Detection and Clinical Significance of Circulating Tumor Cells in Patients Undergoing Radical Cystectomy for Urothelial Bladder Cancer

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Abstract

We studied cytokeratin 7 (CK7) mRNA in circulating cells of patients undergoing radical cystectomy. We found that CK7-positive patients were at higher risk for developing disease recurrence and death, years after surgery. We conclude that CK7 testing is a useful adjunct to define the prognosis of patients undergoing radical cystectomy and to identify candidates for systemic therapy.

Introduction: Estimation of prognosis is patients undergoing radical cystectomy is often unreliable, as occult disease remains undetected by conventional diagnostic tools. The purpose of this study was to evaluate the feasibility and the clinical significance of a polymerase chain reaction assay to detect cytokeratin 7 (CK7) mRNA expression in peripheral blood cells of patients undergoing radical cystectomy for clinically nonmetastatic bladder cancer. **Patients and Methods:** From 2005 to 2009, 59 patients undergoing radical cystectomy and pelvic lymph node dissection were prospectively investigated. Peripheral blood was collected prior to surgery, and a nested polymerase chain reaction assay was developed to identify patients with circulating cells expressing CK7 mRNA. Preoperative, histopathologic data and clinical outcome were compared with CK7 findings. **Results:** CK7 expression was detected in 23 (38.9%) of 59 patients and correlated to T stage and lymph node status. After a median follow-up of 42 months, 29 patients experienced a recurrence, whereas 36 died. The presence of CK7-positive cells was significantly associated with an increased risk for recurrence and decreased survival as compared with patients who were CK7-negative ($P < .001$ and $P < .001$, respectively; hazard ratios of 8.77 and 5.2 for recurrence and overall death, respectively). The detection of CK7-positive cells was an independent predictor of recurrence and death in a multivariable analysis. **Conclusion:** The detection of CK7 mRNA in the circulating cells of patients undergoing radical cystectomy for urothelial cancer identifies those with significantly increased risk of cancer recurrence and death.

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Introduction

Radical cystectomy (RC) with pelvic lymph node dissection (PLND) is considered the treatment of choice for locally invasive bladder cancer (BC). However, more than one-half of the patients with pathologically node-negative BC will face disease progression and die after surgery.[1] This suggests the presence of occult disease that cannot be detected by imaging and conventional pathology. Contemporary cancer diagnostics are gradually relying upon new methods and biological sources for obtaining prognostic information. One promising source is the peripheral blood.

Detection of circulating tumor cells (CTC) in the bloodstream of patients with a solid tumor plays a role in early diagnosis of metastatic disease,[2] as well as to define prognosis and response to systemic treatment in patients with established metastatic disease.[3,4] Several methods have been explored.[5] Most of the evidence results from studies based on immunomagnetic detection technologies. Among those, the CellSearch system (Veridex, Raritan, NJ) is

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based on a positive selection of CTC by an epithelial cell adhesion molecule (EpCAM), and has received US Food and Drug Administration approval for the definition of prognosis in patients with metastatic colon, breast, and prostate cancer.[3,6,7] On the other hand, several studies have successfully employed a variety of reverse transcriptase-polymerase chain reaction (RT-PCR) methods to detect epithelial transcripts as surrogate reporters of CTC.[4,8,9] Studies directly comparing multiple detection methods have shown that RT-PCR results in higher diagnostic sensitivity.[10,11]

Among markers specific for urothelial lineage, cytokeratin 7 (CK7), is highly expressed in several variants of urothelial carcinoma, and commonly used in immunohistochemistry for the identification of metastatic tissue of urothelial origin.[12,13] Therefore, we developed a nested RT-PCR assay for detecting CK7 transcripts from peripheral blood cells of patients undergoing RC. Finally, we explored any correlation between CK7 status and the clinical outcome of our patients.

Patients and Methods

Patients

Starting in 2005, all patients undergoing an RC with PLND for urothelial cancer at our institution were considered potentially eligible for the present study. Eligibility criteria included prior transurethral resection with urothelial carcinoma histology. All subjects were 18 years or older, had Eastern Cooperative Oncology Group performance status ≤ 2, and had to exhibit a recent (within 6 weeks of cystectomy) total body computed tomography (CT) excluding distant metastases and upper urinary tract cancer. Patients excluded from enrollment were those in whom neoadjuvant chemotherapy was either planned or performed, receiving investigational medications, and not suitable for an RC with PLND. At follow-up, all patients were monitored quarterly for the first 2 years, every 6 months until the fifth year, and annually afterwards. For staging purposes, a bone scan at 12 months and a chest and abdominal CT scan to be performed after 6, 12, 18, and 24 months, and yearly thereafter, were scheduled. However, patients with less than a yearly chest and abdominal CT scan were excluded from analysis. Decisions regarding treatment in the adjuvant setting or at the time of recurrence/progression were at discretion of the treating physician. Cells were obtained from the peripheral blood of 8 healthy volunteers without a history of urothelial cancer and were tested as negative controls. In accordance with the precepts of the Helsinki Declaration, written informed consent to the use of biologic material and agreement to participate to the study were obtained from all patients and healthy volunteers prior to sampling and surgery (ClinicalTrials.gov identifier: NCT02345473).

CTC Isolation

Blood Sample Collection and Processing. For each subject, a 4 mL peripheral blood sample was drawn within 24 hours before surgery, and collected into EDTA collection tubes. Ficoll-Hypaque separated nucleated cells were recovered and suspended in 500 µl of TRIzol reagent (Life Technologies, Carlsbad, CA) for total RNA extraction according to the manufacturer's instruction. Before storage at −80°, total RNA amount and integrity (OD_{260}/OD_{280} nm absorption ratio > 1.6) were determined spectrophotometrically (NanoDrop 1000, Thermo Scientific, Waltham, MA).

RT Reaction and Nested PCR. Complementary DNA (cDNA) was obtained from a 20 µL PCR mixture containing 2 µg of total RNA, 25 pmol of random primers, 2.5 mM of each triphosphate deoxynucleotide, 2 µL of 10× RNA PCR Buffer, 1 µL of Multi Scribe Reverse Transcription (all from Life Technologies), and 3.2 µL of Rnase free water. The mixture was incubated at 25°C for 10 minutes, 37°C for 120 minutes, heated to 85°C for 5 seconds, and then chilled on ice. Two sets of CK7 primers (MWG Biotech, Ebersberg, Germany) were designed for the PCR protocol to obtain a final 218 bp PCR product. The primer's sequences were as follows: outer sense, 5′-CGTGCGCTCTGCCTATGG-3′; outer antisense, 5′-GCGGTTAATTTCATCTTCGT-3′; inner sense, 5′-TCCGCAGGTCACCATTAAC-3′; inner antisense, 5′-GCTGCTCTTGGCCGACTTCT-3′. During the first PCR round, 25 cycles of amplification corresponded to 30 seconds of denaturation at 94°, 30 seconds annealing at 60°, and 30 seconds extension at 72°. For the second PCR round, 1 µL aliquot of the first-round PCR product was amplified for 27 cycles under the same conditions, using the outer set of CK7 primers. Adequacy of cDNA synthesis was confirmed by a non-nested RT-PCR of β-actin. A no-template control, omitting any cDNA template, was included in all PCR reactions. Ten µL of second-round PCR products were subjected to 3% agarose gel electrophoresis and stained with ethidium bromide. The present PCR methodology generated a dichotomous result for each sample: CK7-positive or CK7-negative. Each sample was tested twice; a third run was performed when the results were inconclusive.

Cell Line Serial Dilutions and Cell Spikes. A J82 bladder cancer cell line (ATCC, Manassas, VA), highly expressing CK7, was used to set up an in vitro sensitivity test of the nested RT-PCR. The sensitivity of RT-PCR to detect CK7 mRNA was evaluated by adding known numbers of J82 cells (10^6, 10^5, 10^4, 10^3, 10^2, 10, 1, and 0) in the peripheral blood obtained from healthy volunteers, before RNA extraction.

Statistical Analysis

Patients' baseline characteristics according to treatment group were reported as frequency and percentage or median and interquartile range and were compared with the Pearson χ^2 test or the Fisher test or the Mann-Whitney *U* test for categorical and continuous variables, respectively. The Kaplan-Meier method and log-rank test statistic were used to compare probabilities of survival in the 2 treatment groups. Relative risks of death (cancer-specific and overall) and disease recurrence were calculated by univariate and multivariate Cox proportional hazards model, adjusting for the effects of other covariates known to be of prognostic importance. Assumption of proportional hazard was tested using the tests of the nonzero slope. Results are expressed as hazard ratios (HR) with their 95% confidence intervals (95% CIs). All the analyses were performed using Stata Statistical Package Release 14 (StataCorp, College Station, TX). A *P* value $< .05$ was considered as being statistically significant.

Results

Sensitivity of the Nested RT-PCR for CK7

The sensitivity of RT-PCR to detect CK7 mRNA was evaluated by adding known numbers of J82 cells (10^6, 10^5, 10^4, 10^3, 10^2, 10,

1, and 0) in mononuclear cells suspensions obtained from the peripheral blood of healthy volunteers, and examined for CK7 expression. Using our nested RT-PCR protocol, we could consistently detect CK7 mRNA starting from a dilution of 10 J82 cells in 10^6 mononuclear cells, corresponding to a single positive cell in more than 10 mL of a peripheral blood sample.

Patient Characteristics and CK7 Expression

From January 2005 to June 2009, 138 patients underwent RC for urothelial cancer at our institution and were screened for inclusion in the present study. A total of 66 patients were noneligible, whereas 7 patients were excluded after surgery for sample inadequacy. Accrual was closed after a planned population of 65 patients was included in the study. As 6 patients were nonadherent to the follow-up schedule, the analysis was conducted on a final population of 59 patients. Accrual is detailed in Figure 1. Adherence to recommended follow-up protocol and a chest and abdominal CT scan were recommended after 6, 12, 18, and 24 months, and yearly thereafter. In addition, 8 healthy volunteers were enrolled as a control group.

As summarized in Table 1, CK7 expression was detected in 23 (38.9%) of 59 patients undergoing RC, suggesting the presence of viable epithelial cells in the bloodstream of patients with positive samples. With the exception of hydronephrosis, there were no significant differences between positive and negative patients in terms of known preoperative prognostic factors for BC. At routine histopathology, statistically significant differences were observed in relation to pathologic T stage and lymph node status. Specifically, CK7 expression was found in 6 (100%) of 6 T4 patients, and in 9 (81.8%) of 11 patients with positive lymph nodes. Based on postoperative pathologic features, clinical course of the disease, overall health status, and individual preferences, a total of 22 patients received adjuvant chemotherapy; 7 of 36 were CK7-negative, and 15 of 23 were CK7-positive. Of the total, only 2 patients received early adjuvant chemotherapy; the rest were treated at the time of disease recurrence.

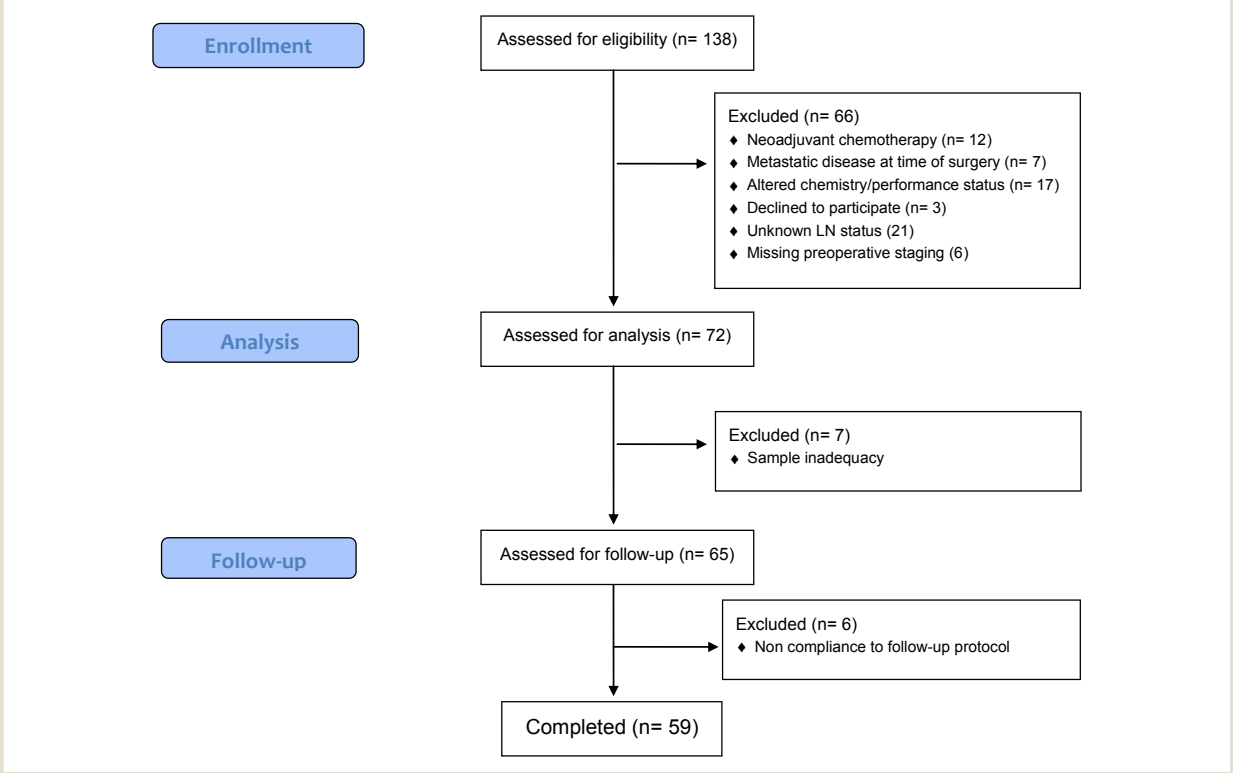
Correlation of CK7 Expression to Clinical Outcome

Median follow-up was 42 months (range, 3-129 months). Major findings are shown in Table 2. During the period of observation, 29 (49.1%) of 59 patients experienced either a local or distant recurrence, whereas 36 (61%) of 59 patients died. In the case of 27 (45.7%) of 59 subjects, death was directly correlated with BC. Median time to recurrence was 37 months (95% CI, 17-37 months), whereas median survival was 42 months (95% CI, 21-80 months). Importantly, we could not find any CK7-positive cells among blood samples from the 8 healthy volunteers. Associations of recurrence and death to CK7 status were further analyzed.

Recurrence

The association between CK7 status and recurrence is summarized in Table 3 and Figure 2A. At 5 years, the estimated probability

Figure 1 Enrollment Flow Diagram



Abbreviation: LN = lymph node.

Table 1 Baseline Patient's Characteristics According to CK7 Findings

	All Sample (N = 59)	CK7		
		Negative (N = 36)	Positive (N = 23)	
	Median (IQR)	Median (IQR)	Median (IQR)	*P* Value
Age, y	67 (62.0-74)	68.0 (63.7-74.5)	64.0 (62-74)	.28
BMI	24.3 (21.4-26.6)	24.2 (21.4-26.7)	24.9 (21.2-26.4)	.89
	N (%)	N (%)	N (%)	
Gender				1
Female	5 (8.47)	3 (8.33)	2 (8.70)	
Male	54 (91.53)	33 (91.67)	21 (91.30)	
Smoking history				.65
No	21 (35.39)	12 (33.33)	9 (39.13)	
Yes	38 (64.41)	24 (66.67)	14 (60.87)	
ASA score				.0837
2	18 (30.51)	8 (22.22)	10 (43.48)	
3-4	41 (69.49)	28 (77.78)	13 (56.52)	
Diabetes				.49
No	49 (83.05)	31 (86.11)	18 (78.26)	
Yes	10 (16.95)	5 (13.89)	5 (21.74)	
Cardiovascular history				.1148
No	39 (66.10)	21 (58.33)	18 (78.26)	
Yes	20 (33.90)	15 (41.67)	5 (21.74)	
Time from last TUR and CK7 measurement				.61
Mean ± SD	38.4 ± 13.3	39.1 ± 2.2	37.3 ± 13.7	
Range	17-72	19-72	17-66	
Hydronephrosis				.0468
No	42 (91.19)	29 (80.56)	13 (56.52)	
Uni/bilateral	17 (28.81)	7 (19.44)	10 (43.48)	
Clinical T stage				.005
T1	18	12	4	
T2	27	19	8	
T3	12	3	9	
T4	2	0	2	
Pathologic T stage				.0107
T0-Ta-T1	19 (32.20)	14 (38.89)	5 (21.74)	
T2	13 (22.03)	9 (25.00)	4 (17.39)	
T3	21 (35.59)	13 (36.11)	8 (34.78)	
T4	6 (10.17)	0 (0.00)	6 (26.09)	
Pathologic N stage				.002
Neg	48 (81.36)	34 (94.44)	14 (60.87)	
Pos	11 (18.64)	2 (5.56)	9 (39.13)	
Lymphovascular invasion				.7783
Neg	27 (45.76)	17 (47.22)	10 (43.48)	
Pos	32 (54.24)	19 (52.78)	13 (56.52)	
Concomitant CIS				.3842
Neg	37 (62.71)	21 (58.33)	16 (69.57)	
Pos	22 (37.29)	15 (41.67)	7 (30.43)	

Abbreviations: ASA = American Society of Anesthesiologists; BMI = body mass index; CIS = carcinoma in situ; CK7 = cytokeratin 7; IQR = interquartile range; SD = standard deviation; TUR = transurethral resection.

Table 2 Outcome According to CK7 Findings

Status	All Sample N (%)	CK7	
		Negative N (%)	Positive N (%)
Alive	23 (38.9)	22 (61.1)	1 (4.3)
Recurred	29 (49.1)	8 (13.5)	21 (35.5)
Dead	36 (61)	14 (38.8)	22 (95.6)
Cancer-specific	27 (45.7)	9 (25)	18 (78.2)
Noncancer-specific	9 (15.2)	5 (13.8)	4 (17.3)

Abbreviation: CK7 = cytokeratin 7.

of developing a recurrence was 0.23 (95% CI, 0.12-0.41) and 0.91 (95% CI, 0.75-0.98; $P < .01$) for CK7-negative and -positive patients, respectively. On average, patients positive for CK7 before surgery were at much higher risk for developing disease relapse (HR, 8.77; $P < .001$) compared with the CK7-negative group. In univariate analyses, although CK7 status was the strongest predictor of recurrence, T stage and LN status were also significantly correlated. In a Cox proportional hazards analysis, CK7 remained the strongest predictor for disease recurrence. Specifically, CK7-positive patients were 7 times more likely to develop a relapse compared with the remaining population. Pathologic stage was also strongly correlated at multivariate analysis.

Death

As for recurrence, we also found a strong association between CK7 status and death (Table 3 and Figure 2, B and C). At 5 years, the estimated probability of dying for any cause was 0.38 (95% CI, 0.25-0.56) and 0.86 (95% CI, 0.70-0.96; $P \leq .01$) for CK7-negative and -positive patients, respectively. In univariate analyses, CK7-positive patients were much more likely to die (HR, 5.2; $P < .001$) compared with the CK7-negative group, when all causes of death were considered. In a Cox proportional hazards analysis, CK7 expression was the strongest predictor for death. Specifically, CK7-positive patients were 8.8 and 11.4 times more likely to die for any cause or for BC, respectively.

Discussion

The present study was designed to evaluate the feasibility and the clinical significance of a PCR assay to detect CK7 mRNA expression in peripheral blood cells of patients undergoing RC for clinically nonmetastatic BC. Circulating CK7 mRNA positive cells were found in patients with bladder urothelial cancer of varying stages, and the detection rates increased with stage. Positive cells were detected in 26.3% of patients with non-muscle invasive tumors (pT0−Ta-T1), 30.7% of pT2 patients, 38% of pT3 patients, and 100% of pT4 patients. Further, blood samples were CK7-positive in 29.1% of N0, as compared with 81.8% of patients with node-positive disease. Thus, the search for an appropriate CK7 sequence was positive among patients that were more likely to develop disease progression.

For the first time, to our knowledge, we attempted long-term clinical correlations and found that CK7 mRNA was a predictor of a worse clinical scenario. Specifically, patients with positive CK7 before RC were much more likely to develop metastatic disease (7.8 times) and to die shortly (5 times), as compared with the CK7-negative counterpart. This was independent from all other traditional prognostic factors in multivariate analysis, including stage and performance status. In our opinion, these results are particularly relevant considering that all patients were staged as nonmetastatic at baseline. Thus, the strong correlation that we found in our study leads to the hypothesis that pre-cystectomy detection of CK7-positive cells may be of prognostic importance and may reflect the presence of otherwise undiagnosed occult disease.

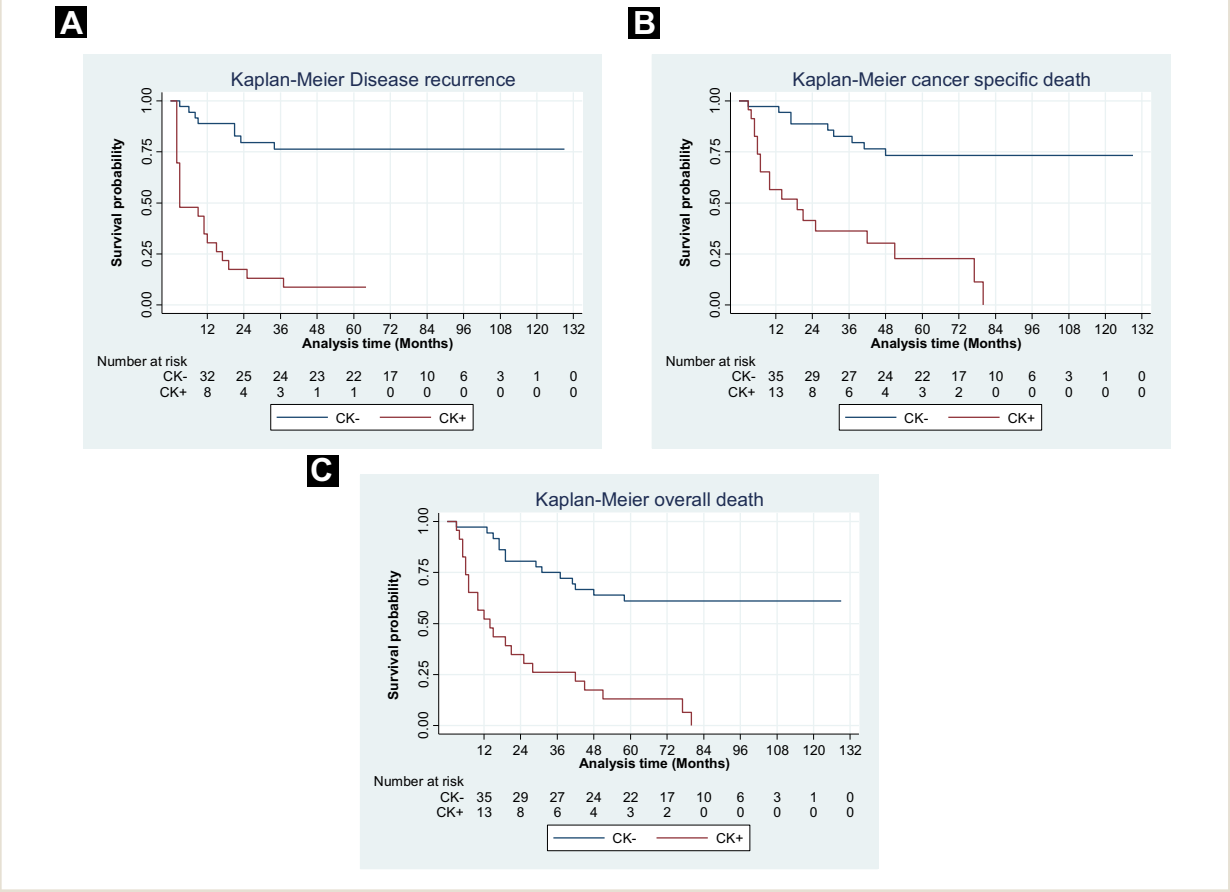
In the present study, CK7 mRNA was chosen for the detection of CTC for several reasons. CK7 is not found in the stroma or in preparations from leucocytes.[14,15] Several reports have shown that CK7 is highly expressed across different invasive variants of

Table 3 Disease Recurrence, Cancer-specific Death, and Overall Death Prediction by Univariable and Multivariable Cox Regression Analyses

	Disease Recurrence			Cancer-specific Death			Overall Death		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Univariable analysis									
Pathologic T stage (≤2 vs. ≥3)	3.51	1.61-7.66	.002	3.61	1.85-8.34	.001	3.44	1.73-6.85	<.001
Nodal status (neg vs. pos)	3.72	1.63-8.48	.002	3.28	1.39-7.73	.007	2.8	1.28-6.13	<.005
Cr. in situ (neg vs. pos)	0.76	0.35-1.64	.49	0.81	0.37-1.78	.61	0.68	0.34-1.37	.28
Lymphovasc. inv. (neg vs. pos)	1.8	0.83-3.88	.13	1.8	0.80-4.01	.15	1.42	0.72-2.78	.3
Hydronephrosis (no vs. yes)	1.8	0.86-3.88	.111	2.03	0.94-4.39	.07	1.83	0.86-3.88	.114
ASA score (2 vs. 3+4)	0.76	0.35-1.64	.49	0.86	0.38-1.92	.71	0.76	0.35-1.64	.49
CK7 status	8.77	3.78-20.34	<.001	6.41	2.8-14.60	<.001	5.2	2.61-10.3	<.001
Adjuvant chemotherapy	3.19	0.75-13.5	.11	2.89	0.66-12.9	.11	2.34	0.55-9.89	.25
Multivariable analysis									
Pathologic T stage (≤2 vs. ≥3)	2.69	1.14-6.34	.024	2.60	1.08-6.26	.032	2.73	1.24-6.03	.013
Nodal status (neg vs. pos)	2.18	0.90-5.24	.80	1.92	0.77-4.78	.16	1.21	0.49-2.98	.67
CK7 status	7.17	2.84-18.1	<.001	4.67	1.89-11.4	<.001	4.00	1.82-8.80	<.001

Abbreviations: ASA = American Society of Anesthesiologists; CI = confidence interval; CK7 = cytokeratin 7; Cr. = carcinoma; HR = hazard ratio; lymphovasc. inv. = lymphovascular invasion.

Figure 2 Kaplan-Meier Plots Describing Recurrence (A), Cancer-specific (B), and Overall (C) Survival in CK7-negative or -positive Patients



Abbreviation: CK7 = cytokeratin 7.

urothelial carcinoma, including micropapillary, plasmacytoid, and nested; within these tissues, when compared with other urothelial specific markers, such as uroplakin III, MUC-1, MUC-2, CK20, p16, p63, and thrombomodulin, CK7 shows the highest immunohistochemical expression.[12,16] Thus, CK7 is recognized as a useful adjunct in the identification of metastatic urothelial carcinoma.[13,17] These data are consistent with the fact that CK7 mRNA is present in a high copy number in BC cells. In a gene expression profiling study, DNA microarrays were used to identify differentially expressed genes during the neoplastic transition of normal bladder urothelium to primary tumors. The study revealed that CK7 had the highest change with a 20-fold mRNA increase in primary bladder tumors compared with normal tissue.[15] We believe that the high level of CK7 mRNA expression in urothelial cancer cells may have been critical for CTC detection in our study.

These results of the present study are in line with previous reports in which a similar approach was adopted for the detection of urothelial CTC. Uroplakins (Ia and II) have commonly been used as molecular markers for this purpose. In a study by Li et al, circulating UP II mRNA-positive cells were detected only in 3 of 10 patients with metastatic tumors and in 0 of 50 patients with nonmetastatic tumors[18]; using a nested RT-PCR assay, the detection rate was increased to 28.6% in patients with muscle invasive tumors and 40.0% in node-positive patients.[19] Cytokeratin 20 was found positive in 12 of 49 samples of a mixed sample of patients with UC of the upper or lower urinary tract undergoing either radical or endoscopic surgeries.[20] Finally, MUC-7 was detected in 37.9% and in 77.7% of patients with noninvasive and invasive disease, respectively.[21] A combination of nested RT-PCR for UPII and CK20 was also tested in a heterogeneous population of 108 subjects with urothelial BC. On multivariable analysis, both markers were predictive of recurrence only in patients with nonmuscle invasive bladder cancer (NMIBC).[22] Importantly, in none of the previous studies, adequate follow-up could establish the clinical relevance of their findings. More recently, the CellSearch assay was used for CTC detection in a series of studies in patients undergoing RC for nonmetastatic UBC. One of the most relevant found CTC in 23 of 100 patients.[23] Although no correlation between CTC status and stage could be found, after a median follow-up of 16 months, the presence of even a single CTC was associated with a worse clinical outcome.

In patients with NMIBC undergoing RC, the risk of finding overt metastases at the time of surgery is low (5%-15%);

nonetheless, shedding of tumor cells into the circulation may occur from early stages of tumor formation.[24] A recent study was published on a homogeneous cohort of 102 patients with T1G3 disease treated conservatively. Importantly, CTC were found in 20% of patients and independently predicted both disease recurrence and progression.[25] In our cohort, 19 patients who underwent RC had a final stage of NMIBC. Among those, 5 (26.3%) were found positive for CK7. Probably owing to the small number of patients in our subset, we could not find a statistically significant correlation between CK7 expression and occurrence of metastatic disease ($P = .07$) or cancer-specific death ($P = .06$). Although both data are favorable, specifically designed trials, as well as larger series, are required to confirm the prognostic role of CTC in patients with high-risk NMIBC.

The present study has limitations, the most important being sample size. Owing to a lack of reference articles at the time of study design, no sample size calculation was performed. Although small, the sample size is not underpowered for analysis; nonetheless, it would be risky to generalize our results to a large-scale population in which more variables come into play. We believe the present should be considered a pilot study, providing very interesting data regarding CK7 mRNA as a potential predictor of clinical outcome. When PCR methods are used, cases of false positivity have been described as a result of contamination or illegitimate transcription. In the present study, our nested RT-PCR has shown to be highly specific as suggested both by the negative results obtained from healthy volunteers, and by the correlation of CK7 detection with disease status and clinical outcome. Finally, there may be concerns related to the nature of a single-center study in which no external validation was performed. Although assay reproducibility was checked by validating variables, such as timing of sample collection, storage, handling, nucleic acid extraction methods, and reaction conditions, a multicenter collaborative study would have been the best setting to clinically validate our results. This may be the focus of future trials.

Conclusions

Our results may be relevant in the current scenario in which the identification of patients who need more aggressive therapy, before or after RC, is only based on clinical criteria that may not reflect the entire biology of BC.[26] Despite prospective trials supporting the use of neoadjuvant chemotherapy for clinical T2-4N0M0 stage BC,[27,28] clinicians are seldom reluctant to recommend neoadjuvant chemotherapy because of concerns related to the correct identification of candidates.[29] In the present study, we have identified CK7 as a powerful predictor of clinical outcome in our patients. We believe CK7 mRNA determination could support a better selection of candidates for systemic therapies, and, following the path of breast and prostate cancer,[30] could be tested in BC as a multifunctional biomarker for future drug development and trial design.

Clinical Practice Points

- More than one-half of the patients with node-negative BC after RC will face disease progression and die after surgery. This suggests the presence of undiagnosed disease at the time of surgery that cannot be detected by imaging and conventional pathology.
- Detection of circulating tumor cells in the bloodstream of patients with a solid tumor plays a role in early diagnosis of metastatic disease, as well as to define prognosis.
- CK7 is a marker specific for urothelial lineage, and is highly expressed in several variants of urothelial carcinoma. To detect CK7 transcripts in the peripheral blood cells of patients undergoing RC, we developed a nested RT-PCR assay against CK7. We also explored any correlation between CK7 status and the clinical outcome of our patients.
- Patients with CK7-positive circulating cells were at higher risk for recurrence and death as compared with CK7-negative patients ($P < .001$ and $P < .001$; HR, 8.77 and 5.2 for recurrence and overall death, respectively). The detection of CK7-positive cells was an independent predictor of recurrence and death in a multivariable analysis.
- CK7 mRNA determination could support a better selection of candidates for systemic therapies and could be tested in BC as a multifunctional biomarker for future drug development and trial design.

Disclosure

The authors have stated that they have no conflicts of interest.

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