



# Head-to-head comparison of serum and urine cytokeratin-19 fragments (CYFRA 21-1) for bladder cancer diagnosis

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**Background:** Some studies have investigated the diagnostic accuracy of cytokeratin-19 fragments (CYFRA 21-1) for bladder cancer, but no study has compared the diagnostic accuracy of serum and urine CYFRA 21-1 in a head-to-head manner. The aims of this study were to (I) compare the diagnostic accuracy of serum and urine CYFRA 21-1 and (II) investigate whether the diagnostic accuracy can be improved if serum and urine CYFRA 21-1 were used together.

**Methods:** In this prospective study, we enrolled patients with bladder cancer symptoms that were admitted to our hospital between January 1, 2016, and December 31, 2016. Serum and urine CYFRA 21-1 were detected using a Maglumi 2000 Plus immunoassay analyser. The diagnostic accuracy of the serum and urine CYFRA 21-1 was assessed using a receiver operating characteristic (ROC) curve analysis and multivariable logistic regression.

**Results:** A total of 234 subjects were enrolled in the study, 152 with bladder cancer and 82 with non-bladder cancer. The areas under the ROC curve (AUCs) for serum and urine CYFRA 21-1 were comparable (0.74 vs. 0.74,  $P=0.91$ ). The AUC for the serum and urine CYFRA 21-1 combination was 0.83, which was significantly higher than that of the serum ( $P=0.04$ ) and urine ( $P=0.03$ ) CYFRA 21-1 alone.

**Conclusions:** Both the serum and urine CYFRA 21-1 have comparable diagnostic accuracy for bladder cancer. The diagnostic accuracy can be improved if the serum and urine CYFRA 21-1 are used together.

**Keywords:** Bladder cancer; cytokeratin-19 fragment; diagnostic accuracy test; sensitivity; specificity

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## Introduction

Bladder cancer is the ninth leading cause of death in males. It is estimated that 429,800 new cases were diagnosed and 165,100 deaths occurred in 2012 worldwide (1). The prognosis of bladder cancer is poor, especially for patients at advanced stage (2). The patient outcomes can be improved if the diagnosis can be established in a timely and accurate manner. Currently, the cystoscopy or voided urine cytology (VUC) are gold standards for bladder cancer diagnosis. However, cystoscopy is an expensive, invasive and

uncomfortable tool. In addition, the diagnostic accuracy of cystoscopy is largely affected by the experience of the operator. VUC has a high diagnostic specificity for bladder cancer; however, its sensitivity is only 0.37 (3). Therefore, it is of great value to diagnose bladder cancer using non-invasive biomarkers (4).

Over the past years, many serum or urine biomarkers have been identified (5), such as nuclear matrix protein 22 (NMP22), qualitative or quantitative bladder tumour antigen (BTA) and cytokeratin-19 fragment (CYFRA 21-1). Among the available biomarkers, CYFRA 21-1 is promising,

**Table 1** Characteristics of the subjects

Variables	Bladder cancer	Non-bladder cancer	P value
Sample size	152	82	NA
Age (years)	63±13	64±13	0.73
Gender (M/F)	115/37	64/18	0.68
Tumour stage (Tris/T1/T2/T3/T4)	15/58/20/37/22	NA	NA
Differentiation (low/moderate/high)	63/15/74	NA	NA
Serum CYFRA 21-1 (ng/mL)	3.79±2.55	1.95±1.00	<0.01
Urine CYFRA 21-1 (ng/mL)	221±234	66±82	<0.01
Voided urine cytology (positive/negative)	37/115	0/82	<0.01

Continuous variables are presented as the mean ± standard deviation and compared using a Mann-Whitney U test. Categorical variables are presented as the absolute number and compared using a Chi-square test or Mann-Whitney U test. NA, not applicable.

as a meta-analysis shows that the area under the summarized receiver operating characteristic (sROC) for the serum and urine CYFRA 21-1 are 0.88 and 0.87, respectively (6). To the best of our knowledge, no previous studies have compared the diagnostic accuracy of serum and urine CYFRA 21-1 in a head-to-head manner. Therefore, we performed a head-to-head comparison study to (I) compare the diagnostic accuracy of serum and urine CYFRA 21-1 for bladder cancer and (II) investigate whether the diagnostic accuracy can be improved if the serum and urine CYFRA 21-1 are used together.

## Methods

### Subjects

This is a prospective study performed between January 1, 2016 and December 31, 2016, in Daping Hospital. The study cohort includes 152 bladder cancer patients and 82 controls. All the subjects were suspected of bladder cancer and have received cystoscopy to determine if the patient had bladder cancer. Among the 82 controls, 40 had cystitis, 15 had urolithiasis, 14 had a urinary tract infection, 7 had kidney carcinomas and 6 had a benign bladder tumour.

The serum and random urine samples were obtained from the subjects within 24 hours after admission. CYFRA 21-1 levels in the serum and urine were measured within 24 hours after collection. Both the serum and urine CYFRA 21-1 were detected using a Maglumi 2000 Plus immunoassay analyser (Shenzhen, China). Test results of both the serum and urine CYFRA 21-1 were blinded to the clinician. Clinical details of the subjects were blinded to the technicians who were responsible for the CYFRA 21-1 determination.

This study was approved by the Ethics Committee of

Daping Hospital (NO: 52). All the included subjects or their legal representatives provided informed consent.

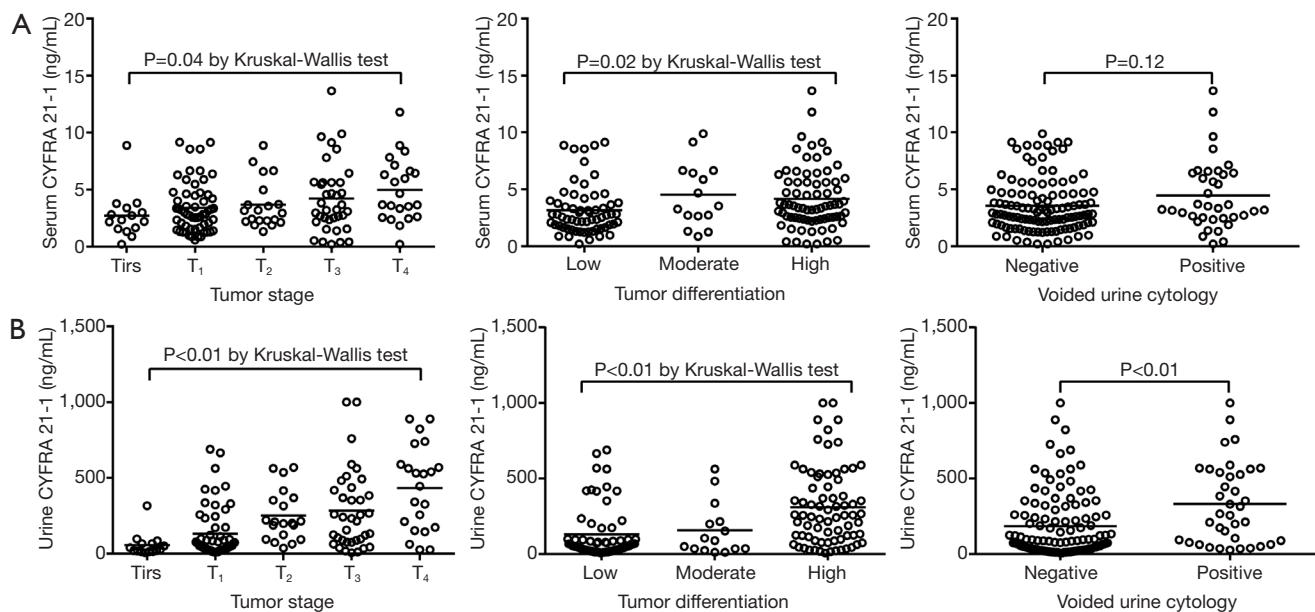
### Statistical analysis

Because the continuous data in this study were not normally distributed (tested by Kolmogorov-Smirnov test), we used a Mann-Whitney test or Kruskal-Wallis test to compare the continuous variables. Dunn's post hoc procedure was used for multiple comparisons and the significance level of the test was set at 0.05/n, where n represents the number of pairwise comparisons. Categorical data were compared using a Chi-square test. The diagnostic value of the serum and urine CYFRA 21-1 were assessed using a ROC curve analysis. A multivariable logistic regression model was constructed to combine the serum and urine CYFRA 21-1 as a single indicator. The threshold with the maximum Youden index was set as the optimal threshold and the corresponding sensitivity and specificity were calculated. The areas under the ROC curves (AUCs) were compared using Delong's approach (7). All analyses were performed in SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and Sigmaplot 12.0 (Systat Software, Inc., San Jose, CA, USA). A P value less than 0.05 was defined as statistically significant.

## Results

### Characteristics of the subjects

Table 1 lists the characteristics of the subjects. Age and gender were comparable between the subjects with or without bladder cancer. Significantly higher serum and urine CYFRA 21-1 were observed in patients with bladder cancer (P<0.01).



**Figure 1** Association between both serum (A) and urine (B) CYFRA 21-1 and the tumour stage and differentiation. Each dot represents a subject and horizontal lines represent the mean values. Kruskal-Wallis H test and Mann-Whitney U test were used for data comparison.

### *Association between CYFRA 21-1 and clinical characteristics*

As shown in *Figure 1*, both the serum and urine CYFRA 21-1 were significantly increased with advanced of tumour stage and differentiation ( $P<0.05$  for both). If the Tris stage was excluded from the analysis, the urine CYFRA 21-1 was significantly increased as the stage advanced ( $P<0.01$ ), but we failed to observe a significant difference in the serum CYFRA 21-1 between the T1 stage and the T4 stage ( $P=0.08$ ). For the post hoc analysis, only the following pairs of comparisons were statistically significant: urine CYFRA 21-1 and stage, Tris *vs.* T2, Tris *vs.* T3, Tris *vs.* T4, T1 *vs.* T2, and T1 *vs.* T3, and in terms of the urine CYFRA 21-1 grade: low *vs.* high and moderate *vs.* high. Using a Spearman's correlation analysis, we found that both the serum and urine CYFRA 21-1 were positively correlated with tumour stage and differentiation ( $P<0.05$  for both). Bladder cancer patients with positive VUC had significantly higher urine CYFRA 21-1 ( $P<0.01$ ), but serum CYFRA 21-1 differences in patients with positive or negative VUC were not statistically significant ( $P=0.12$ ).

### *Diagnostic accuracy of serum and urine CYFRA 21-1 for bladder cancer*

*Figure 2* is an ROC curve describing the diagnostic

accuracy of both the serum and urine CYFRA 21-1 for bladder cancer. The AUCs (95% CI) for serum and urine CYFRA 21-1 were 0.74 (0.68–0.81) and 0.74 (0.68–0.80), respectively. The differences between the AUCs of serum and urine CYFRA 21-1 were not statistically significantly ( $P=0.91$ ). The AUC (95% CI) for the combination of serum and urine CYFRA 21-1 was 0.83 (0.78–0.88), which was significantly higher than that of serum ( $P=0.04$ ) and urine ( $P=0.03$ ) CYFRA 21-1 alone.

*Table 2* lists the thresholds, as well as the corresponding sensitivities and specificities, of both the serum and urine CYFRA 21-1.

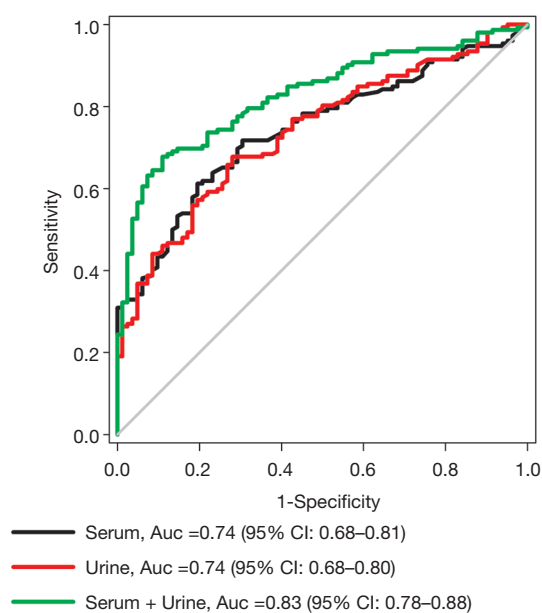
### **Discussion**

In this study, we compared the diagnostic value of the serum and urine CYFRA 21-1 for bladder cancer and found that (I) the diagnostic accuracy of both the serum and urine CYFRA 21-1 was fair, as their AUCs were only 0.74; (II) both the serum and urine CYFRA 21-1 increased with advanced tumour stage, indicating that they are potential prognostic factors for bladder cancer; and c) the diagnostic value of serum and urine CYFRA 21-1 was comparable, and combinational use can improve the diagnostic accuracy of bladder cancer.

Although many studies have investigated the diagnostic

accuracy of serum or urine CYFRA 21-1 for bladder cancer (8-12), our study shows its strengths. First, to the best of our knowledge, this is the first study to do a head-to-head comparison of the diagnostic accuracy of CYFRA 21-1 in a cohort. Second, this is a double-blind study. The laboratory technicians were blinded to the clinical details of the subjects and the clinicians who made the diagnosis were blinded to the serum and urine CYFRA 21-1 test results. Therefore, incorporation bias and review bias were avoided (13). Third, all subjects in this study received cystoscopy. Therefore, partial verification bias is avoided (13,14). Fourth, all the subjects enrolled in this study had signs or symptoms of bladder cancer. Therefore, the study cohort had good representativeness.

Because the sensitivity and specificity are greatly affected by the threshold that was adopted, both indicators have limitations in estimating the overall diagnostic accuracy



**Figure 2** Receiver operating characteristic (ROC) curve for CYFRA 21-1. AUC, area under the ROC curve.

of an index test. It is widely accepted that AUC is a global indicator that reflects the overall diagnostic accuracy of an index test (15). The AUC ranges from 0.5 to 1, with a higher value indicating a stronger diagnostic performance. In this study, we found that AUCs for both the serum and urine CYFRA 21-1 were 0.71, indicating that the diagnostic accuracy of the serum and urine CYFRA 21-1 was fair. The difference between the AUCs for serum and urine CYFRA 21-1 was not significant, indicating that the diagnostic accuracy of the serum and urine CYFRA 21-1 was comparable. Our results were consistent with a previous meta-analysis (6), which reported that the areas under the summary ROC curve for serum and urine CYFRA 21-1 were 0.88 and 0.87, respectively. The AUCs in our study were lower than those reported by some previous studies (10,11). This inconsistency may be due to differences in the disease spectrum and disease prevalence of the cohorts. It is well known that the AUC of an index test is greatly affected by the disease spectrum and prevalence of the studied cohort (16,17).

We used a logistic regression model to incorporate the serum and urine CYFRA 21-1 into a model. ROC curve analysis was used to estimate the diagnostic accuracy of this model. We found that the AUC of the model was 0.83, which was significantly higher than that of the serum and urine CYFRA 21-1. This result indicates that using the serum and urine CYFRA 21-1 together can improve the diagnostic accuracy of bladder cancer. Therefore, for a suspicious bladder cancer patient, both the serum and urine CYFRA 21-1 should be determined to improve the diagnostic accuracy of bladder cancer.

Taken together, our study indicated that the diagnostic accuracy of the serum and urine CYFRA 21-1 was comparable and that using them together can improve the diagnostic accuracy of bladder cancer. Due to the small sample size and single-centre design, further studies with larger sample sizes are needed to further estimate the diagnostic accuracy of serum and urine CYFRA 21-1.

**Table 2** Diagnostic accuracy of the serum and urine CYFRA 21-1 and their combination at optimal thresholds

Markers	AUC (95% CI)	Threshold (ng/mL)	Sensitivity (95% CI)	Specificity (95% CI)
Serum CYFRA 21-1	0.74 (0.68–0.81)	2.28	0.72 (0.64–0.79)	0.70 (0.57–0.79)
Urine CYFRA 21-1	0.74 (0.68–0.80)	62.74	0.68 (0.60–0.75)	0.72 (0.61–0.81)
Serum and urine CYFRA 21-1	0.83 (0.78–0.88)	0.66	0.68 (0.60–0.75)	0.89 (0.80–0.95)

AUC, area under the ROC curve; ROC, receiver operating characteristic.

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## Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2018.01.08>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committee of Daping Hospital (NO: 52). All the included subjects or their legal representatives provided informed consent.

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