



Bladder Cancer

Urinary CYFRA 21.1 Is Not a Useful Marker for the Detection of Recurrences in the Follow-Up of Superficial Bladder Cancer

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Abstract

Objectives: The objective of this prospective study is to establish an appropriate cutoff value of urinary CYFRA 21.1 assay and to assess its utility combined with voided cytology and/or haemoglobin dipstick in the follow-up of patients with superficial bladder cancer.

Methods: From December 2000 to November 2003, 446 patients in follow-up for superficial bladder cancer (Ta–T1) after transurethral resection of the bladder (TURB) were included in a prospective study. Voided urine specimens were collected 7–14 d before cystoscopy and/or TURB for CYFRA 21.1 (one sample), haemoglobin dipstick (one sample), and cytology (three samples). All samples were processed for CYFRA 21.1 and haemoglobin dipstick according to manufacturer instructions. A control group (n = 185) was obtained from patients in follow-up after transurethral resection of superficial disease (without recurrences within the following 6 mo). There were 125 recurrent transitional tumours detected by cystoscopy (34 TaG1; 53 TaG2/T1G1–2; 23 Ta–1G3/Tis, and 15 T2–4). Receiver operator characteristic (ROC) curves were constructed and cutoff values were chosen. Sensitivity, specificity, PPV (positive predictive value), NPV (negative predictive value), and their 95% confidence intervals were calculated.

Results: ROC curve analysis based on the previously reported cutoff value of 4 ng/ml for CYFRA 21.1 demonstrated a sensitivity and specificity of 43% and 68%, respectively. At a cutoff value of 1.5 ng/ml, sensitivity was 73.8% with a low specificity (41%). Further lowering of the cutoff point below 1.5 ng/ml did not demonstrate a significant increase in sensitivity. Therefore, this value was chosen as the most sensitive CYFRA 21.1 cutoff point during the rest of the study. Specificity increased when all the patients treated with pelvic radiotherapy or with UTI, urethral catheterisation, and intravesical instillations within 3 previous months were not included in our analysis. CYFRA 21.1 plus cytology and the combination of CYFRA 21.1, cytology, and haemoglobin dipstick demonstrated the highest overall sensitivities, and detected 91.3% of Ta–1G3 tumours and 93.3% of T2–4 tumours. However, there were one muscle-invasive tumour, two T1G3/Tis, three T1G2, and nine T1G1 neoplasms with negative combination of cytology and CYFRA 21.1 (1.5 ng/ml). All these tumours were smaller than 2 cm in size; most were single tumours. Nevertheless, there were 16 tumours larger than 0.5 cm (0.5–2 cm), and multiple neoplasms were endoscopically detected in 14 patients. Similar results were obtained through the combination of CYFRA 21.1 (cutoff: 1.5 ng/ml), cytology, and haemoglobin dipstick.

Conclusions: In our experience the low sensitivity of urinary CYFRA 21.1, even using lower cutoff values and/or a combination with cytology and/or haemoglobin dipstick, makes its application not very useful as a surveillance tool for superficial bladder carcinoma.

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1. Introduction

Cystoscopy and cytology are the standard modalities to monitor tumour recurrence and progression of superficial urothelial carcinoma [1]. Cystoscopy is invasive, causes discomfort, and is costly [2]. Cytology from voided urine lacks the diagnostic sensitivity necessary to rule out cancer [3]. These characteristics have prompted the search for more reliable non-invasive markers of bladder cancer [4]. Almost all markers have lower specificity than cytology, particularly when they are used independently, yielding more false-positive results in patients with concurrent benign bladder conditions [5].

Currently there is not a “dream marker”; however, several urinary tests such as BTASat, BTAtak, NMP22, telomerase, HA and Hase, Immunocyt, Quanticyt, FDP, BLCA4, HA (Hyaluronan), FISH, and CYFRA 21.1 have enough potential for future clinical use [4,6]. Moreover, the sensitivity of the markers can be improved significantly if they are used in combination with cytology or if a panel of markers is used [4]. Recently, Shariat et al [7] reported that urinary levels of soluble e-cadherin might add information to cytology in the detection of bladder cancer. Some authors have emphasised the importance of high sensitivity of urine markers in considering surveillance for superficial bladder cancer. Although these tests generally show relatively low specificity, yielding a high number of false-positive determinations, persistent negative results might postpone and reduce the number of cystoscopies [8–10].

The cytokeratins are the intermediate filament proteins characteristic of epithelial cells. In human cells, 20 different cytokeratin isotypes have been identified. The urothelium shows alterations in the expression and configuration of cytokeratin isotypes related to stratification and differentiation. In transitional cell carcinoma, changes in cytokeratin profile may provide information of potential diagnostic and prognostic significance [11]. CYFRA 21.1 assay measures fragments of cytokeratin 19 and has been routinely used as a serum tumour marker for diagnosing and monitoring nonsmall cell lung cancer [12,13]. Additionally, high levels of CYFRA 21.1 have been recorded in other solid tumours including gastric, head and neck, ovarian, breast, and prostate. Recently, Andreadis et al [14] have reported that serum CYFRA 21.1 increased in patients with metastatic bladder cancer, and appears to be a potentially sensitive and useful indicator for monitoring treatment response.

Urothelial tumour may exfoliate in urine-lysed cells with probable concomitant discharge of intra-

cellular components, including cytokeratin 19 [15]. The use of the CYFRA 21.1 assay on voided urine has been examined by several investigators using different cutoff points. The results of the different studies have been variable [13–22], although most reports suggest that CYFRA 21.1 may be a promising urine marker of bladder cancer.

The objective of this prospective study is to establish an appropriate cutoff value of urinary CYFRA 21.1 assay, and to assess its utility combined with voided cytology and/or haemoglobin dipstick in the follow-up of patients with superficial bladder cancer.

2. Patients and methods

From December 2000 to November 2003, 446 patients in follow-up for superficial bladder cancer after TURB were included in a prospective study. Local Ethics Committee approval and patient consent were obtained for this study. The follow-up schedule included urine determinations and cystoscopy every 3 mo for the first 2 yr, every 6 mo for the next 2 yr, and yearly in following years. Upper tract tumours were excluded by using a predefined schedule that included renal ultrasonography (and or intravenous urogram [IVU]) at the beginning and thereafter annually. Additionally, we performed renal ultrasonography or IVU in patients with positive cytologies despite bladder tumours in cystoscopy.

All the recurrent tumours were staged pathologically after TURB according to the TNM classification; grade was classified according to the World Health Classification (WHO) classification. Tumour size (<0.5, 0.5–3, or >3 cm in diameter), pattern of growth (papillary vs. solid), and focality (single vs. multiple) were recorded from the surgical and clinical data. To facilitate comparative analysis, tumours were grouped on the basis of a combination of stage and grade, according to EAU guidelines.

Voided urine specimens were collected 7–14 days before cystoscopy and/or TURB for CYFRA 21.1 (one sample), haemoglobin dipstick (one sample), and cytology (three samples determined by one pathologist). Pooled urines for measurement of CYFRA were transported to the laboratory, centrifuged at 1.5 rpm for 10 min at 4 °C and stored at –20 °C until processing. All samples were processed for CYFRA 21.1 and haemoglobin dipstick according to manufacturer instructions. Briefly, CYFRA 21.1 was determined by a commercial enzyme-linked immunosorbent assay kit (ELISA) with two different monoclonal antibodies to recognise cytokeratin 19 fragments; results were expressed as ng/ml. Cytology was performed by microscopic examination of three voided urine specimens (exfoliated cells from cancerous bladder epithelium). Two cytology categories were defined as negative and positive for malignancy (only the presence of malignant cells was considered positive). At the time of cystoscopy, urologists were blinded to CYFRA 21.1 assay results.

Characteristics of the patients are summarised in Table 1. A control group ($n = 185$) was obtained from patients in follow-up after transurethral resection of superficial disease. Patients in the control group did not have benign conditions (urinary

Table 1 – Characteristics of our series

Type of case (%)		Number (%)	Mean of urinary CYFRA 21.1 (SD)	Min–max
Controls (41.5%) ^a		185	3.3 (13.8)	0.1–187
Recurrences 125 (28%)	TaG1	34 (27.2)	2.9 (3.6)	0.1–13.4
	TaG2/T1G1–2	53 (42.4)	46.7 (139.7)	0.2–877
	Ta-1G3/Tis	23 (18.4)	13.04 (22.1)	0.1–98.2
	T2–4	15 (12)	92.2 (193.6)	0.6–707
Benign conditions 136 (30.5%)	BCG instillations	81 (59.6)	9.9 (11.3)	0.4–63
	MMC instillations	20 (14.7)	7.1 (11.1)	0.2–37.2
	UTI	17 (12.5)	58.1 (148.7)	0.2–507
	Urethral catheterisation	9 (6.6)	21.16 (32.6)	1.3–92.9
	Radiotherapy ^b	9 (6.6)	3.4 (3.1)	0.3–9.3
Total		446	15.79 (69.8)	0.1–877

SD = standard deviation; BCG = Bacillus Calmette-Guérin; MMC = mitomycin C; UTI = urinary tract infection.

^a No recurrence; no benign conditions.

^b Prostate brachytherapy and external beam radiotherapy for prostate cancer.

tract infection [UTI], pelvic radiotherapy, etc) and were not treated with intravesical therapies or urethral catheterisation within at least 12 mo before CYFRA 21.1 was done. Moreover, they did not have recurrent tumours for at least the following 6 mo after test was performed. There were 125 recurrent tumours detected by cystoscopy; 66 (52.8%) were single tumours and 59 (47.2%) multiple tumours. All the tumours were transitional cell carcinomas: 34 TaG1; 53 TaG2/T1G1–2; 23 Ta-1G3/Tis; 15 T2–4. The mean size of the tumours was 1.63 ± 1.6 cm (0.3–8 cm); 44 tumours (35.2%) were <0.5 cm, 65 (52%) from 0.5 to 3 cm, and 16 (12.8%) >3 cm. All the patients with benign conditions ($n = 136$) were included: intravesical therapy (Bacillus Calmette-Guérin [BCG] and mitomycin C [MMC] instillations) in the last year and urinary tract infections or urethral catheterisations if these conditions occurred during the follow-up period. Additionally, we recorded nine patients who have been treated with external radiotherapy [1] or brachytherapy [8] for prostate cancer, regardless of the time from therapy. Patients with benign conditions who presented recurrent tumours within 6 mo during the follow-up were not included in the study to avoid confusion because of the overlapping of several factors in CYFRA 21.1 results. Median time from UTI, BCG instillations, MMC instillations, urethral catheterisation, and radiotherapy to CYFRA 21.1 determination was 2, 30, 45, 15, and 420 d, respectively.

Receiver operator characteristic (ROC) curves were constructed and cutoff values were chosen. Initially, we studied the cutoff points recently reported (5.4 ng/ml and 4 ng/ml). Afterwards, we analysed better sensitivity cutoff points, searching for an optimal value with higher sensitivity and lower lack of specificity. Thus, we compared this cutoff point with those previously published (4 ng/ml). Sensitivity, specificity, and their 95% confidence intervals were calculated. Sensitivity was considered as the number of cases with a concentration of marker exceeding the cutoff that was positive by biopsy (TURB). Specificity was defined as the number of cases with a marker concentration less than the cutoff, and negative cystoscopy and biopsy (TURB).

Sensitivity was also calculated for stage and grade combination. Positive predictive value (PPV) was calculated as the number of positive cystoscopies at the point when the concentration of the marker exceeds the cutoff level. Negative predictive value (NPV) was calculated as the number of negative cystoscopies at the point when the concentration of the marker was lesser than the cutoff level. All analyses were performed with the Statistical Package for the Social Sciences, version 10 for Windows (SPSS Inc, Chicago, IL, USA).

3. Results

Using the previously reported cutoff value of 4 ng/ml for CYFRA 21.1 [13], ROC curve analysis demonstrated a sensitivity and specificity of 43% and 68%, respectively. At a cutoff value of 1.5 ng/ml, sensitivity was 73.8% with a low specificity (41%; Fig. 1). Further lowering of the cutoff point below 1.5 ng/ml did not demonstrate a significant increase in sensitivity. Therefore, this value was chosen as the most sensitive cutoff point for urinary CYFRA 21.1. In an attempt to improve specificity, we explored the possibility to decrease the influence of benign conditions. In this way, we found that most cases with positive CYFRA 21.1 (cutoff: 1.5 ng/ml) occurred within the first 3 mo after a benign condition. However, some patients who had received pelvic radiotherapy and some of the patients treated with BCG or MMC had false-positive results after 3 mo. Therefore, specificity increased when all the patients treated with radiotherapy (prolonged alteration of CYFRA 21.1) or with UTI, urethral catheterisation, and intravesical instillations within the 3 previous months were not included in our analysis (Fig. 2).

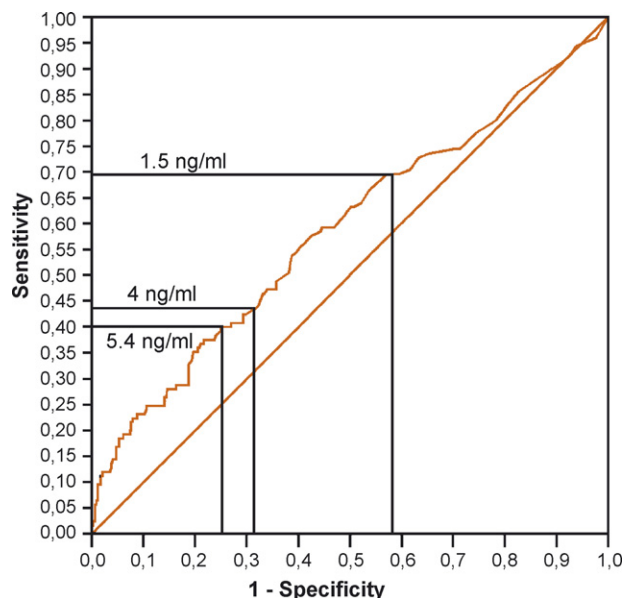


Fig. 1 – Receiver operator characteristic curves analysis in our series with a calculated area under the curve of 0.58. CYFRA cutoffs: 1.5 ng/ml (S: 0.696; 1-S: 0.572), 4 ng/ml (S: 0.43; 1-S: 0.311), and 5.4 ng/ml (S: 0.4; 1-S: 0.25).

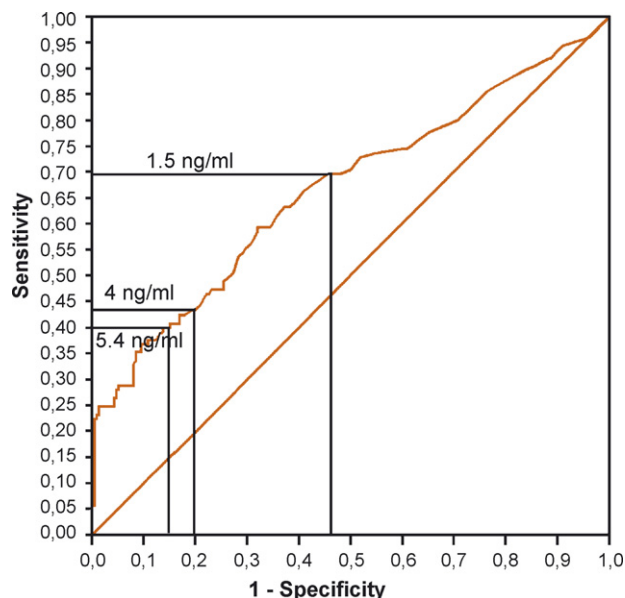


Fig. 2 – Receiver operator characteristic curves analysis in our series excluding cases with benign conditions occurring within 3 mo before CYFRA 21.1 determination and patients treated with brachytherapy or external beam radiation (area under the curve of 0.66). CYFRA cutoffs: 1.5 ng/ml (S: 0.696; 1-S: 0.458), 4 ng/ml (S: 0.43; 1-S: 0.193) and 5.4 ng/ml (S: 0.4; 1-S: 0.137).

Table 2 shows overall sensitivity and specificity, positive and negative predictive values of cytology, and haemoglobin dipstick of CYFRA 21.1 (cutoffs of 4 and 1.5 ng/ml), and of the combinations of CYFRA 21.1 with cytology and/or haemoglobin dipstick. Combinations were considered to be positive when one or both of the tests were positive. After deleting the samples from patients with benign conditions (specificity 2), sensitivity remained unchanged, because patients with benign conditions who

presented recurrent tumours within 6 mo during the follow-up had been excluded from the present study. Table 3 shows sensitivity results of urinary tests to detect tumour recurrences, according to stage, grade, tumour size, and focality. CYFRA 21.1 plus cytology and the combination of CYFRA 21.1, cytology, and haemoglobin dipstick demonstrated

Table 2 – Cytology, haemoglobin dipstick, and CYFRA 21.1 (cutoffs: 4 and 1.5 ng/ml) performance and 95% confidence intervals [in parentheses]

	Sensitivity	Specificity 1 ^a	Specificity 2 ^b	PPV	NPV	
Cytology	33.6 (25.02–45.2)	98.9 (97.7–100)	100	100	72.6 (67.4–77.8)	
Dipstick	33.6 (25.3–41.8)	88.8 (84.5–91.4)	97.6 (95.6–99.7)	89.36 (80.55–98–2)	71.38 (66.2–76.6)	
CYFRA (4 ng/ml)	43.2 (34.5–51.8)	68.9 (64–73.8)	80.2 (74.8–85.5)	56.2 (46.3–66.2)	70.54 (64.8–76.3)	
CYFRA (1.5 ng/ml)	69.6 (61.5–77.6)	42.7 (37.6–48.1)	54.25 (47.5–60.9)	47.3 (40.1–54.5)	75.1 (68.3–82)	
CYFRA (4 ng/ml) +	Cytology	56 (47.3–64.7)	61.6 (56.4–66.7)	77.8 (72.2–83.4)	59.8 (50.9–68.7)	75 (69.3–80.7)
	Dipstick	54.4 (45.7–63.1)	64.2 (59.1–69.3)	78.3 (72.7–83.8)	59.6 (50.6–68.6)	74.4 (68.7–80.2)
CYFRA (1.5 ng/ml) +	Cytology	74.4 (62.7–82.05)	40.2 (34.1–44.5)	53.3 (46.6–60)	48.4 (41.4–55.5)	77.9 (71.2–84.7)
	Dipstick	74.4 (62.7–82.05)	40.2 (35.5–45.9)	53.3 (46.6–60)	48.4 (41.4–55.5)	77.9 (71.2–84.7)
CYFRA (4 ng/ml) + cytology + dipstick	60.8 (52.2–69.4)	58.9 (53.7–64.2)	75.9 (70.2–81.7)	59.8 (51.3–68.4)	76.7 (70.9–82.4)	
CYFRA (1.5 ng/ml) + cytology + dipstick	76.8 (69.4–84.2)	39.3 (33.2–43.6)	52.8 (46.1–59.5)	48.9 (41.9–55.9)	79.4 (72.7–86.1)	

PPV = positive predictive value; NPV = negative predictive value.

^a Overall specificity.

^b Urinary tract infection, urethral catheterisation, radiotherapy, and intravesical instillations within the 3 mo prior to inclusion in the study were not included.

Table 3 – Sensitivity of urinary tests (CYFRA 21.1 cutoffs: 4 and 1.5 ng/ml) according to stage, grade, size, and focality of recurrent tumours

	Stage/grade	Total	Number		Size		
			Single	Multiple	<0.5 cm	0.5–3 cm	>3 cm
Cytology	TaG1	3.2	0 (12)	5.3 (22)	5.6 (20)	0 (14)	—
	TaG2/T1G1–2	28.6	20 (24)	34.5 (29)	11.1 (19)	32.1 (31)	100 (3)
	Ta–1G3/Tis	61.9	64.3 (16)	57.1 (7)	20 (5)	72.7 (13)	80 (5)
	T2–4	73.3	71.4 (14)	100 (1)	—	71.4 (7)	75 (8)
Dipstick	TaG1	8.8	0 (12)	13.6 (22)	10 (20)	7.1 (14)	—
	TaG2/T1G1–2	30.2	41.7 (24)	20.7 (29)	5.3 (19)	38.7 (31)	100 (3)
	Ta–1G3/Tis	47.8	37.5 (16)	71.4 (7)	40 (5)	53.8 (13)	40 (5)
	T2–4	80	78.6 (14)	100 (1)	—	71.4 (7)	87.5 (8)
CYFRA (4 ng/ml)	TaG1	20.6	0 (12)	31.8 (22)	25 (20)	14.3 (14)	—
	TaG2/T1G1–2	45.3	41.7 (24)	48.3 (29)	26.3 (19)	51.6 (31)	100 (3)
	Ta–1G3/Tis	47.8	56.3 (16)	28.6 (7)	20 (5)	46.2 (13)	80 (5)
	T2–4	80	78.6 (14)	100 (1)	—	57.1 (7)	100 (8)
CYFRA (4 ng/ml) + cytology	TaG1	29.4	0 (12)	45.5 (22)	40 (20)	14.3 (14)	—
	TaG2/T1G1–2	56.6	54.2 (24)	58.6 (29)	36.8 (19)	64.5 (31)	100 (3)
	Ta–1G3/Tis	69.6	75 (16)	57.1 (7)	40 (5)	76.9 (13)	80 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)
CYFRA (4 ng/ml) + dipstick	TaG1	26.6	0 (12)	40.9 (22)	35 (20)	14.3 (14)	—
	TaG2/T1G1–2	54.7	54.2 (24)	55.2 (29)	31.6 (19)	64.5 (31)	100 (3)
	Ta–1G3/Tis	69.6	68.8 (16)	71.4 (7)	60 (5)	69.2 (13)	80 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)
CYFRA (4 ng/ml) + cytology + dipstick	TaG1	32.4	0 (12)	50 (22)	45 (20)	14.3 (14)	—
	TaG2/T1G1–2	62.3	62.5 (24)	62.1 (29)	42.1 (19)	71 (31)	100 (3)
	Ta–1G3/Tis	78.3	75 (16)	85.7 (7)	80 (5)	76.9 (13)	80 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)
CYFRA (1.5 ng/ml)	TaG1	50	33.3 (12)	59.1 (22)	60 (20)	35.7 (14)	—
	TaG2/T1G1–2	71.7	66.7 (24)	75.9 (29)	63.2 (19)	74.2 (31)	100 (3)
	Ta–1G3/Tis	78.3	75 (16)	85.7 (7)	80 (5)	69.2 (13)	100 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)
CYFRA (1.5 ng/ml) + cytology	TaG1	50	33.3 (12)	59.1 (22)	60 (20)	35.7 (14)	—
	TaG2/T1G1–2	77.4	70.8 (24)	82.8 (29)	63.2 (19)	83.9 (31)	100 (3)
	Ta–1G3/Tis	91.3	87.5 (16)	100 (7)	80 (5)	92.3 (13)	100 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)
CYFRA (1.5 ng/ml) + dipstick	TaG1	52.9	33.3 (12)	63.6 (22)	65 (20)	35.7 (14)	—
	TaG2/T1G1–2	77.4	70.8 (24)	82.8 (29)	68.4 (19)	80.6 (31)	100 (3)
	Ta–1G3/Tis	87	81.3 (16)	100 (7)	80 (5)	84.6 (13)	100 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)
CYFRA (1.5 ng/ml) + cytology + dipstick	TaG1	52.9	33.3 (12)	63.6 (22)	65 (20)	35.7 (14)	—
	TaG2/T1G1–2	81.1	75 (24)	86.2 (29)	68.4 (19)	87.1 (31)	100 (3)
	Ta–1G3/Tis	91.3	87.5 (16)	100 (7)	80 (5)	92.3 (13)	100 (5)
	T2–4	93.3	92.9 (14)	100 (1)	—	85.7 (7)	100 (8)

Values in parentheses indicate number of patients in each subgroup according to tumour parameters.

the highest overall sensitivities, and detected 91.3% of Ta–1G3 tumours and 93.3% of T2–4 tumours.

Additionally, we analysed the pathologic characteristics associated with recurrence and progression (stage, grade, number, and size) of the tumours with negative value of CYFRA 21.1 (cutoffs: 1.5 and 4 ng/ml) and CYFRA combinations (CYFRA 21.1 plus cytology, CYFRA 21.1 plus cytology plus haemoglobin dipstick). We focused our analysis on the CYFRA 21.1 (cutoff: 1.5 ng/ml) and cytology combination because of its high sensitivity. In relation to stage

and grade, most of the patients with negative CYFRA 21.1 (cutoff: 1.5 ng/ml) and cytology combination had TaG1 neoplasms. However, there were one muscle-invasive tumour, two T1G3/Tis, three T1G2, and nine T1G1 neoplasms with negative results. All tumours with negative CYFRA 21.1 (cutoff: 1.5 ng/ml) and cytology combination were smaller than 2 cm in diameter, and most of them were single tumours. Nevertheless, there were 16 tumours larger than 0.5 cm (0.5–2 cm), and multiple neoplasms were endoscopically detected in 14 patients. Similar

Table 4 – Histologic characteristics (stage, grade, tumour size and focality) of recurrent tumours in patients with false-negative urinary tests (CYFRA 21.1 and CYFRA 21.1 combinations; CYFRA 21.1 cutoff: 1.5 ng/ml)

	Stage/grade	Number		Size	
		Single	Multiple	<0.5 cm	≥0.5 cm
CYFRA (1.5 ng/ml)	TaG1	8	9 (3)	8	9
	TaG2/T1G1-2 (1TaG2, 10 T1G1, 4 T1G2)	8	7 (4)	7	8
	Ta-1G3/Tis	4	1	1	4
	T2-4	1	—	—	1
CYFRA (1.5 ng/ml) + cytology	TaG1	8	9 (3)	8	9
	TaG2/T1G1-2 (9 T1G1, 3 T1G2)	7	5 (3)	7	5
	Ta-1G3/Tis	2	—	1	1
	T2-4	1	—	—	1
CYFRA (1.5 ng/ml) + cytology + dipstick	TaG1	8	8 (3)	7	9
	TaG2/T1G1-2 (9 T1G1, 1 T1G2)	6	4 (3)	6	4
	Ta-1G3/Tis	2	—	1	1
	T2-4	1	—	—	1

With regard to multiple tumours, values in parentheses indicate number of patients with more than three three tumours. No patients with tumours >3 cm had negative tests.

results were obtained through the combination of CYFRA 21.1 (cutoff: 1.5 ng/ml), cytology, and haemoglobin dipstick (Table 4).

4. Discussion

The onset of invasion in urothelial tumours occurs with the disruption of the basement membrane and discharge of intracellular components in the bladder-like cytokeratins. On this basis, Pariente et al [15] proposed cytokeratin 19 as a potential marker that might be detected in urine with the use of the ELISA-CYFRA 21.1 assay. However, variable results have been reported [21], with a sensitivity of the CYFRA 21.1 assay ranging between 64.8% [16] and 96.9% [15] and a specificity between 67.2% and 97.2% [10,15]. For Nisman et al [21], this variability can be attributed to different patient populations and differences in the method of urine collection and storage (centrifugation, 24-h urine collection, and temperature). Although some authors have recommended the use of ratio of urine CYFRA 21.1 to urine creatinine, this determination does not improve the accuracy of the CYFRA 21.1 in isolation. Additionally, a centrifugation step has not increased the accuracy of the test.

Different cutoff values have been used for urinary CYFRA 21.1. Pariente et al [15] determined a threshold of 4 ng/ml for urinary CYFRA 21.1 as the value providing the highest sensitivity (96.9%) at an acceptable specificity (67.2%) in a series of 48 bladder tumours (only 12% had grade 1 tumours). Sanchez-Carbayo et al [22] used an optimal cutoff of 5.4 ng/ml, and found a sensitivity of 81% and a

specificity of 97.2% in a group of 82 patients with bladder carcinoma (27% of Ta tumours). These authors reported that a combination of CYFRA 21.1 with a cutoff value of 5.4 ng/ml and NMP22 (cutoff: 14.6 U/ml) yielded an increase of the overall sensitivity (88.3%).

The first objective for a urinary marker in the follow-up of bladder cancer is the reduction of the number of cystoscopies [9]. In this way, some authors believe that the most important quality of a urinary marker is a high sensitivity for the detection of high-grade tumours and Tis. Since the clinical task of a diagnostic test for bladder cancer demands high sensitivity, only those operating points on the ROC curve that have high sensitivity values are clinically pertinent [23]. A relatively low specificity is not a significant disadvantage, because all patients with a positive test are referred for cystoscopy to confirm the diagnosis and persistent negative results might postpone and reduce the number of cystoscopies [10,21,24]. Additionally, Sharma et al [25] reported that a role of some urinary markers in patients with recurrent disease might be to schedule sedative procedures for those showing positive values, when we expect a necessary biopsy, and to reserve flexible cystoscopy for those with negative values.

We investigated CYFRA 21.1 and CYFRA 21.1 combinations in the follow-up of superficial bladder cancer, lowering the cutoff point to improve sensitivity. Additionally, with the aim of reducing unnecessary cystoscopies, our study examined the false-positive results in patients with benign conditions to establish relative exclusion criteria for CYFRA 21.1 assay [21]. These exclusion criteria

(UTI, urethral catheterisation, pelvic radiotherapy, brachytherapy, and intravesical therapy within 3 mo prior to urinary tests) significantly improved the specificity (12–17%) of CYFRA and CYFRA combinations. Although all patients were in follow-up for transitional cell carcinoma and many had a cystoscopy (urethral manipulation) every 3 mo, CYFRA levels were not influenced, because the assay was performed before cystoscopy. Moreover, we demonstrated that the influence of urethral catheterisation was not seen for over a month.

About 40% of patients in our study had negative combinations of CYFRA 21.1 and cytology and/or haemoglobin dipstick. We failed to reproduce the good results previously reported with high sensitivity values. Moreover, some of the tumours missed with CYFRA 21.1 and CYFRA combinations in our series can be dangerous. Nisman et al [21] reported that a delay in the diagnosis of small superficial low-grade tumours using CYFRA 21.1 (4.9 ng/ml) might not be critical, because they rarely invade or metastasise; however, in our study some of the tumours with high risk of progression were not detected.

5. Conclusions

In our experience the low sensitivity of urinary CYFRA 21.1, even using lower cutoff values and/or a combination with cytology and/or haemoglobin dipstick, makes its application not very useful as a surveillance tool for superficial bladder carcinoma.

Conflicts of interest

In the present study there was no financial support which might be considered as constituting an apparent conflict of interest.

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