recurrences. In the case of positive urine cytology as sole finding, CT urogram and random bladder biopsies were performed. The voided urine specimens were divided into two aliquots. One was prepared for cytopathological examination and the other was frozen and stored at –20˚C for the CYFRA 21-1 assay. Urinary cytology was performed independently by board-certified cytopathologists using standard diagnostic criteria (5). The cytology results were determined according to the Papanicolaou classification and were divided into negative, suspicious and positive, corresponding to the classes I-II, III and IV, respectively. In this study classes III and IV were considered pathological.

Urine cytokeratin 19 fragments were measured using commercially available solid-phase 'two-site sandwich' immunoradiometric assay (ELISA-CYFRA 21-1; CIS Bio International, Gif-sur Yvette, France). The concentration of CYFRA 21-1 was expressed in μg/l. The normalization of CYFRA 21-1 for urine creatinine concentration was not performed, since it did not improve the accuracy of the assay (11).

All patients underwent transabdominal bladder US examination using an AcusonXP10 unit with electronic curved array 3.0/5.0 MHz sector transducers. Patients were asked to present with a full bladder. The bladder was investigated with transverse and longitudinal scans with the patient in the supine position. Echogenic exophytic lesions that protruded from the bladder wall and could be separated from the prostate were diagnosed as bladder tumors. The US examinations were performed by a board-certified urologist who had acquired experience in ultra-sonography or with a board-certified radiologist.

Statistical analysis. Associations between categorical variables were evaluated with Fisher's exact test or Chi-square test. Correlation between numerical variables was analyzed by a linear nonparametric (Spearman) correlation test. For analyses of the sensitivity and specificity relation of the CYFRA 21-1 assay, receiver operating characteristic (ROC) curves were constructed and used as a tool for determination of an optimal cut-off value. The performance characteristics of different diagnostic approaches were determined according to statistical methods for the analysis of rates and proportions. Comparison of the sensitivity of the two assays was based on paired observations for which the McNemar test was appropriate. Statistical calculations were performed using SPSS®, version 10 (SPSS Inc., Chicago, IL, USA) and WinPepi, version 7.4 (13). A value of $p<0.05$ was considered significant.

Results

Recurrent tumors were found in 21 out of 154 patients. Of 21 recurrent tumors, 17 were LG and two were HG transitional cell carcinoma. In two cases, papillary urothelial tumors of low malignant potential were detected. The stage distribution was as follows: CIS in one patient, Ta in 18 and T1 in two patients. The majority of tumors (77%) were less than 10 mm. Only one patient had a large (30 mm) tumor. Multiple lesions (≥3) were found in one patient.

Each modality used alone missed high-risk urothelial tumors. Urine cytology detected suspicious cells in 14 samples, among which 4 were true positive findings. Among 17 tumors missed by cytology, one was LG T1 tumor, and the remaining 16 were LG Ta tumors, including one patient with multifocal lesions. US detected 11 tumors; 10 tumors were overlooked, including one CIS, one T1 HG tumor, one LG T1 tumor and multifocal recurrence in one patient.

Table III. *Performance characteristics of urine cytology, abdominal US and CYFRA 21-1 used in different combinations.*

	Cytology + US (95% CI)	Cytology + CYFRA 21-1 (95% CI)	US + CYFRA 21-1 (95% CI)	Cytology + US + CYFRA 21-1 (95% CI)
Sensitivity (%)	61.9 (40.9-79.3)	76.2 (54.9-89.4)	85.7 (65.4-95.0)	90.5 (71.1-97.4)
Specificity (%)	96.2 (93.3-97.9)	67.6 (62.0-72.7)	68.3 (62.7-73.4)	67.2 (61.6-72.4)
NPV (%)	97.2 (95.3-98.4)	97.5 (94.9-98.8)	98.5 (96.2-99.5)	99.0 (96.7-99.7)
PPV (%)	54.2 (37.9-69.6)	14.5 (11.2-18.7)	16.4 (13.2-20.1)	16.7 (13.8-20.0)

CI, Confidence interval; NPV, negative predictive value; PPV, positive predictive value.

Based on the data from 311 observations, an ROC curve was constructed. For CYFRA 21-1 concentrations, the area under the curve was 0.72±0.06 and the inflection point was 4.3 μg/l.

The urine concentration of CYFRA 21-1 was higher than 4.3 μg/l in 106 samples, and only 15 were true-positive results. Six tumors were missed using the CYFRA 21-1 assay. One was CIS and 5 were LG tumors, less than 5 mm in size. Abnormal urinary cytology, abnormal abdominal US, and elevated urine concentration of CYFRA 21 were all significantly associated with the presence of recurrent bladder tumor (p=0.001, p<0.001 and p<0.001, respectively).

The performance characteristics of the three modalities used alone and in various combinations are presented in Tables II and III. The McNemar test showed a significant difference in favor of CYFRA 21-1 assay *versus* cytology (p=0.003). The difference between US and cytology, as well as between CYFRA 21-1 and US was not significant (p=0.07 and p=0.34, respectively). The combination of all three tests had a sensitivity of 90.5%, being significantly higher then the sensitivity of any single modality (cytology, p<0.0001; US, p=0.008), with the exception of the CYFRA 21-1 assay (p=0.07). In 197 observations (63.3%), all three tests were normal and only 2 LG Ta tumors less than 3 mm in diameter were missed using the 3-mode combination.

There were 41 observations in patients after intravesical BCG treatment. The rate of false-positive results with CYFRA 21-1 assay among these patients comprised 46.3%, compared to 28.2% in the group that had not been treated with BCG (p=0.03).

Discussion

The high propensity for recurrences of NMIBC dictates cystoscopic surveillance at defined intervals. Although flexible cystoscopy has greatly reduced the morbidity associated with cystoscopy, it remains an invasive and uncomfortable examination. Various non-invasive tests have been investigated, including urine cytopathology, immunostaining and fluorescent *in situ* hybridization (FISH) of exfoliated cells or measuring the concentration of soluble components in the urine such as cytokeratins. In addition, bladder US has been used for the detection of recurrent bladder tumors.

In this pilot study we have explored for the first time the role of the combined three noninvasive tests in the diagnosis of recurrent bladder carcinoma. We found that abnormal cytology, a high level of CYFRA 21-1 and abnormal abdominal US are all indicative of the possibility of tumor recurrence. Nevertheless, the sensitivity and specificity of each of these modalities alone was not sufficient to replace cystoscopy.

Previous studies have shown that urinary cytology has a sensitivity of only 10%-40% in the detection of LG tumors. However, cytology is still a widely used test because of its higher sensitivity in detecting HG tumors and CIS (5). Since most recurrent tumors were small and of LG, cytology alone recognized only 4 out of 21 lesions (sensitivity of 19.1%).

CYFRA 21-1 has shown promise as a diagnostic marker (7, 14). Previous studies have reported that the CYFRA 21-1 assay has a sensitivity between 70% and 88% and a specificity between 43% and 95% in the detection of recurrent bladder tumors (8, 11, 15, 16). In our current study, the results of the CYFRA 21-1 assay on 311 urine samples were used to construct an ROC model for the detection of recurrent tumors. The optimal cut-off level defined from this model, 4.3 μg/l, yielded a sensitivity of 71.4% and a specificity of 68.6%, lower than those (75.7% and 72.9) reported in our previous study (11). The different results reflect the differences in the size and grade of recurrent tumors found during surveillance. In the current study, when compared with primary tumors, 90% of the tumors were LG and 29% of the tumors were smaller than 5 mm. Five out of the six patients that had false-negative CYFRA 21-1 results had LG tumors, less than 5 mm in diameter.

Bladder US may detect even small exophytic bladder tumors. Previous studies resulted in sensitivity between 50% and 74% and specificity between 83% and 90% in the detection of recurrent tumors (17-20). In our study, transabdominal US missed 10 tumors, including CIS, an HG T1 tumor and a multifocal recurrence. US had a sensitivity of 52.4% and a specificity of 99.7%. This is in accordance with previous observations that US has not proven itself as

an accurate tool for detecting small flat tumors and CIS (10). Therefore, the use of US cannot be recommended for the surveillance of patients with a history of HG cancer or CIS.

Several studies have demonstrated that cytology combined with US (19, 20) or a urine tumor marker (21-23) may enhance the performance of each of these tests. Most studies reported a synergistic effect, but the sensitivity was still below 80% . In our study, the combined use of cytology with US or with CYFRA21-1 assay resulted in a sensitivity of 61.9% and 76% , respectively.

Fernandez-Gomez *et al*. (8) using cytology combined with the CYFRA 21-1 assay (cut-off 1.5 ng/ml) and/or hemoglobin dipstick in the follow-up of patients with superficial bladder cancer reported the combined sensitivity of three tests to be 74.4% . Cytology contributed only 4.8% to the sensitivity of the CYFRA 21-1 test, as in our study, and it was not improved by the addition of hemoglobin dipstick. It should be noted that the preparation of urine samples in that study and in ours were different. We measured the CYFRA 21-1 concentration in uncentrifuged urine, while in the study of Fernandez-Gomez *et al.,* supernatants of centrifuged urine were explored. Earlier, we reported a significant decrease of CYFRA 21-1 in the urine samples after centrifugation. This may be the main reason for the significantly higher optimal cut-off level used in our study compared to that in the study of Fernandez-Gomez *et al*.

Several authors have expressed their opinion, as have patients, that any test used for early diagnosis of tumors should accurately detect at least 90% of the tumors (24). We were able to detect 90.5% of the recurrent tumors using a combination of cytology, urine CYFRA 21-1 and bladder US. Of 311 observations, the combined triple test was negative in 197 (63.3%). Only two LG tumors, 2-3 mm in size, were missed by this combined approach. Such tumors may be followed without treatment and could be detected later by one of the noninvasive tests (25). However, since a combination of multiple tests is accompanied by a lower specificity, cystoscopy is often required to verify positive results, in many cases only to reveal that the results were falsely positive. In our study, the overall specificity of cytology and US used alone or in combination was 96.6% , 99.7% and 96.2% , respectively. The addition of the CYFRA 21-1 assay caused a decrease in specificity to 67.2% . The positive predictive value (PPV) of the combined triple test was 16.7% , implying that for each true-positive result there will be five false-positive results. On the other hand, the use of this combined test allowed us to avoid 63.3% of the cystoscopies that would not find any significant tumor in any case.

In this series, we observed a relatively low recurrence rate (13.6%). This low rate may be explained by the relatively short follow-up period (6-12 months) and the high proportion of LG tumors (75.3%) and pTa stage (77.9%) among the primary bladder tumors.

Bladder inflammation and intravesical immunotherapy with BCG lower the specificity of the CYFRA 21-1 test (8, 11, 16). In the present study, the rate of false-positive results among patients who had received BCG therapy was significantly higher as compared to patients who had not received such treatment (46.3% *versus* 28.2%). Therefore, the CYFRA 21-1 assay should not be part of surveillance in patients who have been treated with BCG.

Conclusion

The combined use of US, urinary CYFRA 21-1 and cytology appears to be an effective, noninvasive approach for the detection of recurrent bladder tumors and may, after validation by a larger scale prospective trial, substitute cystoscopy in more than half of the patients under surveillance. The specificity of combined testing can be improved by the exclusion of patients who have been previously treated with intravesical BCG.

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