Low Utility of CYFRA 21-1 Serum Levels for Diagnosis and Follow-Up in Bladder Cancer Patients

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We evaluated serum levels of soluble fragments of cytokeratin 19 (CYFRA 21-1) by immunoassay (ES-700; Roche Diagnostics, Mannheim, Germany) to assess its usefulness in the diagnosis and follow-up of bladder cancer. The study included 39 patients with a diagnosis of transitional cell carcinoma (group 1) and 190 patients (group 2) with no evidence of tumor. In group 2, 180 patients had a history of bladder cancer, and 10 had benign prostatic hyperplasia. Significant differences in CYFRA 21-1 concentrations between groups 1 and 2 ($P < 0.01$) were noted. However, CYFRA 21-1 levels did not significantly differ between the patients with benign prostatic hyperplasia and those with bladder cancer ($P = 0.274$). CYFRA 21-1 values were higher in invasive

bladder cancer compared to that detected in superficial stages ($P = 0.011$). Setting the optimal cutoff value at 2.5 ng/mL resulted in a sensitivity of 43.6% and a specificity of 82.1%. No statistical differences were found when we compared disease-free time among the 66 patients with recurrences (30.7 months with levels < 2.5 ng/mL vs. 41.2 months with levels > 2.5 ng/mL; $P = 0.248$). The risk of recurrence in patients with levels lower than 2.5 ng/mL (0.79) was no different ($P = 0.097$) from that found in patients with higher levels (1.69). CYFRA 21-1 serum levels do not provide enough sensitivity to justify its application in routine protocols for the detection and follow-up of bladder cancer. *J. Clin. Lab. Anal.* 19:167–171, 2005. © 2005 Wiley-Liss, Inc.

Key words: CYFRA 21-1; serum; bladder cancer; diagnosis; follow-up

INTRODUCTION

There are about 263,000 new cases of bladder cancer and 115,000 deaths from this disease each year worldwide. About 90% of these cancers are transitional cell carcinomas (1). At the time of presentation, 75–85% of patients have symptomatic hematuria. Diagnostic tools for patients with bladder cancer symptoms include echography, urinary cytology, cystoscopy, and eventually urography. Unfortunately, more than 70% of superficial tumors recur despite treatment, and therefore long-term follow-up of these patients is required after transurethral resection of the tumor is performed (2).

Researchers have attempted to replace cystoscopy with cytopathology and flow cytometry as diagnostic tools, but these methods have insufficient sensitivity for well or moderately differentiated tumors (3). For these reasons, a more objective noninvasive marker of bladder

transitional cell carcinoma would be useful. Ideally, this new marker would also help the urologist assess the need for cystoscopy, which is an expensive and uncomfortable examination, or increase the interval between endoscopic evaluations (4,5).

Recently many investigators have developed new, noninvasive tests to replace traditional diagnostic methods for detecting bladder cancer (6,7). One of these methods involves the detection of cytokeratins, which are intermediate filaments expressed by all epithelial

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cells, and which appear to be useful markers of epithelial differentiation. Cytokeratin 19 is an acid-type cytokeratin of 400 amino acids with a low molecular weight (40 kD). During cell lysis it is released as soluble fragments that may be detected using the CYFRA 21-1 assay in serum or urine. The CYFRA 21-1 assay measures cytokeratin fragments of cytokeratin 19 with the aid of two specific monoclonal antibodies: BM 19.21 and KS 19.1. Both epitopes are recognized by the monoclonal antibodies in the C-terminal helical region of the molecule (8,9).

The aim of this study was to evaluate the diagnostic usefulness of serum CYFRA 21-1 levels in patients with bladder cancer. We analytically evaluated the CYFRA 21-1 assay kit by testing its utility for detecting bladder cancer and reducing routine cystoscopy during follow-up of patients who were under surveillance after undergoing transurethral tumor resection.

MATERIALS AND METHODS

Patients, Samples, and Study Design

A total of 229 patients were enrolled in the study. Demographic data, such as age and sex, were registered. Group 1 included 39 bladder cancer patients with a mean age of 68.9 years (standard deviation (SD)=7.9; range=50–85). Group 2 consisted of 190 subjects with no evidence of tumor (180 of these patients had previously treated bladder cancer but were free of disease at the time of the study, and 10 had benign prostatic hyperplasia). Group 2 patients had a mean age of 69.3 years (SD=9.7; range=41–89 years). The male-to-female ratio for groups 1 and 2 was 33/6 and 154/36, respectively. Between November 1993 and July 2000, we followed up the 180 patients with a history of bladder tumors but no evidence of the disease at study entry. Serum analysis of CYFRA 21-1 was performed when the patients entered in the study. Subjects with a previous diagnosis of malignancy other than bladder cancer of any histology were excluded from the study. All of the subjects who took part in the study gave their informed consent.

Histopathological data were also recorded. In group 1 the cases were staged as superficial carcinoma (Ta or T1) or muscle invasive disease (T2–T4) according to TNM criteria (10). Serum samples were collected and centrifuged at 1,500 rpm for 8 min at 4°C, aliquotted, and then frozen at –20°C until CYFRA 21-1 was measured. The serum samples were always analyzed during the first month after they were frozen. All samples were assayed in duplicate. Serum CYFRA 21-1 values were determined using a commercial kit (Roche Diagnostics, Mannheim, Germany). For the quantitative determination of cytokeratin 19 fragments in vitro, the principle of

the test was based on a two-step sandwich assay (11) using streptavidin-biotin technology on an automated analyzer (ES-700; Roche Diagnostics). The sample or standard (35 µL) and incubation solution (700 µL) mixed with biotinylated antibody (KS 19.1) were incubated in streptavidin-coated polystyrene tubes for 30 min. After washing, the retained antibody was incubated with horseradish peroxidase conjugated antibody (BM 19.21) for 30 min. The unbound conjugate was removed by another washing procedure. After the addition of a 700-µL substrate-chromogen solution, and incubation for 60 min, the absorbance was measured photometrically at 422 nm. The results are expressed in ng/mL.

The distribution of the CYFRA 21-1 values in serum were asymmetric; therefore, we evaluated statistical inferences using nonparametric tests. We assessed differences between the two means using the Mann-Whitney U-test. When three or more groups were considered, differences were evaluated using a Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA). Sensitivity, specificity, positive predictive value, negative predictive value, and global accuracy were obtained by receiver operating characteristics (ROC) analysis. We used different cutoffs, applying our own experience and suggestions by other authors (12). All of the data were analyzed with the use of the SPSS 11.0 statistical software package.

RESULTS

No significant differences between groups were observed regarding age or sex distribution. Table 1 shows a descriptive analysis of CYFRA 21-1 serum concentrations among all groups of patients with and without bladder cancer and for various parameters of urothelial cancers. A significant difference was noted between groups 1 and 2 in the CYFRA 21-1 serum values ($P<0.01$). However, the mean CYFRA 21-1 serum levels in group 2 patients with benign prostatic hyperplasia ($n=10$), (2.22 ± 1.31 ng/mL) were not significantly different than those in group 1 patients with bladder cancer ($P=0.274$). A statistical analysis of CYFRA 21-1 serum levels according to pathological staging and grading showed no significant differences among the patients with bladder cancer. Nevertheless, the mean CYFRA 21-1 serum values were higher in patients with invasive bladder transitional cell carcinoma ($n=15$) than those observed in superficial stages ($P=0.011$).

Table 2 shows the sensitivity, specificity, positive predictive value, negative predictive value, and global accuracy of CYFRA 21-1 serum values taken at different cutoffs. The results of CYFRA 21-1 serum

TABLE 1. Descriptive analysis of CYFRA 21-1 serum concentrations (ng/mL) among all groups of patients with and without bladder cancer and in various parameters of urothelial cancers

	N	Mean	SD	MSE	Median	P ₂₅ –P ₇₅
Grade						
I	11	2.4	2.1	0.6	1.9	1.3–2.2
II	14	3.7	5.1	1.4	2.2	1.7–2.8
III	14	8.5	13.2	3.5	2.6	1.9–10.3
Stage						
Ta	11	2.2	1.5	0.4	2.1	1.1–2.5
T1	13	2.4	1.8	0.5	1.9	1.9–2.2
T2	2	11.8	13.0	9.2	11.8	2.6–21.0
T3	4	7.0	9.3	4.7	2.6	2.1–16.4
T4	9	9.9	15.6	5.2	2.9	1.6–14.0
Primary/superficial	2	2.9	0.5	0.4	2.9	2.5–3.2
Primary/invasive	8	7.8	8.3	2.9	3.0	2.6–17.4
Recurrent/superficial	22	2.3	1.7	0.4	1.9	1.4–2.2
Recurrent/invasive	7	11.2	17.8	6.7	1.9	1.5–21.3
Overall group 1 (tumors)	39	5.0	8.8	1.4	2.1	1.7–3.0
Overall group 2	190	1.9	1.2	0.1	1.6	1.2–2.0

SD, standard deviation; MSE, mean standard error; P₂₅ percentile 25; P₇₅, percentile 75.

TABLE 2. Diagnostic characteristics of CYFRA 21-1 serum values with different cut-offs from ROC analysis

n = 229	cutoff: 2.1 ng/mL	cutoff: 2.5 ng/mL	cutoff: 5.4 ng/mL
Sensitivity (%)	21/39 (54)	17/39 (43.6)	7/39 (17.9)
Specificity (%)	145/190 (76.3)	156/190 (82.1)	190/190 (100)
PPV (%)	21/66 (31.8)	17/51 (33.3)	7/39 (18)
NPV (%)	145/163 (89)	156/178 (87.6)	190/190 (100)
Accuracy (%)	166/229 (72.5)	173/229 (75.6)	197/229 (86)

levels in patients from groups 1 and 2 were used to construct the ROC model for the detection of bladder carcinoma. Using the cutoff level of 2.1 ng/mL that was defined by this model, the sensitivity of the assay for the detection of primary bladder tumors was 53.8% and the specificity was 75.3%. Setting the optimal cutoff value at 2.5 ng/mL resulted in a sensitivity of only 43.6% and a specificity of 82.1% (area ROC of 0.697). Using another cutoff (3.3 ng/mL) suggested in the literature (12), sensitivity was only 20.5% and specificity increased to 88.9% for the detection of bladder tumors. With a cutoff value of 3.3 ng/mL, the diagnosis for bladder transitional cell carcinoma was negative in 31 patients with cancer. In addition, there was a 10% rate of false-positive results for patients with benign prostatic hyperplasia. Furthermore, with a cutoff value of 2.1, the rate of false-positive results reached 30%.

In Table 3 we present a comparison of diagnostic utility between CYFRA 21-1 serum levels and cytology, setting the optimal cutoff value at 2.5 ng/mL, for patients in group 1 according to the grade and stage of disease.

Cytokeratin detection to evaluate recurrences in patients with a previous bladder cancer history (66

TABLE 3. Diagnostic characteristics of CYFRA 21-1 compared to urinary cytology taking a cutoff of 2.5 ng/mL according to the grade and stage in bladder cancer patients

	Sensitivity (%)	
	CYFRA 21-1	Cytology
Grade		
I	2/11 (18.2)	3/11 (27.3)
II	6/14 (42.9)	7/14 (50.0)
III	9/14 (64.3)	12/14 (85.7)
Stage		
Ta	4/11 (36.4)	3/11 (27.3)
T1	2/13 (15.4)	6/13 (46.2)
T2	2/2 (100)	2/2 (100)
T3	3/4 (75)	3/4 (75)
T4	6/9 (66.7)	8/9 (88.9)

recurrent tumors after follow-up) did not reveal any utility for our purposes. The mean CYFRA 21-1 serum levels (1.98 ± 1.27 ng/mL; 40.1 months follow-up) in patients with recurrence from group 2 (n = 66) did not significantly differ ($P = 0.137$) from those in patients

without recurrence ($n=114$; 1.74 ± 1.11 ng/mL; 35.6 months follow-up). Indeed, with a cutoff of 2.5 ng/mL, we found no statistical differences when we compared disease-free time among the patients with recurrences (41.2 months with levels <2.5 ng/mL vs. 30.7 with levels >2.5 ng/mL; $P=0.248$). Statistical differences were not found between serum CYFRA 21-1 levels and recurrence risk ($P=0.097$); patients who had CYFRA 21-1 values lower than 2.5 ng/mL were associated with an odds ratio of 0.79 (IC 95%; 0.625–1.002) and patients with higher CYFRA 21-1 values were associated with an odds ratio of 1.69 (IC 95%; 0.855–3.322). We noted very similar results when we compared patients for the first year follow-up (35 recurrences vs. 145 no recurrences), using the same cutoff (CYFRA 21-1 <2.5 ng/mL, odds ratio=0.92 (IC 95%; 0.778–1.094) vs. CYFRA 21-1 >2.5 , odds ratio=1.46 (IC 95%; 0.558–3.803; $P=0.427$). Using other cutoffs (2.1 and 3.3 ng/mL), we observed the same recurrence risk in follow-up patients.

DISCUSSION

CYFRA 21-1 is an immunoassay that measures soluble fragments of cytokeratin 19 in serum and urine. In this line, various assays that measure the concentration of soluble cytokeratins have been suggested for the detection of bladder tumors (13–15). We found in the literature a great variability in the sensitivity results for this assay, ranging between 64.8% and 96.9% (16,17), which can be attributed to the different patient populations used in the studies. CYFRA 21-1 has been routinely used for years as a serum tumor marker for diagnosis of non-small-cell lung cancer, and as a recurrence marker for that condition (12,18). In recent studies (19,20), researchers evaluated the precision and accuracy of CYFRA 21-1 in terms of its analytical performance in urine, since this type of sample is in direct contact with the tumor and appears to be indicated for a better bladder cancer diagnosis. They concluded that urinary CYFRA 21-1 provides a high overall sensitivity for transitional cell carcinoma and useful sensitivity for low-grade disease, which may enable earlier detection and the design of treatments tailored to the individual. van der Gaast et al. (12) reported an acceptable sensitivity when they compared subgroups of patients in many different clinical situations. Their reference values for CYFRA 21-1 serum levels show that 99.6% of presumably normal subjects had CYFRA 21-1 concentration less than 3.3 ng/mL.

In contrast, we focused on and compared only two groups of patients (those with bladder cancer and those without evidence of disease). In the second group we investigated whether CYFRA 21-1 in serum could be a

marker with a good sensitivity for diagnosis as well as for follow-up, especially in predicting the risk of recurrence. Taking a cutoff of 2.1 ng/mL, we found a negative predictive value of 89%, which is a good result. On the other hand, we found that the likelihood to find a negative cystoscopy in a diagnosis of bladder cancer reached 68%, as suggested by the positive predictive value. We obtained similar results when we applied the other cutoffs used in this study.

It is important to note that there are no tumor markers with sensitivity and specificity near 100%. The selection of a cutoff for a tumor marker depends on the desired objective and, of course, the performance characteristics of the assays used. Therefore, if we take these considerations in account, the CYFRA 21-1 assay could be useful for urologists treating patients who have hematuria and are suspicious for bladder cancer. This observation may lead to the inclusion of this biomarker in routine protocols for clinical monitoring of patients; however, it is improbable that urologists would recommend a surgical treatment without making a previous endoscopic evaluation.

Moreover, we should not forget that inflammation in benign urological conditions and nonbladder malignancies can lead to misinterpretations of the test. In this context, positive results do not indicate the presence of bladder cancer. In our study, with the cutoff set at 2.1 ng/mL, the sensitivity for CYFRA 21-1 in serum was 54%. The achievement of the maximum specificity would minimize the false-positive results and raise the cutoffs, which might reduce the number of cystoscopies used; however, occult bladder cancer might be missed. Other common clinical situations in urological patients, such as hematuria and urinary tract infections, might also confound test interpretation.

When we compared our results with previous individual evaluations of CYFRA 21-1 (19), we found that the previous studies suggested the use of urinary biomarker combinations. The combination of two tumor markers (cytokeratin with a nuclear matrix protein) is desirable to maximize sensitivity (which was very poor in our study), lower the cutoffs as much as possible, and prevent the loss of some primary or recurrent process. It should be noted that previous works assumed the existence of a risk for no detection of some bladder cancer cases. In this sense, the cytologic examination can achieve a high grade of sensitivity and specificity for higher grades of bladder carcinomas; in contrast, for lower grade lesions, false-positive and false-negative rates can be higher than 10% (20). Indeed, the use of exfoliative cytology by non-experts in a general pathology practice setting has been criticized. However, in our study cytology showed better sensitivity than CYFRA 21-1 for any grade and stage.

In conclusion, the measurement of cytokeratin 19 in serum does not yet have enough sensitivity to justify its use in diagnosing bladder cancer. In our experience the detection of this tumor marker does not allow one to predict the risk of recurrence, and therefore it apparently is not useful in routine clinical practice.

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