Diagnostic utility of urinary BLCA-4 tests for bladder cancer: a meta-analysis

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Abstract: To evaluate the diagnostic utility of urinary BLCA-4 tests for bladder cancer. We performed a meta-analysis of literature from PubMed, EmBase, and CBM. Studies that evaluated urine BLCA-4 tests, used cystoscopy or histopathology as the reference standard, and constructed a 2 × 2 contingency table were included. Two reviewers independently evaluated trial eligibility and methodological quality and performed data extraction. Random effect models were used to perform the meta-analysis. Seven studies with 877 subjects, including 312 in the case group and 565 in the control group, were included in the meta-analysis. High heterogeneity was present among the studies. The pooled sensitivity and specificity for the urine BLCA-4 tests were 0.85 (95% CI, 0.81–0.88) and 0.97 (95% CI, 0.95–0.98), respectively, and the area under the curve for the urine BLCA-4 tests was 0.9806. Urinary BLCA-4 can be used as a quick, simple and noninvasive method for the screening of bladder cancer.
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1. Introduction

As the second-most common urological cancer in the developed world, over 70,000 new cases of bladder cancer are diagnosed each year in the United States alone (Siegel et al. 2012; Siegel et al. 2012, 2012). In 75-85% of patients, bladder cancer presents as an invasive cancer of the nonmuscle tissues. The progression rate is highly variable (1-45% at 5 years). Good prognosis is heavily dependent on early detection, with the 5-year recurrence rate ranging from 31% to 78% (Sylvester et al. 2006).

The primary methods used in the screening and surveillance of bladder cancers are cystoscopy, which is the gold standard approach (Babjuk et al. 2011; Goldberg et al. 2008; van der Aa et al. 2010) , and urine cytology, which is commonly employed in combination with cystoscopy. Although useful in patients with a history of bladder cancer, cystoscopy may be overused in low-grade cancers and may miss certain tumors, including papillary tumors and carcinomas. As a less-expensive and less-invasive method than cystoscopy, urine cytology has been shown to detect certain high-grade carcinomas with high sensitivity. However, urine cytology has poor sensitivity at detecting low-grade tumors, and it has poor intra- and interobserver reproducibility (Lokeshwar et al. 2005; Raitanen et al. 2002). Although the prostate-specific antigen (PSA) test shows better sensitivity for bladder tumors than urine cytology, it typically has lower specificity than urine cytology without necessarily offering additional useful information for disease management.

Researchers are actively searching for an ideal bladder tumor marker to overcome the above disadvantages of currently available detection approaches. Such a marker should be inexpensive, show high specificity and sensitivity, and allow the rapid and convenient (e.g., urine-based) screening of the target population. Recently, a nuclear matrix protein specific to bladder cancer tissues, BLCA-4, was identified, which showed potential utility as a urine-based bladder tumor marker (Van Le et al. 2004). BLCA-4 affects the pathogenesis of bladder cancer by increasing the levels of thrombomodulin, which maintains blood flow, and of interleukin (IL)-1α and IL-8, which mediate cellular proliferation, invasion, and angiogenesis(Myers-Irvin et al. 2005). According to Van Le et al., BLCA-4 has a sensitivity of 89% and a specificity of 95% for bladder cancer detection (Konety et al. 2000; Van Le et al. 2004). The BLCA-4 level is not influenced by other conditions of the urinary tract, such as a history of cystitis, further supporting its potential use as a specific tumor marker.

In spite of its promising characteristics, the clinical utility of a urine BLCA-4 test remains to be elucidated. To address this issue, we performed a systematic review of studies concerning the diagnostic accuracy (i.e., sensitivity and specificity) of urine BLCA-4 tests for bladder cancer.

2. Material and Methods

Ethics

The study was approved by the Ethics Committee of the third affiliated hospital of Guangzhou medical university and all aspects of the study comply with the Declaration of Helsinki. Ethics Committee of the third affiliated hospital of Guangzhou medical university specifically approved that not informed consent was required because data were obtained from databases.

Search strategy

A systematic review was performed to identify original studies of the diagnostic accuracy of urine BLCA-4 tests for bladder cancer. Two reviewers independently searched the PubMed online, EmBase (Embase covers journals from 1974 to present), and CBM(CBM covers journals from 1966 to present) with the keywords "BLCA-4", "BLCA", "bladder cancer", "Urinary Bladder Neoplasms", "bladder carcinoma", "urinary bladder cancer", "cancer of bladder" and "carcinoma of bladder" on Thursday, June 27, 2013. The search included studies in English or Chinese.

Selection criteria

Articles were selected through a two-step process. In the first step, one reviewer screened the titles and abstracts of articles fulfilling the search criteria by the inclusion and exclusion criteria. Articles that passed the first selection step were admitted to the second step. In the second selection step, two reviewers independently reviewed the full-text of the articles according to the same inclusion and exclusion criteria. Disagreements between the reviewers were discussed until consensus was reached or resolved by discussion with a third party.

The inclusion criteria were: 1) study in any language, 2) detected BLCA-4 in urine, 3) provided a 2 × 2 contingency table, 4) included a per-patient analysis, and 5) used cystoscopy or histopathology as the reference standard. The exclusion criteria were: 1) study in nonhumans, 2) review article, letter, editorial comment, or case report, 3) did not include raw data, and 4) concerned cancer not arising in the bladder. According to the quality criteria for diagnostic studies of the Oxford Centre for Evidence-based Medicine, all of the included studies were of sufficiently high quality: they all clearly identified the different patient groups (e.g., with and without disease) and included a disease-free (true-negative) group.

Data extraction

The following data were retrieved from the articles and stored in a database: author, publication year, patient demographics, detection methods, total number of enrolled patients, and numbers of true-positive, true-negative, false-positive, and false-negative cases, as determined by cystoscopy or histopathology. Statistical parameters that were not presented in the original article were calculated from the sensitivity, specificity, or predictive values or other reference tests given.

Quality evaluation

The quality of the included studies was independently analyzed by two reviewers, according to the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) (Schuetz et al. 2010). The QUADAS is a 14-item instrument that uses a 3-point scoring method ("yes," "no," or "unclear") to assess the quality of studies according to their variations (2 items), bias (9 items), and report quality (3 items). Any causes of variation and bias in the studies were identified.

Homogeneity test

The heterogeneity of the included studies was examined with the likelihood ratio (LR) I^2 and the chi-squared (χ^2) test. The I^2 is calculated based on Cochran's heterogeneity statistic (Q) and the degrees of freedom (df) as $I^2 = (Q - df)/Q \times 100\%$. The LR I^2 reflects the interstudy variation that is due to heterogeneity rather than chance. We considered heterogeneity to be substantial for $I^2 > 50\%$ and for $P < 0.05$ in the $\chi 2$ test. In cases of significant heterogeneity, a random effects meta-analysis was used to analyze the data, and the overall test sensitivity with 95% confidence intervals (CIs) was determined. In cases with confirmed positive results, analysis was performed by a pooled specificity with 95% CIs.

Threshold effect analysis

In some diagnostic accuracy studies, a differential threshold effect may be responsible for a lack of detectable sensitivity or specificity. A differential threshold may be used to define positive test results. Summary ROC curves of the urine BLCA-4 test results in the included studies were constructed. If the points in the ROC space graph aligned in a characteristic shoulder-like pattern, then a threshold effect was suspected. Spearman's rank correlation between the specificity and sensitivity was also calculated to assess the threshold effect, with a high correlation indicating the threshold effect.

Statistical analysis

The Meta-Disc software program (version 1.4) was used to analyze the heterogeneity and the threshold effect, to calculate the pooled weighted specificity and sensitivity, and to generate the summary ROC curve. Results with a two-sided P-value < 0.05 were considered statistically significant.

3. Results

Literature retrieval and study characteristics

Of the 91 articles that were initially identified in the search strategy, 84 studies were removed, including 24 duplicates, 53 studies during the title/abstract review, and 14 studies during the full-text review (Fig1). Seven studies satisfied all of the criteria and were included in

this report (Chen et al. 2005; Feng et al. 2011; Feng et al. 2012; Konety et al. 2000; Konety et al. 2000; Van Le et al. 2005; Zhao 2011). These 7 studies concerned 877 subjects (312 case group, 565 control group). Three studies were conducted in the USA (Konety et al. 2000; Konety et al. 2000; Van Le et al. 2005), and four were performed in China (Chen et al. 2005; Feng et al. 2011; Feng et al. 2012; Zhao 2011). In all seven studies, the BLCA-4 levels were measured by ELISA.

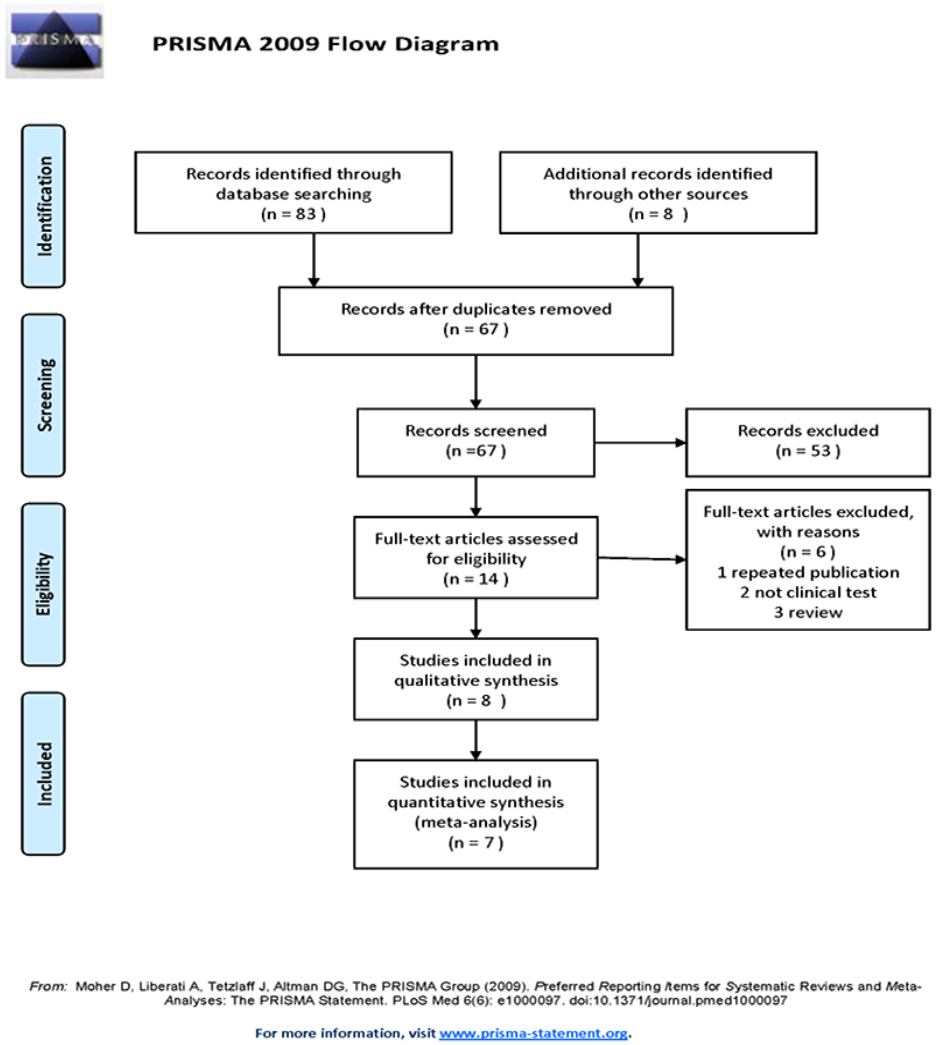

Figure 1. Flow chart of the study selection process

Of the 91 articles that were initially identified in the search strategy, 84 studies were removed, including 24 duplicates, 53 studies during the title/abstract review, and 14 studies during the full-text review . Seven studies satisfied all of the criteria and were included in this report

Quality evaluation

The results of the QUADAS analysis are shown in Table 1. All of the studies represented spectrum of patients (Q1), clearly described the selection criteria (Q3), avoided disease progression bias (Q4), avoided interpretation bias (Q7), described in sufficient detail to permit replication of index test (Q8), interpreted the reference standard and the index test results separately (Q11), provided the same clinical data (Q12), reported uninterruptable or intermediate test results (Q13) and explained the withdrawals from the study (Q14). Studies that used a reference standard did not provide sufficiently detailed methods to permit replication of the test (Q9) and no study used blinding for index test (Q10). Most of the studies accurately reported the selection criteria (Q2) and more than a half of the studies verified by reference standard (Q5) and used the same reference standard (Q6). The rationale for the sample size was not clearly provided in any study. Few studies included consecutive patients, and few included independent assessments of the marker and reference standard tests .

Table 1. Characteristics of studies included in the meta-analysis

Study	Country	Number	Method	Cut-off	Results				QUADAS
					TP	FP	FN	TN	
Konety, 2000[12]	USA	106	ELISA	13 A/μg	53	2	0	51	12
Konety, 2000[14]	USA	307	ELISA	0.04 OD	52	2	38	215	12
Chen, 2005[15]	China	76	ELISA	13 A/μg	33	2	0	41	9
VanLe,2005[16]	USA	140	ELISA	13 A/μg	67	8	3	62	11
Feng, 2011[17]	China	128	ELISA	1.7×10^{-4} A	33	2	8	85	12
Zhao, 2011[18]	China	60	ELISA	13 A/μg	28	2	2	28	10
Feng, 2012[19]	China	60	ELISA	5.5×10^{-4}A	28	0	2	30	12

Study heterogeneity

Significant heterogeneities were detected for sensitivity (2 = 79.42, df = 6, P < 0.01; I2 = 92.4%) and specificity (2 = 17.72, df = 6, P < 0.01; I2 = 66.1%) (Fig 2A and Fig 2B).

Threshold effect

The points of the ROC space did not show a shoulder-like pattern (Fig 3A). Spearman rank correlation analysis of the urine BLCA-4 tests provided a coefficient of 0.64 (P = 0.119). Therefore, a threshold effect was not observed in the included studies.

Summary estimates of the BLCA-4 tests

The random effects model was used to analyze the overall diagnostic accuracy of the urine BLCA-4 tests in the included studies, in terms of their pooled sensitivity (0.85; 95% CI, 0.81–0.88) and pooled specificity (0.97; 95% CI, 0.95–0.98) (Fig 2A and Fig 2B). The pooled diagnostic odds ratio (OR) was 232.09 (95% CI, 113.55–474.39; Fig. 3B), the pooled positive LR was 19.68 (95% CI, 10.55–36.68), and the pooled negative LR was 0.08 (95% CI 0.03–0.26) (Fig 2C and 2D). Due to the high heterogeneity and lack of a threshold effect, the random effects model was used to generate the ROC curve of the urine BLCA-4 tests. The area under the ROC curve (AUC) was 0.9806 and the estimated Q* index was 0.9384 (Fig. 3A).

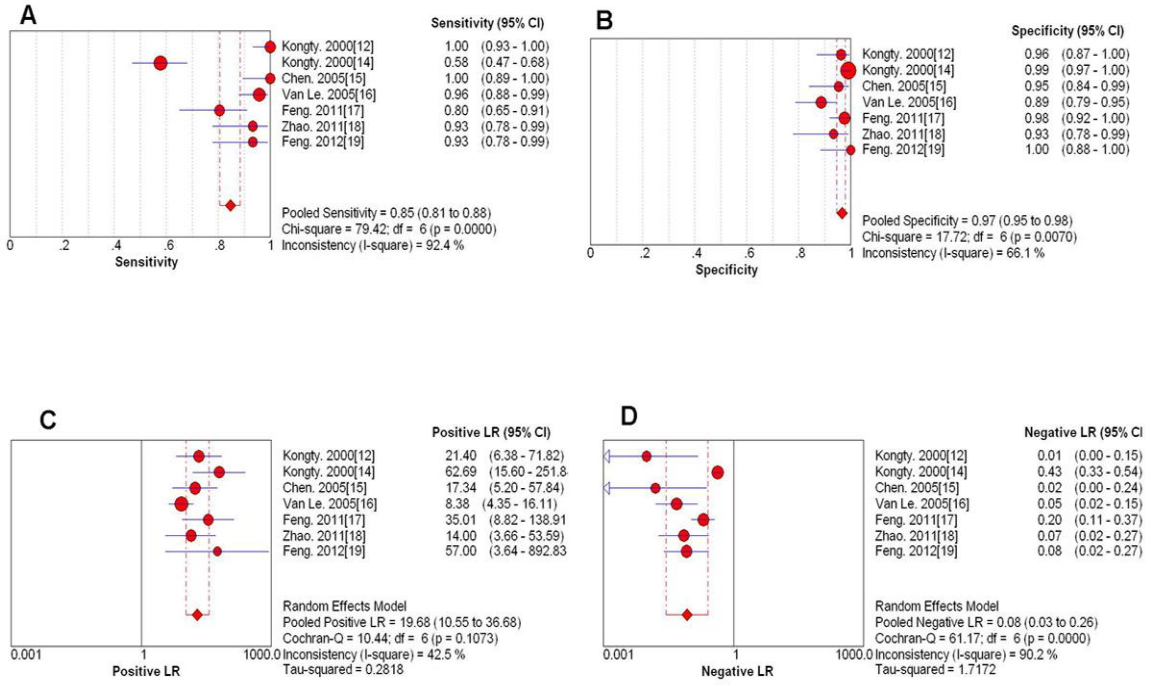

Figure 2 Forest plot of sensitivity, specificity, positive likelihood ratio, negative likelihood ratio of urine BLCA-4 in detecting bladder cancer

Forest plot of sensitivity of urine BLCA-4 in detecting bladder cancer B. Forest plot of specificity of urine BLCA-4 in detecting bladder cancer C. Forest plot of positive likelihood ratio of urine BLCA-4 in detecting bladder cancer D. Forest plot of negative likelihood ratio of urine BLCA-4 in detecting bladder cancer.

A. Area under the curve of the summary receiver operating characteristics and the *Q index of urine BLCA-4 in detecting bladder cancer B. Forest plot of diagnostic odds ratio (dOR) of urine BLCA-4 in detecting bladder cancer.

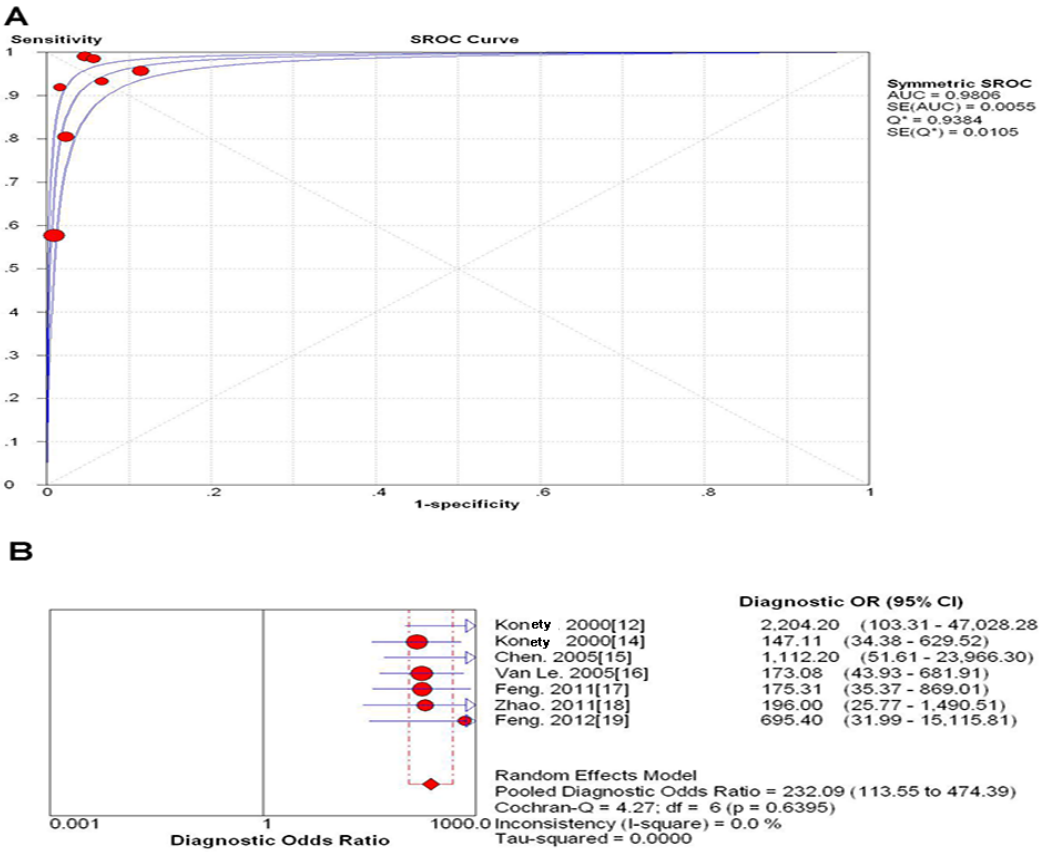

Figure 3. Forest plot of diagnostic odds ratio (dOR) and area under the curve of the summary receiver operating characteristics of urine BLCA-4 in detecting bladder cancer

Subgroup analysis

We performed a subgroup analysis of the different urine BLCA-4 cut-off values used for diagnosis (Table 1). For urine BLCA-4 tests with a cut-off value of 13 absorbance units per microgram protein (A/ g), the specificity, diagnostic OR, positive LR, and Q* index values were higher, the sensitivity and negative LR values were lower, and the AUC estimate was similar compared to the values of tests using other cut-offs.

4. Discussions

To address the clinical utility of urine BLCA-4 tests, we performed a systematic review of the literature concerning the diagnostic accuracy of urine BLCA-4 tests in patients with bladder cancer compared to reference standards (cystoscopy or histopathology). Some studies reported that most patients with bladder cancer (~89%) preferred cystoscopy as the diagnostic method for bladder tumors when the sensitivity of the tumor marker was less than 90%(Satoh et al. 2002; van der Aa et al. 2010). Nevertheless, our results showed that the urine BLCA-4 tests offered simpler, quicker and noninvasive detection of bladder tumors compared to cytology, with a pooled sensitivity of 85% and specificity of 97%. Therefore, urine BLCA-4 tests might offer utility as a compliment to cytology for bladder cancer screening and surveillance. These tests may be applied, for example, between cystoscopies or in combination with cytology to triage patients with certain symptoms. Of course, these suggestions must be evaluated by rigorous prospective studies. The results of this meta-analysis were based on very few studies, none of which evaluated specific test combinations.

To include both systematic and coincidental differences between the studies, we used a bivariate random effects model in our analyses. The value of the LR reflects the degree to which a diagnostic test result will increase or decrease the pre-test probability of the target disorder (in this case, bladder cancer). To estimate the post-test probability, the LR considers both the specificity and sensitivity (Carey et al. 1998; Soloway 2008). Higher LRs (LRs > 10) are estimated to generate larger, more conclusive changes from pre- to post-test probabilities, whereas very small LRs (0.5-2.0) generate negligible changes. Tests with high positive LRs can be used to confirm the presence of a disease (Pewsner et al. 2004). In the present study, a positive urine BLCA-4 test result (positive LR of 19.68) was associated with a conclusive change in the probability of any stage of bladder cancer, whereas a negative result (negative LR of 0.08) indicated a change that was likely to be insignificant.

This study had some limitations. In some studies, the use of healthy patients as a control group may have resulted in higher specificity values for the marker. The studies showed differences in the detection techniques (e.g., reagents, processing and storage times, instruments, operators, etc.), which may have influenced the outcomes. Overall, urine BLCA-4 tests have not been standardized, in part because threshold diagnostic levels for this marker have not been defined.

In conclusion, we performed a meta-analysis of studies of the diagnostic utility of urine BLCA-4 tests for bladder cancer. Urinary BLCA-4 can be used as a quick, simple and noninvasive method for the screening of bladder cancer. However, these findings should be interpreted with caution, in light of the small number of included studies. Further larger well-designed studies with standardized unbiased methods and well-matched controls are needed.

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Conflicts of interest

The authors have declared that no competing interests exist.

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