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Review Article

Cell-free DNA in blood and urine as a diagnostic tool for bladder cancer: a meta-analysis

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Abstract: Objective: To assess the diagnostic performance of cell-free DNA assays in the detection of bladder cancer. Patients and methods: The quality of the studies included in this meta-analysis was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. Statistical analyses were performed using the software RevMan 5.3 and Stata 14.0. We assessed the pooled sensitivity and specificity, positive/negative likelihood ratios (PLRs/NLRs), diagnostic odds ratios (DORs), and corresponding 95% confidence intervals (95% CIs). Summary receiver operating characteristic curve (ROC curve) and area under the curve (AUC) were used to summarize the overall test performance. Heterogeneity and publication bias were also examined. Results: Eleven studies included 802 bladder cancer patients and 668 controls met the eligibility criteria. The overall diagnostic accuracy was measured as follows: sensitivity 0.71 (95% CI = 0.64-0.77), specificity 0.78 (95% CI = 0.70-0.85), PLR 3.3 (95% CI = 2.4-54.5), NLR 0.37 (95% CI = 0.30-0.46), DOR 9 (95% CI = 6-14), and AUC 0.80 (95% CI = 0.77-0.83). Subgroup analysis suggested that ethnicity significantly accounted for the heterogeneity of specificity. The Deeks' funnel plot asymmetry test (P = 0.97) suggested no potential publication bias. Conclusions: Cell-free DNA has a high diagnostic value in bladder cancer.

Keywords: Cell-free DNA, biomarker, bladder cancer, diagnosis

Introduction

Bladder cancer is the fourth most common cancer among men in the western world, with a rate of recurrence of 50%, and 15-40% growing into muscle invasive disease [1, 2]. An estimated 429,800 new cases of bladder cancer and 165,100 deaths occurred in 2012 worldwide [3]. Early diagnosis has an important impact on increasing disease-free survival and reducing mortality in patients with different tumour types. Currently, cystoscopic examination is the gold standard, and most sensitive method, for bladder tumour detection. However, it is invasive and could make patients feel uncomfortable or even upset. Voided cytology, currently the only non-invasive approach, has a low sensitivity for detecting low grade and early-stage tumours [4, 5]. Therefore, a timely and accurate diagnosis method is critically needed for improving treatment outcomes in patients with bladder cancer. Several new molecular markers have been introduced in recent years to improve the diagnosis and management of bladder cancer [6], such as gene alterations [7, 8], Nuclear Matrix Protein Number 22 (NMP 22) [9], telomerase activity [7, 10], urine dipstick [11], as well as BTA-stat and BTA-trak tests [12], but none has proven to be sufficiently sensitive or specific to prevent cystoscopic surveillance [5]. Therefore, development of new non-invasive technologies with enhanced sensitivity and specificity to detect and diagnose bladder cancer is in critical demand.

Ideal tumour markers should have a high degree of sensitivity and specificity for early tumour diagnosis, and should be easy to implement, non-invasive and economical. The existence of cell-free DNA (cfDNA) in blood was discovered as early as the 1940s [13], which was the fragmented DNA originating from cancer cells through the processes of necrosis and apoptosis [14]. It is presumed that cfDNA (150-

200 nucleotides in length) is usually derived from normal or tumour cells through apoptosis or necrosis [15, 16], which mainly are composed of tumorous cfDNA in the cancer patient [17]. It is reported that most patients with malignancies, such as CaP [18, 19] and testicular cancer [19, 20], have increased levels of cfDNA compared with patients with non-malignant diseases and healthy individuals. These cancer-specific alterations can serve as targets for the detection of neoplastic cells in clinical specimens such as readily accessible bodily fluids.

A number of studies reported higher levels of cell-free DNA in plasma or serum of patients with various tumour entities such as ovarian cancer [21], breast cancer [22], lung cancer [23], prostate cancer [18], renal cell carcinoma [24], gastric cancer [25] and oesophageal cancer [26]. Also, many studies have shown that cell-free DNA in urine has potential value for bladder cancer screening [13, 27, 28], but the results across these studies were not consistent. This lack of agreement in the literature might be due to differences in ethnicity, assay methods, sample types, source of controls and methylation gene location. Therefore, the purpose of this meta-analysis is to assess the diagnostic value of cfDNA in bladder cancer screening comprehensively.

Materials and methods

Literature source and search

The medical ethics committee of the Medical College of Henan University of Science and Technology approved the study before clinical data collection. Two authors independently conducted this meta-analysis. PubMed, Embase, and the Cochrane Library and CNKI online databases were comprehensively searched to evaluate all articles published from database inception up to December 1, 2017. Studies from different databases were imported to EndNote for further review. Searches were completed using random combinations of the following key words: cell-free DNA, circulating DNA, plasma DNA, serum DNA, urine DNA, cfDNA (cell-free DNA), bladder neoplasms, bladder carcinoma, bladder cancer, bladder tumour, sensitivity and specificity, diagnosis, and accuracy. We also contacted the authors of the articles when more information was needed.

Inclusion and exclusion criteria

The articles included met the following criteria: (1) articles that evaluated the diagnostic value of cfDNA for bladder cancer in urine or blood samples, (2) patients had bladder cancer confirmed by a gold standard test, (3) articles provided sufficient data, and (4) the literature was considered of a high-quality according to the quality evaluation. Publications were excluded if they met one or more of the following criteria: (1) a lack of sufficient outcomes (e.g. sensitivity, specificity, true positives, false positives, false negatives and true negatives), (2) studies that were not case-control in design; and (3) reviews, technical reports, case reports, meeting documents, comments, letters with invalid data or duplicate publications.

Quality assessment

Two investigators (Zhao and Wang) assessed the quality of the selected studies; based on the guidelines of the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) [29], which was developed by Cochrane collaborative websites. The tool allows for a more transparent assessment of the bias and applicability than the original diagnostic accuracy study. The risk of bias and applicability of the selected literature concerns four key areas: patient selection, index text, reference standard, and flow and timing.

Data extraction

After the quality assessment, the data from all 11 eligible studies were carefully extracted [27, 30-39] in duplicate by two independent investigators (Zhao and Zhang), and the information collected mainly included the following: first author's name, year of publication, country, characteristics of participants (ethnicity, number of cases and controls, sample source, and control type), type of cfDNA, source of specimen, assay methods, definition of cut-off, sensitivity and specificity data, and TP, FP, FN, and TN. When the investigators had any doubts about the data, they contacted the author of the study to ask for the original material. The study was excluded if no response was received.

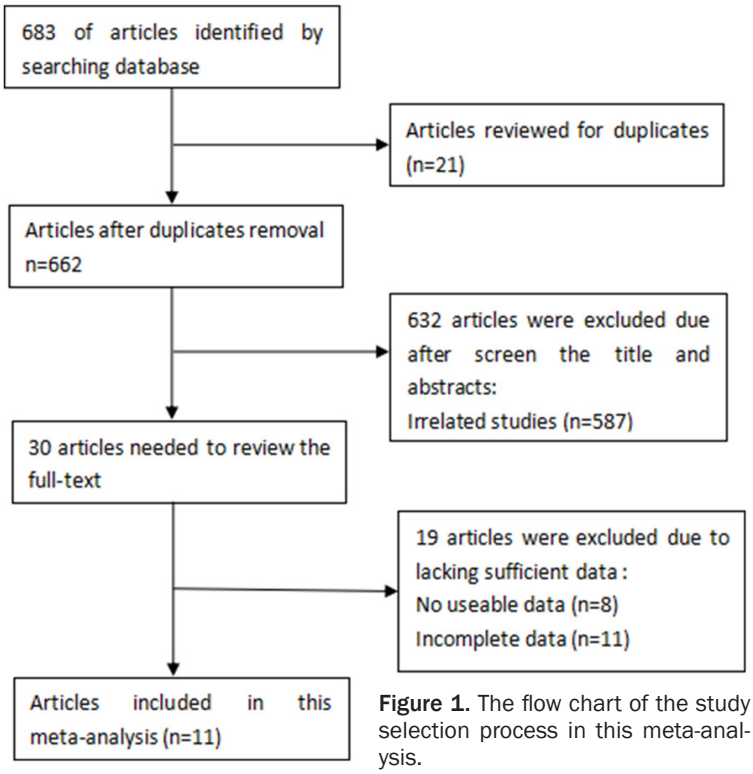

Figure 1. The flow chart of the study selection process in this meta-analysis.

Statistical analysis

RevMan 5.3 (Revman, the Cochrane Collaboration) and Stata 14.0 (Stata, College Station) were used to perform all the meta-analyses. Pooled sensitivity (TP/[TP + FN]) and specificity (TN/[TN + FP]), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) [40], corresponding 95% confidence intervals (CIs), and the confidence and prediction contours of the summary receiver operating characteristic (SROC) curves were calculated using the bivariate meta-analysis model [41]. To analyse the test accuracy, the area under the curve (AUC) was used for grading the overall accuracy as a potential summary of the SROC curve [42, 43].

We used the likelihood ratio test (LRT) I^2 statistic and LRT_Q (X^2) statistics to verify statistical significant heterogeneity among these studies. $P \leq 0.1$ or $I^2 \geq 50\%$ for LRT_Q indicates substantial heterogeneity. If the heterogeneity was significant, we should select a random effect model (DerSimonian and Laird method) [44] and a subgroup analysis was performed for the used literature to minimize the influence of heterogeneity on the results. In addition, Meta-regression analysis [45] was used to explore the sources of heterogeneity based on the characteristics of the included articles. Furthermore, potential publication bias [46] was assessed by the Deeks' test. For each analysis with $P < 0.05$ indicated statistical significance.

Results

Search results and eligible studies

Figure 1 presents the results of the literature retrieval. After initial screening of titles and abstracts, 30 studies, all of which evaluated abnormal concentrations of cfDNA for the diagnosis of bladder cancer, were retrieved. Eight articles were excluded because they lacked useful data, 11 articles were excluded due to insufficient data or because data could not be extracted by reviewing the full text. A total of 11 publications dealing with blood or urine cfDNA samples for the diagnosis of bladder cancer were selected.

Basic information of the enrolled literature

Four of these 11 studies evaluated abnormal concentrations of cell-free DNA in serum [21, 29-31], five studies in urine supernatant [27, 35, 37-39], three studies in urine sediment [30, 35, 36], and three studies assessed the validity of single-gene methylation alterations [30, 33, 36]. All these selected studies, published between 2002 and 2016, included 802 cases of histologically diagnosed bladder cancer and 668 healthy controls or patients with benign urological disease. The sample sources of cfDNA included serum and urine. In addition, the majority of subjects were from Europe, and the rest were from Asia. The details of the included studies and the enrolled patients are presented in **Table 1**.

Quality assessment of literature

The results of the quality assessment using the QUADAS-2 tool [29] are shown in **Figure 2**. The majority of all included articles in the cur-

Table 1. Characteristics of the included studies

First Author	Year	Country	Ethnicity	Case	Control	Control Type	Gene	Methylation	Specimen	Assay Methods	Cutoff	TP	FP	FN	TN	Sensitivity (%)	Specificity (%)
Jian Yu	2007	China	Asia	132	23	Noncancerous urinary disease	11-gene set	Yes	Urine Sediments	PCR	NA	121	3	11	20	91.7	87
Stefan Hauser	2012	*Germany*	Europe	143	84	Healthy and Benign disease	ACTB-106	No	Serum	PCR	NA	131	48	12	36	91.6	43.3
Stefan Hauser	2012	*Germany*	Europe	143	84	Healthy and Benign disease	DNA integrity	No	Serum	PCR	NA	86	20	57	64	59.8	75.8
M.T. Valenzuela	2003	Spain	Europe	86	49	Healthy and Benign disease	p16	Yes	Serum	MSP	NA	19	1	67	48	22.1	98
Jörg Ellinger	2012	*Germany*	Europe	84	79	Healthy	mtDNA-79	No	Serum	PCR	NA	74	2	10	77	88.1	97.5
Jörg Ellinger	2012	*Germany*	Europe	84	79	Healthy	mtDNA-230	No	Serum	PCR	NA	72	3	12	76	85.7	96.2
Jörg Ellinger	2012	*Germany*	Europe	84	79	Healthy	mtDNA-integrity	No	Serum	PCR	NA	38	11	46	68	45.2	86.1
Michael W. Y.	2002	Hong Kong	Asia	22	17	Healthy	RARβ	Yes	Urine Sediments	MSP	NA	15	4	7	13	68.2	76.4
Valentina Casadio	2012	*Italy*	Europe	51	78	Healthy and Benign disease	DNA integrity	No	Urine supernatant	PCR	0.04 (ng/ul)	40	23	11	55	78	70.5
Valentina Casadio	2012	*Italy*	Europe	51	78	Healthy and Benign disease	DNA integrity	No	Urine supernatant	PCR	0.06 (ng/ul)	38	19	13	59	75	75.6
Valentina Casadio	2012	*Italy*	Europe	51	78	Healthy and Benign disease	DNA integrity	No	Urine supernatant	PCR	0.08 (ng/ul)	37	13	14	65	73	83.3
Valentina Casadio	2012	*Italy*	Europe	51	78	Healthy and Benign disease	DNA integrity	No	Urine supernatant	PCR	0.1 (ng/ul)	37	13	14	65	73	83.3
Valentina Casadio	2012	*Italy*	Europe	51	78	Healthy and Benign disease	DNA integrity	No	Urine supernatant	PCR	0.12 (ng/ul)	32	11	19	67	63	85.9
Valentina Casadio	2012	*Italy*	Europe	51	78	Healthy and Benign disease	DNA integrity	No	Urine supernatant	PCR	0.14 (ng/ul)	27	9	24	69	53	88.5
H.W. Chang	2007	*Taiwan*	Asia	46	98	Nonmalignant	cf-DNA	No	Urine supernatant	PCR	0.0645 (ng/mL)	40	26	6	71	86.1	72
H.W. Chang	2007	*Taiwan*	Asia	46	98	Nonmalignant	cf-DNA	No	Urine supernatant	PCR	0.753 (ng/mL)	31	15	15	83	66.7	84.9
H.W. Chang	2007	*Taiwan*	Asia	46	98	Nonmalignant	cf-DNA	No	Urine supernatant	PCR	1.276 (ng/mL)	26	11	20	87	55.6	89.2
H.W. Chang	2007	*Taiwan*	Asia	46	98	Nonmalignant	cf-DNA	No	Urine supernatant	PicoGreen	1.84 (ng/mL)	37	93	9	5	80.4	5.1
H.W. Chang	2007	*Taiwan*	Asia	46	98	Nonmalignant	cf-DNA	No	Urine supernatant	PicoGreen	3.09 (ng/mL)	31	75	15	23	67.4	23.5
H.W. Chang	2007	*Taiwan*	Asia	46	98	Nonmalignant	cf-DNA	No	Urine supernatant	PicoGreen	6.795 (ng/mL)	25	25	21	73	54.3	74.5
Antonin Brisuda	2015	Czech	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	65055 (ng)	28	3	38	31	42.4	91.2
Antonin Brisuda	2015	Czech	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	87991 (ng)	24	2	42	32	36.4	94.1
Antonin Brisuda	2015	Czech Republic	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	52800 (ng)	31	7	35	27	47	79.4

Antonin Brisuda	2015	Czech Republic	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	39124 (ng)	37	9	29	25	56.1	73.5
Antonin Brisuda	2015	Czech Republic	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	19866 (ng)	45	13	21	21	68.2	61.8
Antonin Brisuda	2015	Czech Republic	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	13601 (ng)	53	16	13	18	80.3	52.9
Antonin Brisuda	2015	Czech Republic	Europe	66	34	Healthy and benign urological disorders	cf-DNA	No	Urine supernatant	PCR	8257 (ng)	59	19	7	15	89.4	44.1
Ye-Hwan Kim	2016	*Korea*	Asia	83	115	Healthy and benign urological disorders	TopoIIA cf- DNA	No	Urine supernatant	PCR	19385.71	61	36	22	79	73.8	68.3
Tibor Szarvas	2007	Hungary	Europe	44	20	Nonmalignant urinary diseases	cf-DNA	No	Urine supernatant	PCR	NA	35	4	9	16	79.5	80
Tibor Szarvas	2007	Hungary	Europe	44	16	Healthy	cf-DNA	No	Urine supernatant	PCR	NA	35	3	9	13	79.5	81.25
Tibor Szarvas	2007	Hungary	Europe	44	20	Nonmalignant urinary diseases	cf-DNA	No	Urine sediment	PCR	NA	27	1	13	19	67.5	95
Tibor Szarvas	2007	Hungary	Europe	44	16	Healthy	cf-DNA	No	Urine sediment	PCR	NA	27	2	13	14	67.5	87.5
Jörg Ellinger	2008	*Germany*	Europe	45	55	Benign prostate hyperplasia	DNA fragment	No	Serum	PCR	19.01 ng/ml	43	21	2	34	95.6	62.2
Jörg Ellinger	2008	*Germany*	Europe	44	55	Benign prostate hyperplasia	DNA fragment	No	Serum	PCR	7.09 ng/ml	26	23	18	32	59.1	57.8

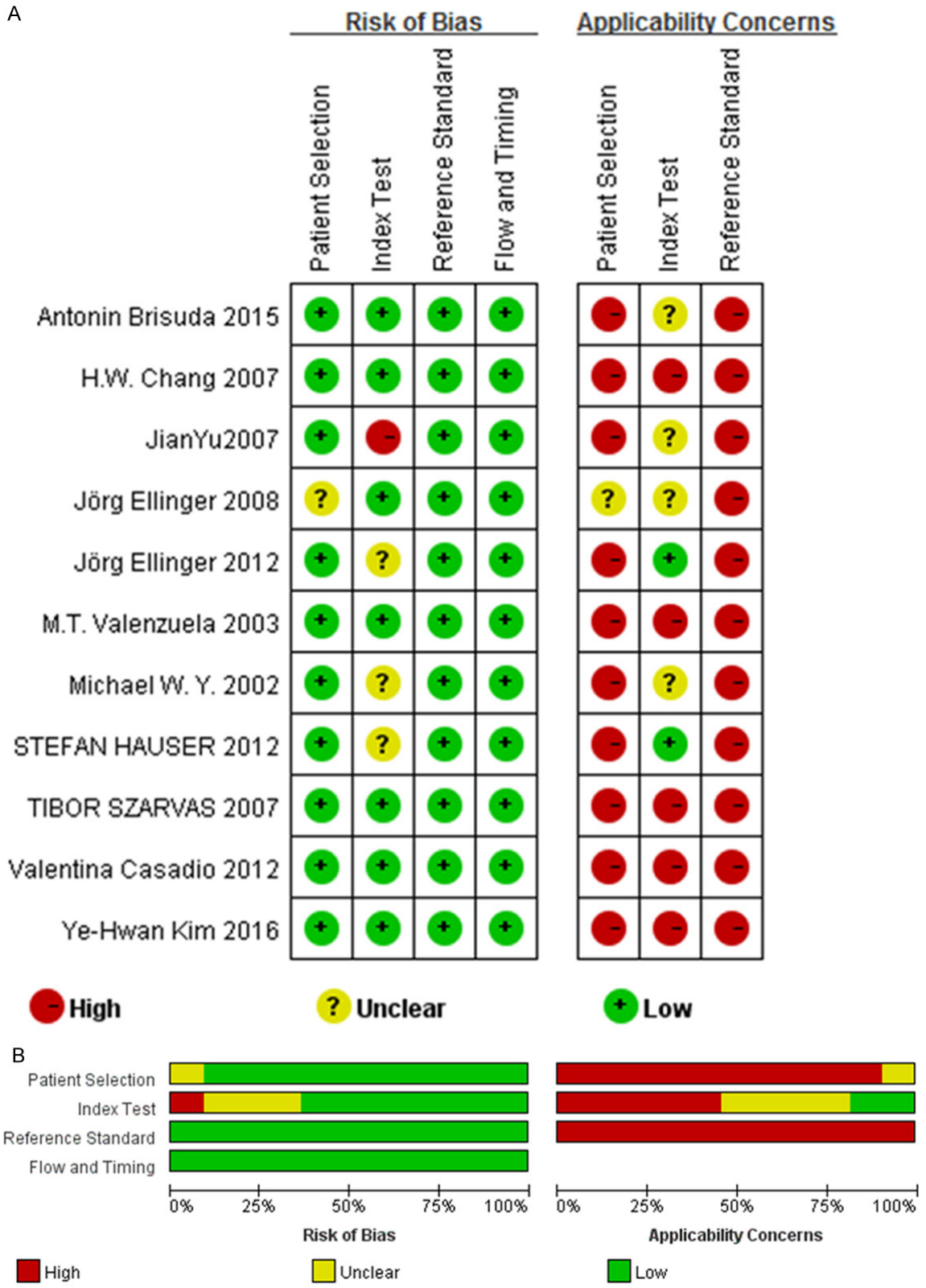

Figure 2. Quality assessments of included studies by using the QUADAS-2 tool. A. Risk of bias summary: review authors' judgments about each risk of bias item for each included study; B. Risk of bias graph: review authors' judgments about each item presented as percentages across all included studies.

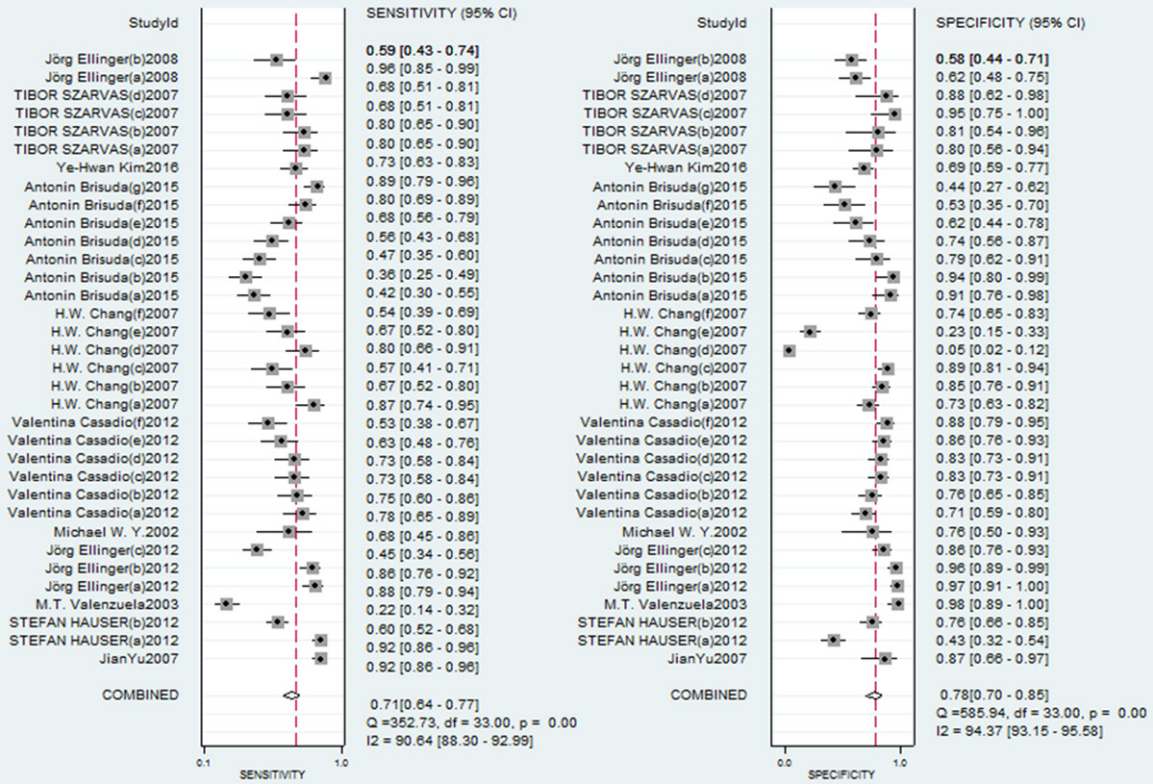

Figure 3. Forest plots of the sensitivity and specificity for cell-free DNA in the diagnosis of bladder cancer. A significant heterogeneity was shown for both sensitivity and specificity data among the included studies (I^2 = 90.64 and I^2 = 94.37, respectively).

rent meta-analysis met most of the QUADAS-2 criteria (items), suggesting that the overall quality of the included studies was moderately high.

Diagnostic accuracy

A sensitivity analysis was conducted to investigate the influence of any single study. No significant difference was found after each study was removed, suggesting that the conclusions are stable. The indicators applied to estimate diagnostic accuracy include sensitivity and specificity, PLR, NLR, and DOR. While a significant heterogeneity was found for both sensitivity and specificity data among the included studies (I^2 = 90.64 and I^2 = 94.37, respectively) (**Figure 3**), thus we calculate the pool estimates in this analysis by using the random effects model. Our results show that the overall sensitivity and specificity of cell-free DNA assays for distinguishing patients with bladder cancer from controls were 0.71 (95% CI = 0.64-0.77) and 0.78 (95% CI = 0.70-0.85), respectively. PLR was 3.3 (95% CI = 2.4-54.5), NLR was 0.37 (95% CI = 0.30-0.46) (**Figure 4**), and DOR was 9 (95% CI = 6-14).

Beyond the observed data, the satisfactory diagnostic performance of cell-free DNA assays for distinguishing cancer patients from healthy individuals was demonstrated by the SROC curve (**Figure 5**). This allowed the evaluation of the trade-off between sensitivity and specificity values in multiple studies. The AUC was 0.80 (95% CI 0.77-0.83), the LRT_I^2 statistic was 99% (95% CI 99-100), the LRT_Q (X^2) was 399.729 ($p < 0.001$), and the Spearman correlation coefficient was -0.061 ($p = 0.777$), indicating considerable heterogeneity among studies caused by non-threshold effects. **Figure 6** shows a Fagan's Nomogram for assessment of the clinical utility of the index test.

Subgroup analysis

We created subgroup analyses based on ethnicity, methylation and specimen type to look for sources of heterogeneity, as shown in **Figure 7**. The subgroup analysis results are shown in **Table 2**.

From the above table, the subgroup analysis based on ethnicity indicates that Europeans

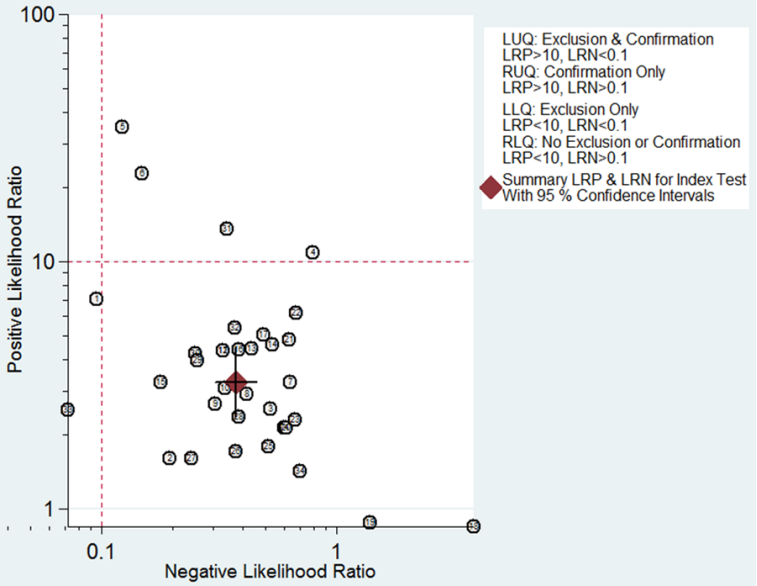

Figure 4. Forest plots of estimated positive likelihood ratio (PLR) and negative likelihood ratio (NLR) for cell-free DNA in the diagnosis of bladder cancer. The results show that the overall sensitivity and specificity of cell-free DNA assays for distinguishing patients with bladder cancer from controls.

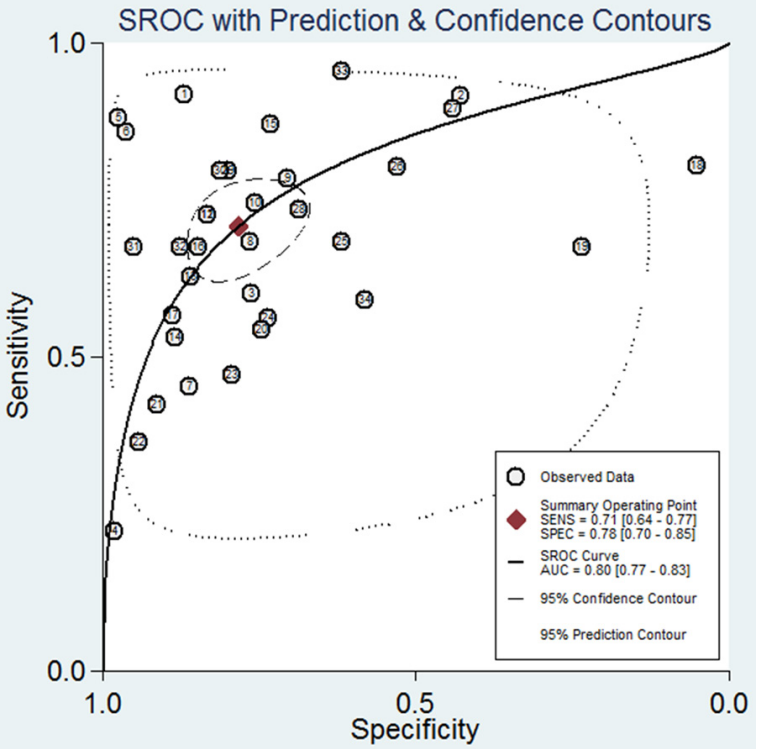

Figure 5. Summary receiver operating characteristic graph of included studies. It displayed a non-typical shoulder arm appearance, indicating the absence of the threshold effect.

and Asians had similar sensitivity values (0.70 versus 0.74, respectively), but Europeans had a higher specificity than that of Asians (0.82 versus 0.65). Furthermore, urine sediment was the most accurate source of specimen with a sensitivity of 0.77, and a specificity of 0.87, indicating that cell-free DNA in urine sediment had the highest diagnostic value for bladder cancer.

Heterogeneity and meta-regression analysis

As mentioned above, obvious heterogeneity from non-threshold effects was present in the selected studies. In order to find the source of the heterogeneity, we used meta-regression analysis to assess covariates used in these studies, including "ethnicity (Asian regions)", "specimen type (serum)", and "methylation". The meta-regression analyses are presented in **Figure 7**. The results reveal that the "ethnicity" covariate may produce major heterogeneity.

Publication bias

The Deeks' test was used to assess the potential publication bias of the included studies. A non-zero slope coefficient is suggestive of significant study bias when $p < 0.10$. The slope coefficient was associated with a p value of 0.97 (**Figure 8**), indicating the absence of significant publication bias in our meta-analysis.

Discussion

Bladder cancer is one of the most common male genitourinary tumours [47-50]. Patients with bladder would clearly benefit from early stage diagnosis, and up to now, cystoscopy remains the most sensitive method for detecting bladder cancer, despite several limitations: it is an invasive procedure;

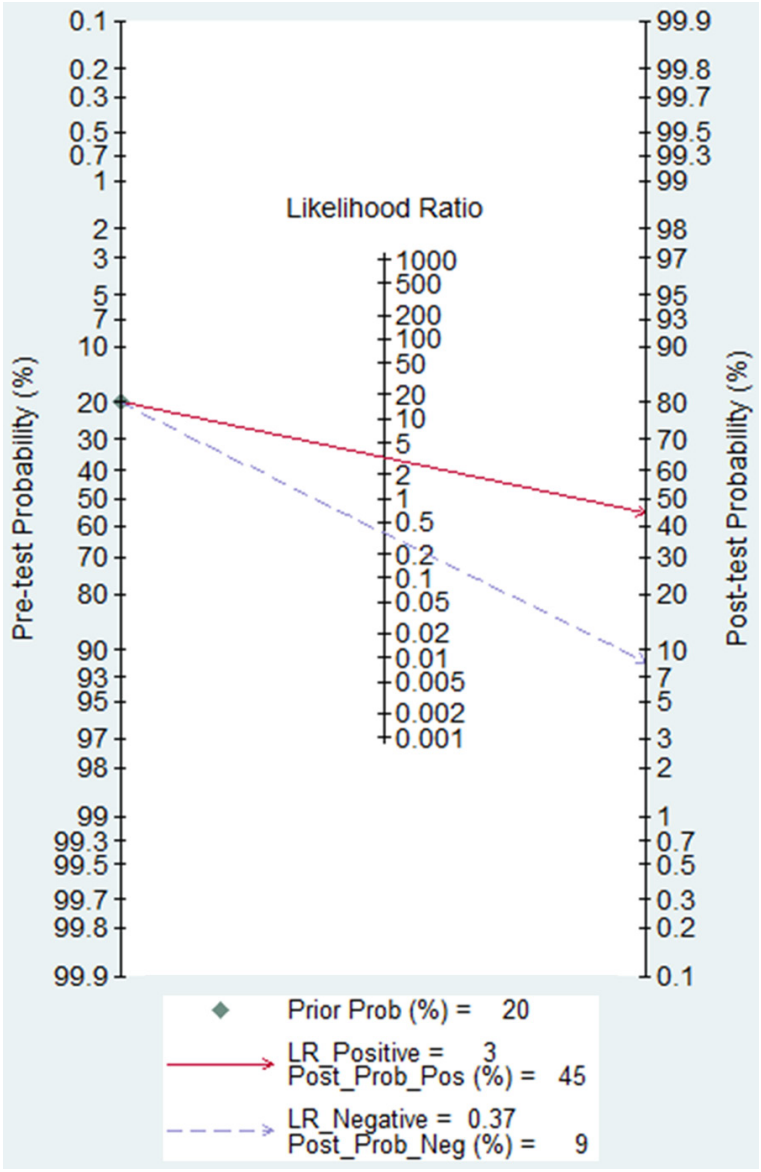

Figure 6. Fagan's nomogram for calculation of post-test probabilities. It suggests a stable value of cell-free DNA in the diagnosis of bladder cancer.

some patients find it uncomfortable, and it requires sedation or anaesthesia. Voided urinary cytology has also been used to detect bladder cancer, but it has a low sensitivity and a high variability. For these reasons, few of the current approaches have a satisfactory performance for clinical use [51, 52]. Thus, novel molecular markers that can help in early diagnosis are still urgently needed. Our work is the first meta-analysis to calculate the overall accuracy of cell-free DNA assays in the detection of bladder cancer.

In our study, pooled sensitivity and specificity, PLR, NLR, and DOR are used to estimate the diagnostic accuracy in the meta-analysis of the diagnostic test. The sensitivity and specificity of the cell-free DNA assays based on the included studies were 0.78 and the specificity value was also 0.78, indicating that a correct diagnosis could often be made through these assays. The SROC curve and the corresponding AUC may be used in the meta-analysis to estimate the overall diagnostic performance. The following evaluation criteria have been suggested: low (AUC: 0.5-0.7), moderate (AUC: 0.7-0.9) or high (AUC: 0.9-1.0) accuracy. Our study shows the AUC calculated for the SROC curves was 0.80, which indicated that the cell-free DNA assay appears to be modestly more accurate than either of these traditional markers, yet few studies have directly compared the diagnostic performance of cell-free DNA and other biomarkers.

To further evaluate diagnostic effectiveness, we also analysed the diagnostic odds ratio (DOR) [40], which is a single indicator of test accuracy. A value of DOR > 10 indicates good discriminatory test performance. In this meta-analysis, the DOR for cell-free DNA assays to discriminate bladder cancer cases from healthy and benign urological disease was 9.0. The DOR of urine sediment assays of cell-free DNA (22.0) was significantly higher than that of serum (16.0) or urine supernatant (6.0) assays. In established studies applying cell-free DNA for cancer diagnoses, the DOR of analyses in lung [53], ovarian [54], and hepatocellular cancer (HCC) [55] were 20.33, 26.05, and 16.35, respectively. This indicated a high value of cell-free DNA assays in the correct diagnosis of bladder cancer, with urine sediment methods being the most accurate.

Furthermore, the likelihood ratio (LR), including PLR and NLR, was also considered to measure

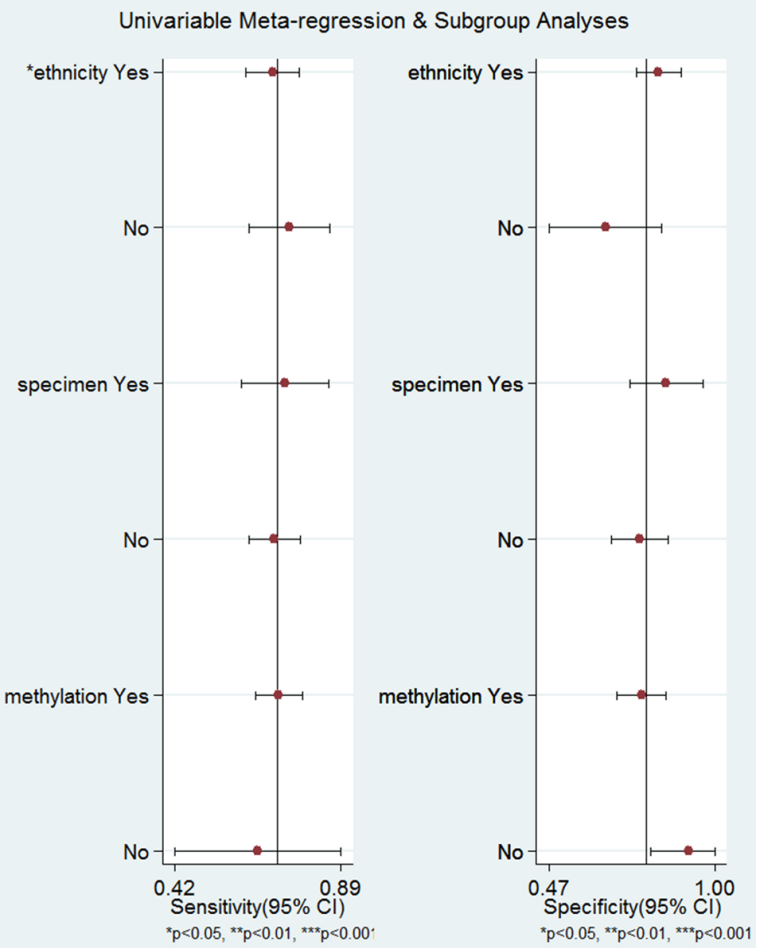

Figure 7. Univariable meta-regression and subgroup analysis. The results reveal that the "ethnicity" covariate may produce major heterogeneity.

the overall diagnostic accuracy [56]. The likelihood of differential diagnosis increased significantly with values of PLR > 10 and NLR < 0.1. In our study, a pooled PLR of 3.3 (95% CI = 2.4-54.5) suggests that patients with bladder cancer have an approximately 3.3-fold higher chance of testing positive using cell-free DNA. The NLR of analyses was found to be 0.37, implying that an error rate of approximately 37% would be present when the TN is determined in the negative test. These data suggest that cell-free DNA assay results should not be used alone as a biomarker to make a bladder cancer diagnosis. In other words, a negative cell-free DNA assay result should be interpreted with caution when cell-free DNA is used independently for the detection of bladder cancer. In addition, LR and post-test probabilities correlated with information provided by clinicians on the likelihood of a patient with a positive or negative result in the test, to actually exhibit bladder cancer. From the Fagan's Nomogram, when 20% was chosen as the pre-test probability, the post-test probability for a cell-free DNA positive result was 45% and a positive likelihood ratio of 3. Similarly, the post-test probability for a cell-free DNA negative result was reduced to 9% with the negative likelihood ratio of 0.37. These outcomes suggest a stable value of cell-free DNA in the diagnosis of bladder cancer.

When interpreting the results for meta-analysis, heterogeneity should be seriously considered as a potential obstacle. Heterogeneity can be derived from threshold and non-threshold effects. When a threshold effect is present, there is also an inverse correlation between sensitivity and specificity, which leads to a typical 'shoulder arm' of the ROC plane distribution. Spearman correlation analysis also suggested a strong positive correlation. In our study, the representation of the sensitivity against the specificity of each study shown in an ROC plane (**Figure 5**), displayed a non-typical shoulder arm appearance, indicating the absence of the threshold effect. Therefore, subgroup analyses were performed to evaluate the factors potentially contributing to the heterogeneity of sensitivity and specificity. The results showed that cfDNA has a higher diagnostic accuracy for bladder cancer in serum than in urine, and that cfDNA is more accurate for bladder cancer detection in Europe than in Asia, indicating that ethnicity and source of specimen may be the main causes of heterogeneity.

Although we made every effort to limit the bias during our meta-analysis, there remains several limitations to this study. First, the number of articles enrolled in our analysis was relatively small, which may have restricted our ability to

Table 2. Summary diagnostic performance of cell-free DNA for bladder cancer

Group	Subgroup	SEN (95% CI)	SPE (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
Overall		0.71 (0.64, 0.77)	0.78 (0.70, 0.85)	3.3 (2.4, 4.5)	0.37 (0.30, 0.46)	9 (6, 14)	0.80 (0.77, 0.83)
Ethnicity	Asian	0.74 (0.64, 0.82)	0.65 (0.40, 0.84)	2.1 (1.1, 4.2)	0.40 (0.25, 0.64)	5 (2, 16)	0.77 (0.73, 0.80)
	Europe	0.70 (0.61, 0.77)	0.82 (0.74, 0.87)	3.8 (2.8, 5.2)	0.37 (0.29, 0.47)	10 (7, 15)	0.83 (0.79, 0.86)
Sample types	Serum	0.74 (0.52, 0.88)	0.85 (0.66, 0.94)	4.9 (2.1, 11.5)	0.31 (0.16, 0.60)	16 (5, 51)	0.87 (0.83, 0.89)
	Urine sediment	0.77 (0.61, 0.88)	0.87 (0.77, 0.93)	5.0 (3.2, 10.7)	0.26 (0.15, 0.47)	22 (8, 60)	0.87 (0.84, 0.90)
	Urine supernatant	0.68 (0.62, 0.74)	0.74 (0.63, 0.82)	2.6 (1.9, 3.6)	0.43 (0.36, 0.51)	6 (4, 9)	0.75 (0.71, 0.79)

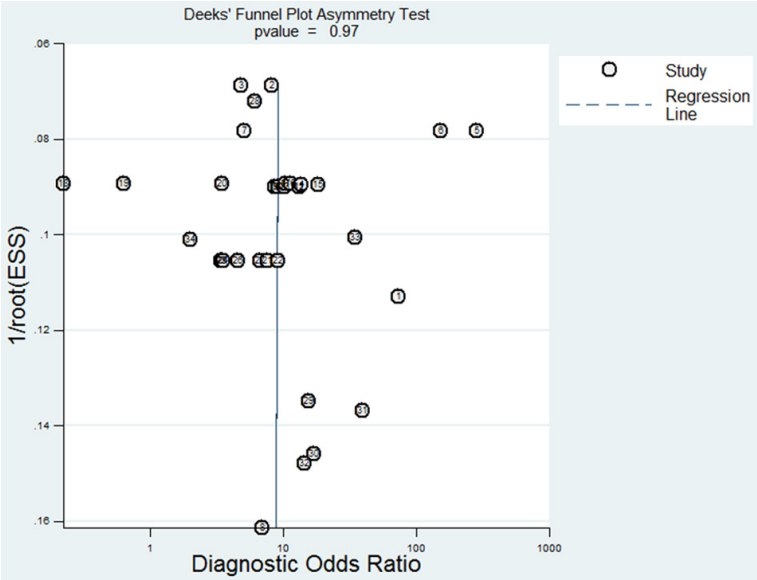

Figure 8. Deeks' funnel plot asymmetry test for publication. Funnel graphs for the assessment of potential publication bias in cell-free DNA assays to distinguish bladder cancer patients vs. controls. ○ = each study in the meta-analysis; center line = regression line.

evaluate the accuracy of cell-free DNA. Second, we could not determine all sources of heterogeneity, because the necessary data were not available from all the selected articles. Probable covariates included tumour size, TNM staging, metastasis, and specific diseases in the control groups. Third, we could not determine the ideal cut-off value for the cell-free DNA test, due to the diversity of cut-off values adopted across studies. Fourth, only English-language or Chinese-language studies were considered, which might have caused bias. Furthermore, prominent heterogeneity may have contaminated our data analysis.

In summary, despite the limitations mentioned above, our meta-analysis suggests that cell-free DNA in blood and urine is a potential diagnostic biomarker for bladder cancer. However, in order to validate this finding, more large-scale and comprehensive clinical studies should be performed before cell-free DNA is applied as a diagnostic marker for bladder cancer in routine clinical practice.

Conclusions

In conclusion, the results of this comprehensive meta-analysis, which is the first meta-analysis on the overall accuracy of cell-free DNA in blood and urine assays in bladder cancer screening, suggest that assaying cell-free DNA in blood and urine has the potential to become an effective adjuvant tool for the early diagnosis of bladder cancer. However, further research must address different strategies to make this approach more sensitive and specific, which is expected to become a key method in the diagnosis and treatment of early-stage bladder cancer in the future.

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Disclosure of conflict of interest

None.

Abbreviations

AUC, area under the curve; BPS, bisulphate pyrosequencing; cfDNA, cell-free DNA; BCa, bladder cancer; DOR, diagnostic odds ratio;

LRT, likelihood ratio test; NLR, negative likelihood ratio; PLR, positive likelihood ratio; RT-qPCR, real-time quantitative PCR; DOR, diagnostic odds ratio; SROC, summary receiver operating characteristic.

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