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Original Article

Diagnostic value of urinary microRNAs as non-invasive biomarkers for bladder cancer: a meta-analysis

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Abstract: *Background:* Bladder cancer (BC) is the fifth most common malignancy worldwide. The expression levels of microRNAs (miRNAs) in urine samples of BC patients have been demonstrated to be different from healthy people. Several studies focusing on the diagnostic value of urinary miRNAs for BC detection have been reported. The aim of this meta-analysis was to access the overall diagnostic accuracy comprehensively and quantitatively. *Methods:* PubMed, Embase, Web of Science, the Cochrane Library, and CNKI were searched without language restrictions for studies about the diagnostic value of miRNAs for BC. The pooled sensitivity, specificity, positive and negative likelihood ratios (PLR and NLR, respectively), diagnostic odds ratio (DOR) were calculated using the random effects model. The summary receiver operating characteristic (SROC) curve was also generated and the area under the curve (AUC) was also reckoned to assess the diagnosis accuracy. Besides, Chi-square test and I^2 test were used to assess the heterogeneity between studies. Publication bias was evaluated by the Deeks' funnel plot asymmetry test. *Results:* Fourteen studies were included in this meta-analysis, with a total of 1,128 BC patients and 1,057 matched controls. The overall sensitivity, specificity, PLR, NLR and DOR of urinary miRNAs for the diagnosis of BC were 0.71 (95% CI: 0.67-0.75), 0.75 (95% CI: 0.70-0.79), 2.8 (95% CI: 2.3-3.4), 0.39 (95% CI: 0.33-0.46) and 7 (95% CI: 5-10), respectively. The area under the SROC curve was 0.79. Subgroup analyses suggested that the ethnicity and miRNA profiling had an obvious influence on the diagnostic accuracy. *Conclusion:* The current analysis suggested that urinary miRNA panels may be a promising noninvasive biomarker in the diagnosis of BC.

Keywords: Urinary, microRNAs, bladder cancer, diagnostic value, meta-analysis

Introduction

Bladder cancer (BC), a kind of malignant tumor developed on bladder mucosa, is the fifth most common malignancy worldwide [1]. In regard to neoplasia of the genitourinary tract, it is the second most common tumor [2]. There are a variety of bladder cancer according to different pathological types, such as urothelial cell carcinoma, squamous cell carcinoma of bladder and bladder adenocarcinoma. Among these forms of cancer in the bladder, urothelial cell carcinoma (UCC) is the most common one, and can be divided into two groups, namely, low grade and high grade [3, 4]. The low-grade UCC are non-muscle invasive and always papillary, whereas the high-grade UCC are often invasive and can be either papillary or non-papillary [5]. The former UCC can be managed by endoscopic resection with intra-vesical chemotherapy [6]. However, the treatment outcome of the latter, that the mortality is around 50% despite radical therapy [7], is disappointing. Consequently, diagnosis of bladder cancer in a low grade can ameliorate the therapy outcomes and is crucial to reduce the mortality rate.

Currently, the standard diagnostic methods to detect BC are cystoscopy and urinary cytology. Although its high sensitivity, cystoscopy is an invasive and relatively expensive procedure [8]. What's more, it's may lead to some discomfort or even some complications, for instance, urinary tract infection. On the other hand, urinary cytology is non-invasive and has a high specificity (90%-95%), but it lacks sensitivity (30%-

40%) [9]. Meanwhile, both of the two methods show an unsatisfactory accuracy in low-grade tumors detection [10, 11]. Therefore, there are ongoing efforts to grope for non-invasive and effective approaches to identify incipient symptoms, and many new urine-based tests have been researched. Quantities of biomarkers in urine, such as bladder tumor antigen (BTA), urine fibrin fibrinogen degradation products (FDP), nuclear matrix protein 22 (NMP22), ImmunoCyt and FISH (Uro Vysion), have been found to be of diagnostic value [12, 13]. In comparison to urinary cytology, those urinary biomarkers have higher sensitivities (50%-70%) and relatively lower specificities (60%-80%) [12, 13]. Despite the fact that those urinary biomarkers can't replace cystoscopy or urinary cytology so far due to their insufficient sensitivity or specificity [14, 15], urine-based diagnostic tools proved to be promising for BC detection with the advantages that urine-based tests are non-invasive, convenient and can be repeated [10]. Besides, physicochemical characters and molecular changes of urine can distinctly reflect the pathophysiological condition of BC as it is the second most common malignancy that involves the urinary system [2]. Thus, more studies are really needed to focus on identification of novel and reliable urinary biomarkers for detecting BC.

MicroRNAs (miRNAs) are small non-coding RNAs, which are involved in various biological processes and act as regulators of gene expression either by messenger RNA degradation or by translation repression [16]. Recently, cumulative evidence has demonstrated that miRNAs play important roles in embryonic development of different cancers [17-21]. For instance, the researches that Calin et al. have done, which characterized the 13q14 deletion in human chronic lymphocytic leukemia, have provided the evidence of implication of 2 miRNAs (miR-15a and miR-16-1) in human cancer [22]. Hence, miRNAs may have potential clinical applicability, for example, serving as biomarkers for cancer diagnosis. In addition, miRNAs have excellent stability in urine against RNase degradation, extreme temperature and pH, multiple freeze-thaw cycle, which facilitate the relevant tests and make miRNAs a promising biomarker in BC detection as well.

Specifically, there have been numerous analyses of expression of many miRNAs in urine samples of BC patients [6, 23-26], and different expression signatures have been observed between normal urothelium and UCC [27, 28]. Notably, lots of studies have demonstrated high expression levels of miR-96 and miR-183 in urine from BC patients [24, 29, 30]. Among them, the analysis Hideki et al. have revealed that the sensitivity and specificity of miR-96 for UCC detection were 70.4% and 90.5%, while the corresponding data were 94.3% and 92.9% as for miR-183 [24]. Enokida et al. also found that the sensitivity of the two miRNAs to distinguish BC from non-BC patients were 71.0% (miR-96) and 74.0% (miR-183), while the specificity were 89.2% (miR-96) and 77.3% (miR-183), respectively [30]. Furthermore, Miah et al. have studied another 3 miRNAs (miR-1224-3p, -135b and -15b) and obtained different results. This study showed that the sensitivity of each individual miRNA ranged from 67.8% to 85.7%, and the combination of miR-1224-3p/-135b/-15b show a higher sensitivity of 94.1% for BC diagnosis [6]. Moreover, miR-126 [23, 31] and miR-200a [26] also appear to be detectable in urine and useful in the diagnosis of bladder cancer. Obviously, these outcomes were inconsistent on the diagnostic accuracy. Additionally, individual study may also limited by small sample size, few kinds of miRNA profiling, etc. Accordingly, a systematic review, which focuses on the overall clinical applicability of miRNAs in BC detection, can provide a more precise result. Therefore, we conducted a meta-analysis to investigate the diagnostic accuracy of miRNAs in BC detection.

Methods

Search strategy

A comprehensive literature search prior to Feb. 7, 2015 was conducted. Sources of studies included PubMed, Embase, Web of Science, the Cochrane Library, Chinese National Knowledge Infrastructure (CNKI), and so on. The searching terms "microRNAs, miRNA, miRs; urinary bladder neoplasms, bladder cancer, urothelial cancer, BC, UCC; sensitivity, specificity, diagnosis, ROC curve" were adopted. We also look up the reference lists of the selected articles to identify any additional eligible studies.

Inclusion and exclusion criteria

Studies were included if they satisfied the following criteria: (1) studies related to the diag-

nostic value of miRNAs for bladder cancer; (2) the diagnosis of BC based on cystoscopy, which was considered as the standard methods for BC diagnosis; (3) studies which measured the expression of miRNAs in urine; (4) both the sensitivity and specificity data were provided or could be calculated.

Correspondingly, studies were excluded based on the criteria below: (1) studies that were abstracts, letters, talks or reviews; (2) studies with duplicate data reported in other studies or without complete data to enable to construct two-by-two tables; (3) studies that focused on miRNAs in tissue or blood.

Data extraction

Two investigators carefully reviewed the full text of the included studies to search for the relevant data. The extracted data elements of each study included: study details (first author, year of publication and country of publication), characteristics of study population (ethnicity, number, age and gender ratio of patients and controls; source of control) and data for diagnostic meta-analysis (specimen, detecting method, miRNAs profiled and miRNAs expression change, sensitivity and specificity data).

Quality assessment

The qualities of included studies were scored by two reviewers independently, using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) criteria [32]. The QUADAS-2 tool contained seven questions, each of which should be answered with "yes" (1 score), "unclear" or "no" (0 score). All questions were given equal weight, resulting in a maximum possible score of 7. Any conflicting evaluation was resolved after fully discussion.

Statistical methods

All statistical analyses were performed using R software. The sensitivity and specificity data of miRNAs associated with the diagnostic value of BC were extracted from each study to evaluate the diagnostic value of urinary miRNAs. Firstly, we calculated the pooled results of sensitivity, specificity, positive and negative likelihood ratio (PLR and NLR, respectively), diagnostic odds ratio (DOR) and their 95% confidence intervals (CIs) by using the random-effect model. Simultaneously, the summary receiver operator characteristic (SROC) curve was generated and the area under the SROC curve (AUC) was calculated. These parameters above enabled our estimate of the overall diagnostic accuracy. Among them, PLR represents the odds of positive test results in BC patients, while NLR reflects the odds of positive results in those without BC. Relatively, DOR is the combination of PLR and NLR (DOR = PLR/NLLR). The higher DOR value is the better discriminatory test performance will be [33]. On the other hand, the SROC curve is widely accepted as a graphical technique to assess the ability of a test to discriminate between those with disease and those without disease [34, 35]. Besides, the heterogeneity between studies was evaluated through Chi-square test and I^2 test. If the tests show a $P < 0.1$ or $I^2 > 50\%$, the existence of significant heterogeneity will be verified [36, 37]. Then meta-regression and subgroup analyses were undertaken to explore the sources of between-study heterogeneity. Furthermore, Deeks' funnel plots were adopted to evaluate the publication bias.

Results

Included studies

A total of 71 relevant studies were initially identified. Afterwards 2 additional studies were found through other sources, and there were no duplicates among these records. The titles, abstracts and key words of the 73 studies were looked up. Then, 55 unqualified studies including letters, reviews, editorials, case reports, irrelevant researches were excluded. As to the rest of 20 literatures, their full-text versions were carefully examined and retrieved. As a result, 6 of them didn't use urine samples: 4 were based on tissue samples [5, 38-40], and 2 were blood samples [8, 41]. According to the exclusion criteria, these studies were excluded from further analysis. Ultimately, 14 high-quality cohort studies were included for this systematic review and meta-analysis [8, 10, 23, 25, 26, 29-31, 42-47].

Baseline characteristics

The main characteristics, along with QUADAS-2 scores of the included articles were summarized in **Table 1**. All the records were published between 2010 and 2013, and 8 of them were

Table 1. Main characteristics of 14 publications included in meta-analysis

First author	Year	Country	Ethnicity	Sample size		Mean age (yr)		Control	MiRNAs profiling	Score
				Case	Control	Case	Control			
Hanke M	2010	Germany	Caucasian	29	11	72	69	HC	miR-126, miR-152, miR-182	5
Hideki E	2010	Japan	Asian	54	42	NA	NA	HC, UTI	miR-96, miR-183	4
Yamada Y	2011	Japan	Asian	100	74	75	36	HC, UTI	miR-96, miR-183	4
Enokida H	2012	Japan	Asian	85	74	NA	NA	HC, UTI	miR-96, miR-183	4
Miah S	2012	UK	Caucasian	68	53	71	58	BUD	miR-135b, -15b, -1224-3p	4
Puerta-Gil P	2012	Spain	Caucasian	37	57	50	50	HC, BUD	miR-452	4
Snowdon J	2012	Canada	Caucasian	8	5	77	63	HC	miR-125b, miR-126	4
Wang G	2012	China	Asian	51	24	74	59	HC	miR-200a	6
Yun SJ	2012	Korea	Asian	207	288	64	64	HC	miR-145, miR-200a	5
Mengual L	2013	Spain	Caucasian	151	126	72	63	HC	miR-187, -18a*, -25, -142-3p, -140-5p, -204	4
Tolle A	2013	China	Asian	20	19	70	49	HC	miR-520, -618, -1255b-5p	3
Wang W	2013	China	Asian	26	26	NA	NA	HC	miR-17-5p	6
Zhou X	2014	China	Asian	112	78	65	62	HC, BUD	miR-106b	4
Eissa S	2015	Egypt	Caucasian	180	180	NA	NA	HC, BUD	miR-210, -10b, -29c	5

NA, not available; HC, healthy control; UTI, urinary tract infection; BUD, benign urological disease.

Table 2. Summary estimates of diagnostic criteria and the 95% confidence intervals

Analysis	Sensitivity (95% CI)	Specificity (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
Ethnicity						
Caucasian	0.69 (0.63-0.74)	0.74 (0.68-0.80)	2.7 (2.1-3.4)	0.42 (0.34-0.51)	6 (4-10)	0.77 (0.74-0.81)
Asian	0.75 (0.67-0.81)	0.75 (0.68-0.81)	3.0 (2.3-3.8)	0.34 (0.63-0.44)	9 (6-13)	0.81 (0.78-0.85)
MiRNA profiling						
Single miRNA	0.69 (0.65-0.73)	0.74 (0.69-0.78)	2.7 (2.2-3.2)	0.41 (0.35-0.48)	6 (5-9)	0.78 (0.74-0.81)
Multiple miRNAs	0.83 (0.67-0.92)	0.81 (0.62-0.92)	4.4 (2.2-8.6)	0.21 (0.11-0.40)	21 (9-48)	0.89 (0.86-0.91)
Overall	0.71 (0.67-0.75)	0.75 (0.70-0.79)	2.8 (2.3-3.4)	0.39 (0.33-0.46)	7 (5-10)	0.79 (0.75-0.82)

CI, confidence interval; LR, likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the curve; UCC, urothelial cell carcinoma.

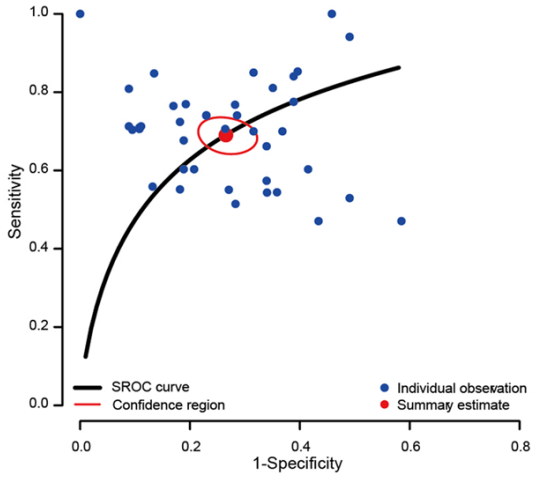

Figure 1. SROC curves of all studies analysis with confidence and prediction regions around mean operating sensitivity and specificity point.

conducted in Asian, while the other 6 were in Caucasian. In this meta-analysis, a total of 1,128 BC patients and 1,057 controls were included. 7 of the included studies used population-based healthy controls, and other controls contained patients with urinary tract infection or benign urological diseases. All diagnosis was confirmed by cystoscopy. Meanwhile, all the 14 articles perform the quantitative real-time polymerase chain reaction (qRT-PCR) method to test the expression level of miRNAs in urine samples. Besides, 35 studies focused on a single kind of miRNAs while there remaining 5 studies discussed the diagnostic value of a set of miRNAs.

Diagnostic accuracy of urinary miRNAs in bladder cancer

Due to the existence of significant heterogeneity between studies in sensitivity and specificity (I^2 = 81.26% and I^2 = 84.42%), the random effect model was adopted. The pooled results of diagnostic criteria and their 95% confidence intervals were listed in **Table 2**. The overall sensitivity, specificity, PLR, NLR and DOR were 0.71

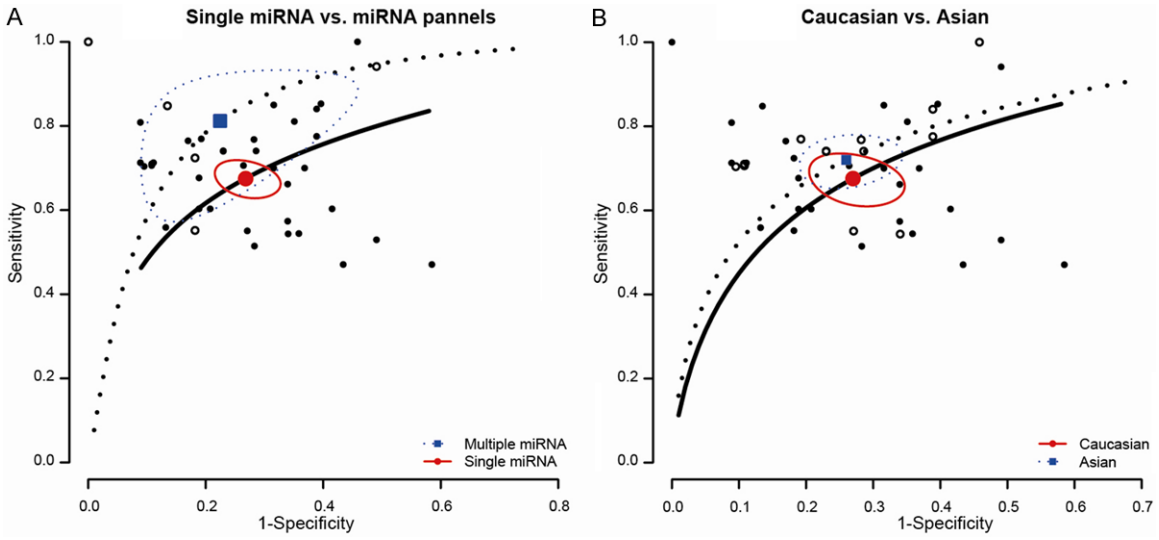

Figure 2. SROC curves of each subgroup analysis with confidence and prediction regions around mean operating sensitivity and specificity point.

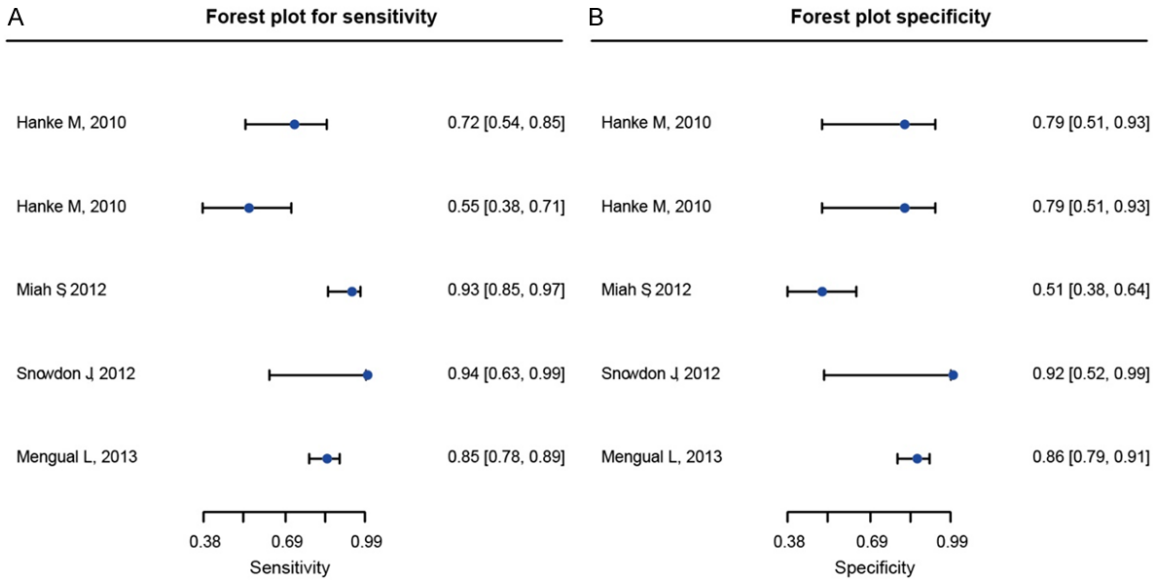

Figure 3. Forest plot showing study-specific (right-axis) and mean sensitivity and specificity for miRNA panels subgroup.

(95% CI: 0.67-0.75), 0.75 (95% CI: 0.70-0.79), 2.8 (95% CI: 2.3-4.6), 0.39 (95% CI: 0.33-0.46) and 7 (95% CI: 5-10), respectively. Moreover, we generated the SROC curve and calculated the area under the curve (AUC), which was 0.79 (95% CI: 0.75-0.82) (**Figure 1**). Altogether, the overall diagnostic accuracy of urinary miRNAs for BC detection was moderately high.

Meta-regression and subgroup analysis

In order to explore the heterogeneity between studies, we conducted subgroup analyses, which were based on ethnicity (Asian or Caucasian) and miRNA profiling (single or multiple). The SROC curves for each group were shown in **Figure 2** and the corresponding diagnostic parameters were listed in **Table 2**. It turned out that both the sensitivity and specificity of miRNA panels (sensitivity: 0.83, specificity: 0.81) were higher than single miRNA (sensitivity: 0.69, specificity: 0.74), which indicated that the diagnostic accuracy of miRNA panels was superior to that of single miRNA. **Figure 3** presented the forest plots of data

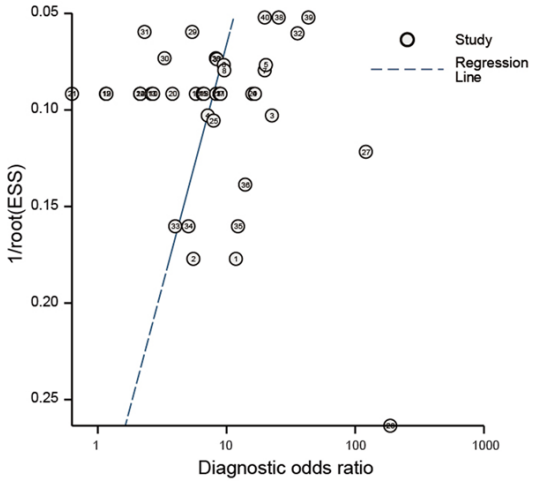

Figure 4. Linear regression test of Deeks' funnel plot asymmetry of overall studies.

from the miRNA panels-based studies and mean sensitivity and specificity.

With regard to another subgroup, the results implied that specificity might come at the cost of sensitivity. To be more specific, the studies based on Caucasian population had a pooled specificity of 0.74 and a sensitivity of 0.69, while studies based on Asian had a relatively higher specificity (0.75) with a little bit lower sensitivity (0.75). All in all, the foregoing results of subgroup analyses suggested that the ethnicity and miRNA profiling had an evident influence on the diagnostic accuracy. Therefore, these factors might be the potential sources of heterogeneity.

To further confirm the results of subgroup analyses, we also conducted the meta-regression. The correlative results indicated that miRNA profiling had a significant influence on both sensitivity and specificity ($P < 0.05$). In consequence, further evidence was provided for the subgroup analysis above.

Publication bias

Finally, the Deeks' funnel plot asymmetry test was conducted to evaluate the publication bias. And either of them had a P value of 0.18 for the slope coefficient, indicating the symmetry in the data and the low likelihood of publication bias (**Figure 4**).

Discussion

As mentioned above, bladder cancer is the fifth most common malignancy worldwide [1], and early detection is important to improve survival of BC patients. Nowadays, cystoscopy and urinary cytology, which are the current standard diagnostic methods to detect BC, lack sufficient diagnostic accuracy of low-grade BC [10, 11]. Thus, novel and reliable biomarkers for BC detection are urgently needed. Notably, present studies have indicated potential diagnostic value of urinary miRNAs for BC [23, 25, 26, 42, 43]. However, there were inconsistencies between studies, not only on the diagnostic accuracy, but also on the kinds of urinary miRNAs that were profiled. For example, miR-96 yielded a sensitivity of 70.4% and a specificity of 90.5% in the study that Hidiki et al. made [29], and a sensitivity of 71.0% and a specificity of 89.2% in Yamada et al.' study [42]. Similarly, there were differences of diagnostic accuracy of miR-200a for BC detection between Wang et al.' study and Yun et al.' study [26, 44]. Except for single miRNA, miRNA panels were also investigated in other studies [23, 31, 43, 45]. What's more, the combination of miR-125b and miR-126 showed a very high diagnostic accuracy to detect BC patients, with a sensitivity of 100% and a specificity of 100% [31]. On the other hand, Mengual et al. developed a panel of six miRNAs (miR-187, -18a*, -25, -142-3p, -140-5p, -204) to discriminate UCC from normal individuals, and the results showed a specificity of 84.8% and a sensitivity of 86.5% [45]. In consideration of different basic characteristics of patients and controls and different selected miRNAs, the above disaccord is accessible. As far as we know, no previous meta-analysis on the overall accuracy of urinary miRNAs in BC diagnosis has been performed. Hence, we performed this meta-analysis to access the overall diagnostic value of urinary miRNAs for BC diagnosis.

As a result, the overall sensitivity and specificity of urinary miRNAs to detect BC were 0.71 and 0.75, respectively. The relatively high sensitivity and specificity revealed a high level of accuracy of urinary miRNAs to detect BC. Meanwhile, the pooled PLR was 2.8 and the pooled NLR was 0.39, which also verified the conclusion above. Furthermore, our meta-analysis presented that the AUC, which was an overall indicator of test performance, was 0.79. In addition, the fact that the DOR value was 7 indicated a good discriminatory test performance. Taken together, these outcomes suggested that urinary miRNAs had a relatively high diagnostic accuracy.

While heterogeneity between studies may affect the results of the meta-analysis, subgroup analyses will help us to explore these influences. Therefore, subgroup analyses, which were based on ethnicity and miRNA profiling, were conducted in our meta-analysis. And it's notable that the diagnostic accuracy of miRNA combination was obviously higher than that of single miRNA. Besides, a meta-regression analysis was performed to confirm the results of subgroup analyses. Through subgroup analyses and meta-regression, we found that miRNA profiling had a significant influence on both sensitivity and specificity. Apart from the analyses mentioned above, there were other potential sources of heterogeneity, such as the difference of the cut-off value applied among the studies. Therefore, further researches are needed to explore heterogeneity and attendant influences.

There are several advantages of the present meta-analysis. First of all, several methods were conducted to reduce the influence of heterogeneity. In addition, we used comprehensive methods, including rigorous literature screening process, to try to avoid publication bias. Moreover, high stability of urinary miRNA was repeatedly reported, which makes our analysis more reliable and repeatable. However, there are still several limitations in our meta-analysis. Firstly, the included studies were based on limited sample size, and there were inconsistence among the included studies. Secondly, few studies were conducted on African populations, and some other language publications (like Japanese and German) may not be included in this study, which may increase the bias of the meta-analysis. Thirdly, most included studies didn't differentiate the grade of bladder cancer, and were short of investigation on the diagnostic value of urinary miRNAs for low-grade BC.

According to the results of the meta-analysis, we can reach the conclusion that urinary miRNAs present a promising noninvasive approach for BC diagnosis. Although the overall diagnostic accuracy was far from ideal, subgroup analysis indicated that miRNA panels had great potential as new diagnostic biomarkers in BC. Nevertheless, there is still a long way to go for their practical usage in BC diagnosis. And large clinical studies are needed to confirm their diagnostic value. What's more, more attention should be paid to the diagnostic accuracy of urinary miRNAs for low-grade BC.

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Disclosure of conflict of interest

None.

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