World Journal of Surgical Oncology

REVIEW

Open Access

The diagnostic accuracy of urine-derived exosomes for bladder cancer: a systematic review and meta-analysis

Chunyue Long[1†], Hongjin Shi[2†], Jinyu Li[1†], Lijian Chen[3], Mei Lv[1], Wenlin Tai[1], Haifeng Wang[2*] and Yiheng Xu[1*]

Abstract

Introduction Urine-derived exosomes could potentially be biomarkers for bladder cancer (BC) diagnosis. This study aimed to systematically evaluate the diagnostic worth of urine-derived exosomes in BC patients through a meta-analysis of diverse studies.

Methods A systematic search was carried out in PubMed, Web of Science, Embase, Cochrane, and CNKI databases to obtain the literature concerning the diagnosis of BC via urine-derived exosomes. A literature retrieval strategy was devised to pick articles and extract needed data from the literature. QUADS-2 was used to evaluate the quality of the included literatures, and the aggregated diagnostic effect was assessed by calculating the area under the aggregated SROC curve. All statistical analyses and plots were conducted with STATA 14.0 and RevMan5.3.

Results A total of 678 articles were retrieved by means of the search strategy of the online database. Through screening, 21 articles were obtained, involving 3348 participants and 77 studies. The meta-analysis of the results indicated that urinary exosomes had a combined sensitivity of 0.75, a specificity of 0.77, and a combined AUC of 0.83 for the diagnosis of BC, suggesting that urine-derived exosomes have a relatively satisfactory diagnostic effect in the detection of BC. Among the subgroups classified by biomarker, long non-coding RNAs (lncRNAs) had the highest comprehensive sensitivity (SEN = 0.78), and miRNAs had the highest comprehensive specificity (SPN = 0.81). In other subgroup analyses, the biomarker panel for multiple exosomes combined diagnosis demonstrated the best diagnostic efficacy, with a combined the area under the curve (AUC) of 0.87.

Conclusions As a novel biomarker, urine-derived exosomes have significant diagnostic prospects in the diagnosis of BC. Nevertheless, their application in clinical settings still demands a considerable number of clinical trials to confirm their clinical feasibility and practicability.

Keywords Urine derived exosomes, Bladder cancer, Diagnosis, Biomarker

†Chunyue Long, Hongjin Shi and Jinyu Li contributed equally to this work.

*Correspondence:
Haifeng Wang
wanghaifeng@kmmu.edu.cn
Yiheng Xu
1510574113@qq.com
Full list of author information is available at the end of the article

Introduction

Bladder cancer (BC) is among the most common malignancies worldwide. According to the 2022 Global Cancer Statistics, BC ranks tenth in incidence globally and is the second most prevalent and lethal male urological malignancy [1]. At initial diagnosis, roughly 75% of patients present with non-muscle-invasive bladder cancer (NMIBC), while the remainder have muscle-invasive bladder cancer (MIBC). Although the five-year survival rate for NMIBC is high, recurrence or metastasis often leads to poor prognoses [2]. Additionally, 10 to 15% of NMIBC patients progress to MIBC within five years, and the five-year survival rate for MIBC is only 5% [3, 4]. Therefore, effective early diagnostic methods are crucial to reduce mortality; however, efficient non-invasive early screening options for bladder disorders are currently lacking.

The gold standard for BC diagnosis is cystoscopy combined with biopathology. However, cystoscopic tissue biopsy is an invasive procedure whose efficacy depends heavily on the operator, leading to variability in sensitivity and specificity [5]. Furthermore, cystoscopy can cause adverse effects such as urinary tract infections, urethral injuries, and difficulties in urination [6, 7]. Since BC is typically asymptomatic in its early stages, cystoscopy is unsuitable for early screening [8, 9]. Currently, urine cytology is the most common non-invasive diagnostic tool for BC, but it has low sensitivity, particularly for low-grade tumors. A recent meta-analysis reported combined sensitivity and specificity for urinary cytology at 0.42 and 1.0, respectively [10]. Additionally, several urine-based biomarkers, such as bladder tumor antigen (BTA), nuclear matrix protein 22 (NMP22), and UroVysion fluorescence in situ hybridization (FISH), have been approved by the U.S. Food and Drug Administration (FDA) for clinical use. However, these biomarkers exhibit limited sensitivity and specificity, with false positives resulting from benign urinary tract conditions like cystitis, hyperplasia, and hematuria [11–13]. Hence, there is an urgent need for innovative non-invasive diagnostic biomarkers with high sensitivity and specificity.

In recent years, exosomes have emerged as promising biomarkers. These membrane-bound nanoparticles, secreted by cells into the extracellular environment, contain bioactive substances such as DNAs, mRNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs, lipids, and proteins. Exosomes, detectable in various body fluids including blood, urine, cerebrospinal fluid, and thoracoabdominal fluid, facilitate intercellular communication and regulate numerous physiological and pathological processes [14–16]. Research indicates that exosomes play a vital role in tumorigenesis, progression, and metastasis through the molecules they transport [17, 18]. The diagnostic value of these exosome-bound substances in tumors is gaining recognition. Studies have shown that BC cells can secrete exosomes into urine, reflecting the state of urothelial cells and serving as biomarkers for screening and monitoring BC patients [19]. Numerous studies have explored the accuracy of urine-derived exosomes for diagnosing BC, yet these studies often lack consistency and evidence-based verification. Therefore, this study aims to systematically review and conduct a meta-analysis to evaluate the accuracy of urine-derived exosomes as biomarkers for BC diagnosis.

Materials and methods

In accordance with the PRISMA guidelines, we carried out this study and formulated a systematic review and diagnostic test accuracy evaluation scheme (McInnes et al. [20]). Before publication, we registered a review of the system with PROSPERO, with the number CRD42024561296 (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42024561296).

Search strategy

To retrieve all relevant literature related to our research objectives, we utilized all keywords concerning "exosomes" and "bladder cancer" extracted from Emtree and Mesh databases. We conducted a comprehensive search of the Chinese and English literature published in PubMed, Web of Science, Embase, Cochrane, and China National Knowledge Infrastructure (CNKI) databases until April 1, 2024. To ensure the reliability of the search, two of our researchers, Long Chunyue and Shi Hongjin, independently conducted the literature search, while another researcher, Xu Yiheng, compared the search results for any differences. To guarantee no relevant articles were overlooked, we manually checked the references of each article and the results of the systematic screening of the online database. For the complete search strategies of PubMed, Web of Science, Embase, and Cochrane databases, refer to Table S1.

Inclusion and exclusion criteria

Choice of research:

Chunyue Long and Hongjin Shi independently evaluated all titles and abstracts to filter out references that failed to comply with the study's content. Subsequently, they reviewed the full results to determine compliance with the inclusion criteria. One investigator made the inclusion assessment, which was validated by the second. Whenever a disagreement emerged, it was resolved through discussion or negotiation with a third researcher.

Inclusion criteria:

The diagnostic value of urinary exosomes in patients with bladder cancer was summarized based on all related studies. ① subjects encompassed patients diagnosed with BC as verified by pathology reports; ② the study was obligated to incorporate both patients with bladder cancer and non-tumor controls; ③ the research ought to regard sensitivity and specificity as outcome indicators, entail the receiver operating characteristic (ROC) curve, and the data needed to be comprehensive. The amounts of true positive (TP), false positive (FP), true negative (TN), and false negative (FN) could be derived from the data.

Exclusion criteria:

①repeated literature; ②systematic reviews and meta-analysis, reviews, conference papers, case reports, animal experiments; ③ research content is not consistent; ④ the quality of the article is too low.

Data collection process

The subsequent information was collected and sorted for each article: the first author, the year of publication, the country, the ethnicity, the biomarkers for relevant identification, the exosome extraction approach, and the biomarker analysis method. Extract the relevant data of FP, FN, TP, and TN. If these data were absent in the study, calculations were made based on sensitivity and specificity. If needed, we reached out to the study authors for additional information. When there was a disagreement among the data collectors, advice was sought from the third author.

Literature quality evaluation

The authors utilized the Reference Quality Assessment tool (QUADS-2) from the Diagnostic Accuracy test in RerMan 5.3 (Nordic Cochrane Centre, Copenhagen, Denmark) to evaluate the risk of bias and clinical applicability of the literature. The bias assessment was categorized into four parts: Patient Selection, Index Test, Reference Standard, Flow and Timing. The clinical adaptability of the first three parts was appraised. Each item was assessed respectively with "yes", "no" and "unclear", and the disputed portion was discussed with the third researcher to reach a consensus.

Statistical treatment

The statistical analyses for this study were all implemented in the STATA 14.0 (STATA Corporation, College Station, TX, U.S.A.) statistical software. At first, relevant indicators including TP, FP, FN, and TN were extracted from every study, and sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) were calculated from these data. The I^2 statistic was utilized to discover the heterogeneity among the studies. If $I^2 < 50\%$ signified that the heterogeneity was not prominent, the fixed effects model was employed for meta-analysis; otherwise, the random effects model was adopted [21]. Employing the "midas" command in the model to assess the merged sensitivity, specificity, corresponding summary receiver operating characteristics (SROC) curve, PLR, NLR, and DOR, the area under the curve (AUC) represents the aggregated diagnostic value. Publication bias was evaluated by the Deeks funnel plot asymmetry test, with $P < 0.10$ denoting a significant difference [22].

Result

Literature search and study selection

A total of 678 articles were retrieved through the search strategy of online databases. Specifically, 107 were from PubMed, 267 from Web of Science, 216 from Embase, 2 from Cochrane, and 83 from CNKI. Additionally, 3 other records were identified through manual search. After employing EndNote, 418 duplicate papers were excluded. Subsequently, 126 reviews, systematic reviews, and conference papers were removed, thereby obtaining 292 papers for preliminary screening. After perusing the titles and abstracts, 255 articles that were inconsistent with this study were excluded, leaving 37 articles. After meticulously reading the full text of all 37 articles in accordance with our inclusion and exclusion criteria and eliminating those with inconsistent research content, poor quality, and the lack of corresponding data, this paper ultimately included 21 compliant literatures from 7 countries, namely China, Iran, South Korea, Japan, the United States, Turkey, and Egypt (see Table 1 and Table 2). The literature search process and research selection based on PRISMA guidelines are depicted in Fig.1.

Quality evaluation

The results of the quality assessment using the QUADAS-2 checklist are presented in Fig. 2 and Table S2. We discovered that the trials to be evaluated had the most significant impact on reducing the risk of quality bias in the articles. Additionally, the lack of clear explanations regarding case selection, process, and progress in some articles was notable. In the applicability section, case selection and tests to be evaluated need to be taken into account. Overall, most of the articles were low-risk literature, suggesting that the overall quality of the included studies was satisfactory.

Table 1 Bibliographic information of included primary studies

No	Country	Author	Year	Title
1	Iran	Abbastabar,M [23]	2020	Tumor-derived urinary exosomal long non-coding rnas as diagnostic biomarkers for bladder cancer
2	China	Bian,B [24]	2022	Urinary exosomal long non-coding RNAs as noninvasive biomarkers for diagnosis of bladder cancer by RNA sequencing
3	China	Chen,C [25]	2022	Urinary Exosomal Long Noncoding RNA TERC as a Noninvasive Diagnostic and Prognostic Biomarker for Bladder Urothelial Carcinoma
4	Egypt	El-Shal,A.S [26]	2021	Urinary exosomal microRNA-96-5p and microRNA-183-5p expression as potential biomarkers of bladder cancer
5	Turkey	Güllü Amuran,G [27]	2020	Urinary micro-RNA expressions and protein concentrations may differentiate bladder cancer patients from healthy controls
6	China	Huang,H [28]	2021	Combination of Urine Exosomal mRNAs and lncRNAs as Novel Diagnostic Biomarkers for Bladder Cancer
7	Korea	Lee,J.S [29]	2022	Alpha-2-macroglobulin as a novel diagnostic biomarker for human bladder cancer in urinary extracellular vesicles
8	China	Lin, H [30]	2021	Urinary Exosomal miRNAs as biomarkers of bladder Cancer and experimental verification of mechanism of miR-93-5p in bladder Cancer
9	China	Liu,C [31]	2023	The value of urinary exosomal lncRNA SNHG16 as a diagnostic biomarker for bladder cancer
10	Japan	Matsuzaki,K [32]	2017	MiR-21-5p in urinary extracellular vesicles is a novel biomarker of urothelial carcinoma
11	China	Qiu,T [33]	2022	Comparative evaluation of long non-coding RNA-based biomarkers in the urinary sediment and urinary exosomes for non-invasive diagnosis of bladder cancer
12	Iran	Sarfi,M [34]	2021	Increased expression of urinary exosomal LnCRNA TUG-1 in early bladder cancer
13	China	Wen,J [35]	2021	Urinary Exosomal CA9 mRNA as a Novel Liquid Biopsy for Molecular Diagnosis of Bladder Cancer
14	China	Xu,Y [36]	2021	A potential panel of five mRNAs in urinary extracellular vesicles for the detection of bladder cancer
15	Iran	Yazarlou,F [37]	2018	Urinary exosomal expression of long non-coding RNAs as diagnostic marker in bladder cancer
16	America	De Long,J [38]	2015	A non-invasive miRNA based assay to detect bladder cancer in cell-free urine
17	China	Gao,Y [39]	2023	Exosomal Long Non-Coding Ribonucleic Acid Ribonuclease Component of Mitochondrial Ribonucleic Acid Processing Endoribonuclease Is Defined as a Potential Non-Invasive Diagnostic Biomarker for Bladder Cancer and Facilitates Tumorigenesis via the miR-206/G6PD Axis
18	China	Zhan,Y [40]	2018	Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer
19	China	Wang X [41]	2024	Urinary exosomal mRNA as a biomarker for the diagnosis of bladder cancer
20	Japan	Murakami T [42]	2024	Cross-sectional and longitudinal analyses of urinary extracellular vesicle mRNA markers in urothelial bladder cancer patients
21	China	Yang FK [43]	2024	The value of urinary exosomal microRNA-21 in the early diagnosis and prognosis of bladder cancer

Meta-analysis of diagnostic accuracy

Table 3 show the diagnostic value of different biomarkers in the included literature, including extraction data for TP, FP, FN, TN, SEN, SPE, PLR, NLR, and DOR.

Included literature and features

To explore the diagnostic accuracy of exosomes in patients with BC, we conducted a meta-analysis based on 77 data from 21 articles, encompassing a total of 3348 participants (including 1833 BC patients and 1515 non-tumor controls). There were 58 studies involving Asian participants and 19 studies involving Caucasian participants. All the studies pertained to exosomes derived from urine, among which 40 studies investigated exosome lncRNA, 16 studies investigated exosome miRNA, 19 studies investigated exosome mRNA, 1 study investigated protein, and another study explored the combined diagnostic efficacy of lncRNA and mRNA. Two methods, namely ultracentrifugation and exosome isolation kit, were employed to extract total exosomes

Table 2 The workflow data from the included studies

No	Study	race	Exosomes Source	Exosome Isolation	Biomarker Analysis
1	Abbastabar, M. [23]	Caucasian	urine	Exosome Isolation Kit	qRT-PCR
2	Bian, B. [24]	Asian	urine	Exosome Isolation Kit	qRT-PCR
3	Chen, C. [25]	Asian	urine	Ultracentrifugation	qRT-PCR
4	El-Shal,A.S. [26]	Caucasian	urine	Exosome Isolation Kit	qRT-PCR
5	Güllü Amuran,G. [27]	Caucasian	urine	Ultracentrifugation	qRT-PCR
6	Huang,H. [28]	Asian	urine	Exosome Isolation Kit	qRT-PCR
7	Lee,J.S. [29]	Asian	urine	Ultracentrifugation/Exosome Isolation Kit	ELISA
8	Lin,H [30]	Asian	urine	Ultracentrifugation	qRT-PCR
9	Liu,C. [31]	Asian	urine	Ultracentrifugation	qRT-PCR
10	Matsuzaki,K. [32]	Asian	urine	Ultracentrifugation	Unclear
11	Qiu,T. [33]	Asian	urine	Ultracentrifugation	qRT-PCR
12	Sarfi,M. [34]	Caucasian	urine	Exosome Isolation Kit	qRT-PCR
13	Wen,J. [35]	Asian	urine	Ultracentrifugation	qRT-PCR
14	Xu,Y. [36]	Asian	urine	Ultracentrifugation	qRT-PCR
15	Yazarlou,F. [37]	Caucasian	urine	Exosome Isolation Kit	qRT-PCR
16	De Long,J. [38]	Caucasian	urine	Ultracentrifugation	qRT-PCR
17	Gao,Y. [39]	Asian	urine	Exosome Isolation Kit	qRT-PCR
18	Zhan,Y. [40]	Asian	urine	Ultracentrifugation	qRT-PCR
19	Wang X [41]	Asian	urine	Ultracentrifugation	qRT-PCR
20	Murakami T [42]	Asian	urine	Exosome Isolation Kit	qRT-PCR
21	Yang FK [43]	Asian	urine	Ultracentrifugation	qRT-PCR

qRT-PCR quantitative reverse transcriptase PCR, *ELISA* Enzyme-linked immunosorbent assay

from urine. There were 39 studies involving ultracentrifugation and 37 studies involving the exosome isolation kit. All the included studies utilized quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) to determine the expression level of exosome ncRNAs, and 1 study used enzyme-linked immunosorbent assay (ELISA) to determine the protein expression level.

Summary meta-analysis

Figure 3 presents a forest map of the sensitivity and specificity of exosomes employed to distinguish between BC patients and non-tumor controls. In the forest plot, individual studies are represented as separate lines, and the point estimates of sensitivity and specificity along with their confidence intervals are plotted. The combined sensitivity (I^2 = 88.05 (85.92—90.18)) and specificity (I^2 = 83.42 (80.17—86.67)) of the heterogeneity test were calculated. A high I2 value indicates significant heterogeneity among the studies. It can be observed from the map that the wide range and substantial variation in the point estimates across studies suggest the presence of heterogeneity. To account for this heterogeneity, the random effect size model was utilized for meta-analysis. This model assumes that the true effect sizes vary among studies and incorporates this variation in the analysis.

The data from the 77 studies are as follows: The sensitivity was 0.75 (95% CI = 0.71—0.78, Fig. 3), the specificity was 0.77 (95% CI = 0.74—0.80, Fig. 3), and the AUC was 0.83 (95% CI = 0.79—0.86, Fig. 4a). The PLR was 3.28 (95% CI = 2.89—3.71, Figure S1), the NLR was 0.33 (95% CI = 0.29—0.37, Figure S1), and the DOR was 10.04 (95% CI = 8.3—12.15, Figure S2). The results imply that urinary exosomes possess superior diagnostic value for BC.

Subgroup analysis

Next, we classified all the studies into meta-analyses in accordance with the type of RNA (lncRNA, miRNA, and mRNA), the total size of the study sample, ethnicity, the method of total exosome extraction, and whether a combined diagnosis was implemented.

Figures S3—S5 present forest maps summarizing the sensitivity and specificity of exosome lncRNAs (lncRNAs, Figure S3), exosome miRNAs (miRNAs, Figure S4), and exosome mRNAs (mRNAs, Figure S5) as biomarkers for differentiating BC patients from non-tumor controls, along with corresponding 95% confidence intervals. The comprehensive sensitivity of exosome lncRNAs was the highest at 0.78 (95% CI = 0.74—0.82). The combined sensitivities of exosome mRNAs and exosome

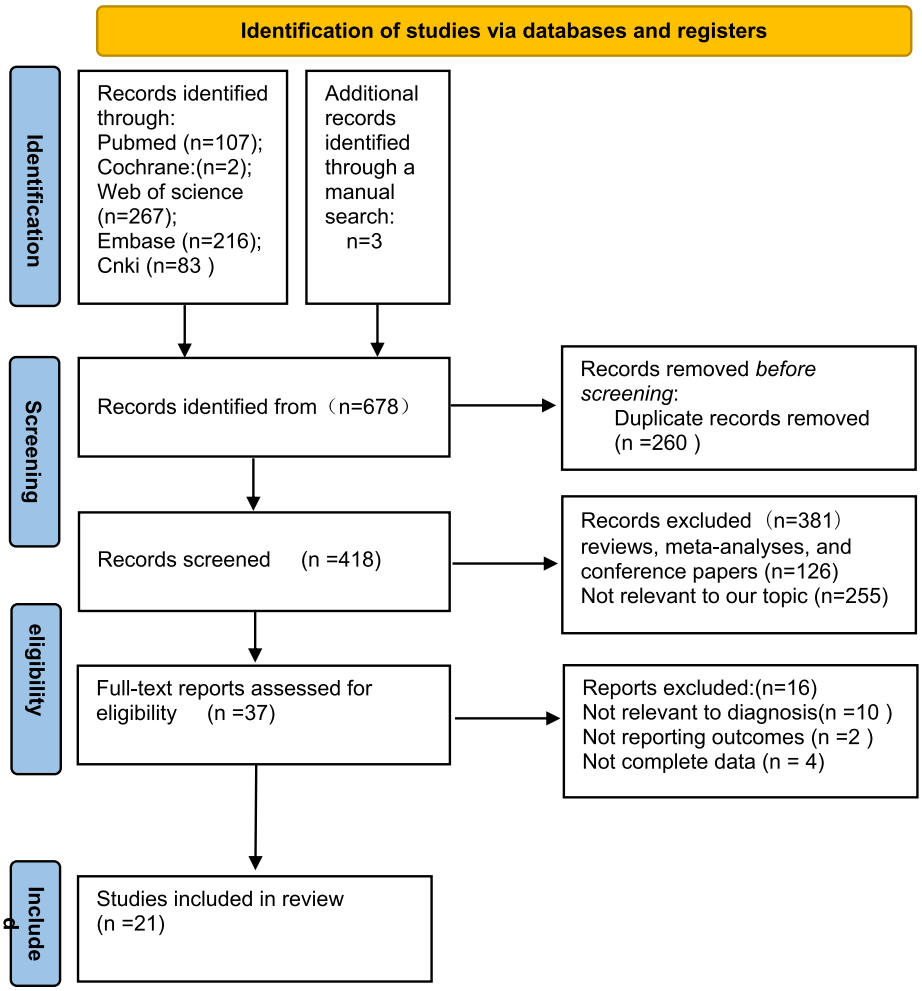

Fig. 1 The literature searches and study selection process for systematic review according to PRISMA

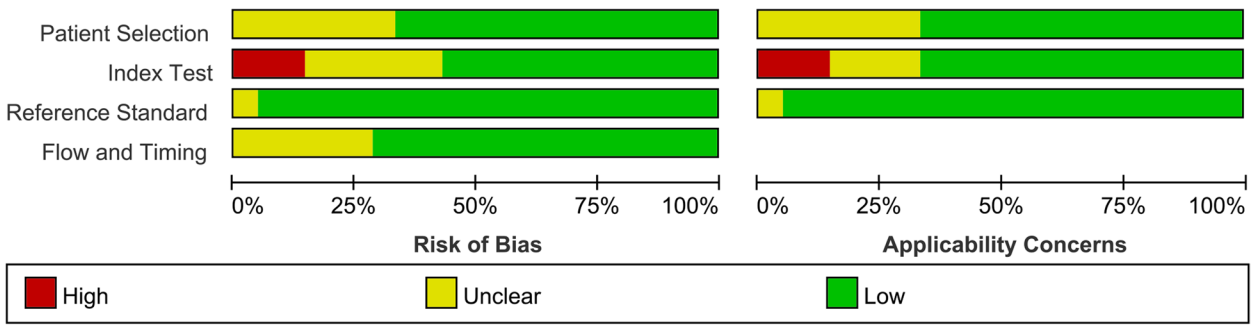

Fig. 2 Quality assessment of the studies included in meta-analysis with the QUADAS-2 checklist

miRNAs were 0.69 (95% CI = 0.63—0.75) and 0.69 (95% CI = 0.60—0.78), respectively. Exosome miRNAs had the highest combined specificity of 0.81 (95% CI = 0.74—0.86), while the specificities of exosome lncRNAs and exosome mRNAs were 0.75 (95% CI = 0.71—0.79) and 0.79 (95% CI = 0.73—0.84), respectively. Figures 4a - d depict the SROC curves of the diagnostic accuracy of biomarkers in the three groups. It can be observed that the area under the curve (AUC) of the panel biomarkers of exosome lncRNAs and exosome miRNAs is 0.83 (95%

Table 3 Data related to study samples from 77 included study in meta-analysis

First author	Biomarker name	Type	Tp	Fp	Tn	Fn	Total	Studied populations	Sen(%)	Spe(%)	PLR	NLR	DOR
Abbastabar, M. [23]	ANRIL	lncRNA	14	1	9	16	40	BC ($n=30$) / HC($n=10$)	0.47	0.90	4.67	0.59	7.88
	PCAT-1	lncRNA	13	1	9	17	40	BC ($n=30$) / HC($n=10$)	0.43	0.90	4.33	0.63	6.88
Bian, B. [24]	MKLN1-AS/S1	lncRNA	46	24	26	4	100	BC ($n=50$) / HC($n=50$)	0.92	0.52	1.92	0.15	12.46
	MKLN1-AS/S2	lncRNA	34	14	29	9	86	BC ($n=43$) / HC($n=43$)	0.79	0.67	2.39	0.31	7.64
	TALAM1/S1	lncRNA	48	26	24	2	100	BC ($n=50$) / HC($n=50$)	0.96	0.48	1.85	0.08	22.15
	TALAM1/S2	lncRNA	39	19	24	4	86	BC ($n=43$) / HC($n=43$)	0.91	0.56	2.07	0.16	12.87
	TTN-AS1/S1	lncRNA	47	24	26	3	100	BC ($n=50$) / HC($n=50$)	0.94	0.52	1.96	0.12	16.97
	TTN-AS1/S2	lncRNA	33	10	33	10	86	BC ($n=43$) / HC($n=43$)	0.77	0.77	3.31	0.30	11.05
	UCA1/S1	lncRNA	46	22	28	4	100	BC ($n=50$) / HC($n=50$)	0.92	0.56	2.09	0.14	14.64
	UCA1/S2	lncRNA	39	4	39	4	86	BC ($n=43$) / HC($n=43$)	0.91	0.91	9.75	0.10	95.11
Chen, C. [25]	TERC	lncRNA	70	14	49	19	152	BC ($n=89$) / HC($n=63$)	0.79	0.78	3.54	0.27	12.89
El-Shal, A. S [26]	miR-96-5p	miRNA	41	4	45	10	100	BC ($n=51$) / nBC($n=49$)	0.80	0.92	9.85	0.21	46.13
	miR-183-5p	miRNA	40	9	40	11	100	BC ($n=51$) / nBC($n=49$)	0.78	0.82	4.27	0.26	16.16
	miR-96-5p,miR-183-5p	miRNA	45	6	43	6	100	BC ($n=51$) / nBC($n=49$)	0.88	0.88	7.21	0.14	52.78
Güllü Amuran, G. [27]	miRNA136–3p	miRNA	26	8	26	33	93	BC ($n=59$) / HC($n=34$)	0.44	0.76	1.87	0.73	2.56
	miRNA139–5p	miRNA	41	17	17	18	93	BC ($n=59$) / HC($n=34$)	0.69	0.50	1.39	0.61	2.28
	miRNA19b1–5p	miRNA	33	18	16	26	93	BC ($n=59$) / HC($n=34$)	0.56	0.47	1.06	0.94	1.13
Huang, H [28]	KLHDC7B	mRNA	55	9	71	25	160	BC ($n=80$) / HC($n=80$)	0.69	0.89	6.11	0.35	17.36
	CASP14	mRNA	62	24	56	18	160	BC ($n=80$) / HC($n=80$)	0.78	0.70	2.58	0.32	8.04
	PRSS1	mRNA	62	20	60	18	160	BC ($n=80$) / HC($n=80$)	0.78	0.75	3.10	0.30	10.33
	KLHDC7B,CASP14,PRSS1	mRNA	58	4	76	22	160	BC ($n=80$) / HC($n=80$)	0.72	0.95	14.40	0.29	48.86
	MIR205HG	lncRNA	62	14	66	18	160	BC ($n=80$) / HC($n=80$)	0.78	0.83	4.43	0.27	16.24
	GAS5	lncRNA	63	32	48	17	160	BC ($n=80$) / HC($n=80$)	0.79	0.60	1.97	0.35	5.56
	MIR205HG,GAS5	lncRNA	54	10	70	26	160	BC ($n=80$) / HC($n=80$)	0.67	0.87	5.15	0.38	13.59
	3 mRNAs&2 lncRNAs	RNAs	71	13	67	9	160	BC ($n=80$) / HC($n=80$)	0.89	0.83	5.30	0.14	38.39
Lee,J.S. [29]	uEV a2M	protein	56	15	8	4	83	BC ($n=60$) / nBC($n=23$)	0.93	0.35	1.43	0.19	7.47
Lin,H [30]	miR-93-5p	miRNA	39	14	37	14	104	BC ($n=53$) / HC($n=51$)	0.74	0.73	2.68	0.36	7.36
	miR-516a-5p	miRNA	48	5	46	5	104	BC ($n=53$) / HC($n=51$)	0.91	0.90	9.24	0.10	88.32
	miR-93-5p&miR-516a-5p	miRNA	45	9	42	8	104	BC ($n=53$) / HC($n=51$)	0.85	0.82	4.72	0.18	25.81
Liu,C. [31]	SNHG16	lncRNA	26	7	35	16	84	BC ($n=42$) / HC($n=42$)	0.62	0.83	3.71	0.46	8.13
Matsuzaki,K. [32]	miR-21-5p	miRNA	27	1	23	9	60	BC ($n=36$) / nBC($n=24$)	0.75	0.96	18.00	0.26	69.00

Table 3 (continued)

First author	Biomarker name	Type	Tp	Fp	Tn	Fn	Total	Studied populations	Sen(%)	Spe(%)	PLR	NLR	DOR
Qiu,T. [33]	RMRP	lncRNA	13	4	16	9	42	BC ($n=22$) / HC($n=20$)	0.59	0.80	2.95	0.51	5.78
	UCA1	lncRNA	15	3	17	7	42	BC ($n=22$) / HC($n=20$)	0.68	0.85	4.55	0.37	12.14
	MALAT1	lncRNA	19	6	14	3	42	BC ($n=22$) / HC($n=20$)	0.86	0.70	2.88	0.19	14.78
	RMRP,UCA1,MALAT1/S1	lncRNA	17	5	15	5	42	BC ($n=22$) / HC($n=20$)	0.75	0.77	3.26	0.32	10.04
	RMRP,UCA1,MALAT1/S2	lncRNA	26	4	19	7	56	BC ($n=33$) / HC($n=23$)	0.79	0.83	4.64	0.26	18.15
	RMRP,UCA1,MALAT1/S3	lncRNA	44	8	35	11	98	BC ($n=55$) / HC($n=43$)	0.80	0.81	4.30	0.25	17.51
Sarfi,M. [34]	TUG-1	lncRNA	23	2	8	7	40	BC ($n=30$) / HC($n=10$)	0.77	0.80	3.83	0.29	13.14
Wen,J. [35]	CA9	mRNA	143	15	75	25	258	BC ($n=168$) / nBC($n=90$)	0.85	0.83	5.11	0.18	28.60
Xu,Y. [36]	MYBL2,TK1,UBE2C,KRT7 &S100A2/S1	mRNA	112	111	131	14	368	BC ($n=126$) / nBC($n=242$)	0.89	0.54	1.94	0.21	9.44
	MYBL2,TK1,UBE2C,KRT7 &S100A2/S2	mRNA	51	49	50	4	154	BC ($n=55$) / nBC($n=99$)	0.93	0.51	1.87	0.14	13.01
Yazarlou,F. [37]	UCA1-201/S1	lncRNA	45	0	24	14	83	BC ($n=59$) / HC($n=24$)	0.75	1.00	/	0.25	/
	UCA1-203/S1	lncRNA	42	6	18	17	83	BC ($n=59$) / HC($n=24$)	0.72	0.75	2.90	0.37	7.87
	MALAT1/S1	lncRNA	38	4	20	21	83	BC ($n=59$) / HC($n=24$)	0.64	0.83	3.82	0.43	8.79
	LINC00355	lncRNA	40	5	19	19	83	BC ($n=59$) / HC($n=24$)	0.68	0.79	3.27	0.40	8.09
	UCA1-201,UCA1-203,MALAT1 &LINC00355	lncRNA	54	2	22	5	83	BC ($n=59$) / HC($n=24$)	0.92	0.92	10.98	0.09	118.80
	UCA1-201/S2	lncRNA	51	22	27	8	108	BC ($n=59$) / nBC($n=49$)	0.86	0.55	1.91	0.25	7.51
	UCA1-203/S2	lncRNA	44	23	26	15	108	BC ($n=59$) / nBC($n=49$)	0.74	0.53	1.58	0.50	3.18
	MALAT1/S2	lncRNA	37	15	34	22	108	BC ($n=59$) / nBC($n=49$)	0.62	0.69	2.03	0.55	3.70
	UCA1-201,UCA1-203 &MALAT1	lncRNA	56	23	26	3	108	BC ($n=59$) / nBC($n=49$)	0.95	0.53	2.02	0.09	21.43
De Long,J. [38]	miR-26a,miR-93,miR-191 &miR-940	miRNA	60	7	38	25	130	BC ($n=85$) / nBC($n=45$)	0.71	0.84	4.54	0.35	13.03
Gao,Y. [39]	RMRP	lncRNA	86	58	76	13	233	BC ($n=99$) / HC($n=134$)	0.87	0.57	2.01	0.23	8.67
Zhan,Y. [40]	MALAT1/S1	lncRNA	75	16	88	29	208	BC ($n=104$) / HC($n=104$)	0.72	0.85	4.69	0.33	14.22
	PCAT-1/S1	lncRNA	75	19	85	29	208	BC ($n=104$) / HC($n=104$)	0.72	0.82	3.95	0.34	11.57
	SPRY4-IT1/S1	lncRNA	67	26	78	37	208	BC ($n=104$) / HC($n=104$)	0.64	0.75	2.58	0.47	5.43
	MALAT1,PCAT-1, SPRY4-IT1/S1	lncRNA	73	15	89	31	208	BC ($n=104$) / HC($n=104$)	0.70	0.86	4.86	0.35	13.87
	MALAT1/S2	lncRNA	63	26	54	17	160	BC ($n=80$) / HC($n=80$)	0.79	0.68	2.42	0.31	7.70
	PCAT-1/S2	lncRNA	57	16	64	23	160	BC ($n=80$) / HC($n=80$)	0.71	0.80	3.56	0.36	9.91
	SPRY4-IT1/S2	lncRNA	70	28	52	10	160	BC ($n=80$) / HC($n=80$)	0.88	0.65	2.50	0.19	13.00
	MALAT1,PCAT-1,SPRY4-IT1/S2	lncRNA	50	12	68	30	160	BC ($n=80$) / HC($n=80$)	0.63	0.85	4.20	0.44	9.65

Table 3 (continued)

First author	Biomarker name	Type	Tp	Fp	Tn	Fn	Total	Studied populations	Sen(%)	Spe(%)	PLR	NLR	DOR
Wang X [41]	TMEFF1	mRNA	25	4	36	35	100	BC ($n=60$) / HC($n=40$)	0.42	0.90	4.20	0.64	6.52
	ACBD7	mRNA	28	2	38	32	100	BC ($n=60$) / HC($n=40$)	0.47	0.96	11.75	0.55	21.28
	SDPR3	mRNA	38	8	32	22	100	BC ($n=60$) / HC($n=40$)	0.64	0.81	3.37	0.44	7.58
	TMEFF1,ACBD7&SDPR3	mRNA	40	10	30	20	100	BC ($n=60$) / HC($n=40$)	0.67	0.76	2.79	0.43	6.43
	TMEFF1&SDPR3	mRNA	39	7	33	21	100	BC ($n=60$) / HC($n=40$)	0.65	0.82	3.61	0.43	8.46
	TMEFF1&ACBD7	mRNA	43	6	34	17	100	BC ($n=60$) / HC($n=40$)	0.71	0.85	4.73	0.34	13.87
	ACBD7&SDPR3	mRNA	40	10	30	20	100	BC ($n=60$) / HC($n=40$)	0.67	0.76	2.79	0.43	6.43
Murakami T [42]	*MDK*	mRNA	149	9	33	87	278	BC ($n=236$) / nBC($n=42$)	0.63	0.79	3.00	0.47	6.41
	KRT17	mRNA	146	8	34	90	278	BC ($n=236$) / nBC($n=42$)	0.62	0.81	3.26	0.47	6.96
	CXCR223	mRNA	125	8	34	111	278	BC ($n=236$) / nBC($n=42$)	0.53	0.81	2.79	0.58	4.81
	GPRC5A	mRNA	144	13	29	92	278	BC ($n=236$) / nBC($n=42$)	0.61	0.69	1.97	0.57	3.48
	SLC2A111	mRNA	165	20	22	71	278	BC ($n=236$) / nBC($n=42$)	0.70	0.52	1.46	0.58	2.53
Yang FK [43]	miR-146a-5p	miRNA	42	7	109	74	232	BC ($n=116$) / HC($n=116$)	0.36	0.94	6.00	0.68	8.81
	miR-93-5p	miRNA	58	34	82	58	232	BC ($n=116$) / HC($n=116$)	0.50	0.71	1.72	0.70	2.45
	miR-663b	miRNA	36	17	99	80	232	BC ($n=116$) / HC($n=116$)	0.31	0.85	2.07	0.81	2.55
	miR-21	miRNA	88	27	89	28	232	BC ($n=116$) / HC($n=116$)	0.76	0.77	3.30	0.31	10.60
	miR-4454	miRNA	89	37	79	27	232	BC ($n=116$) / HC($n=116$)	0.77	0.68	2.41	0.34	7.11

TP true positive, *TN* true negative, *FP* false positive, *FN* false negative, *Sen* Sensitivity, *Spe* Specificity, *NLR* Negative likelihood ratio, *PLR* Positive likelihood ratio, *DOR* Diagnostic odds ratio, *BC* Bladder cancer, *HC* Healthy Control, *nBC* Non-bladder cancer (Patients with benign bladder lesions and healthy Control)

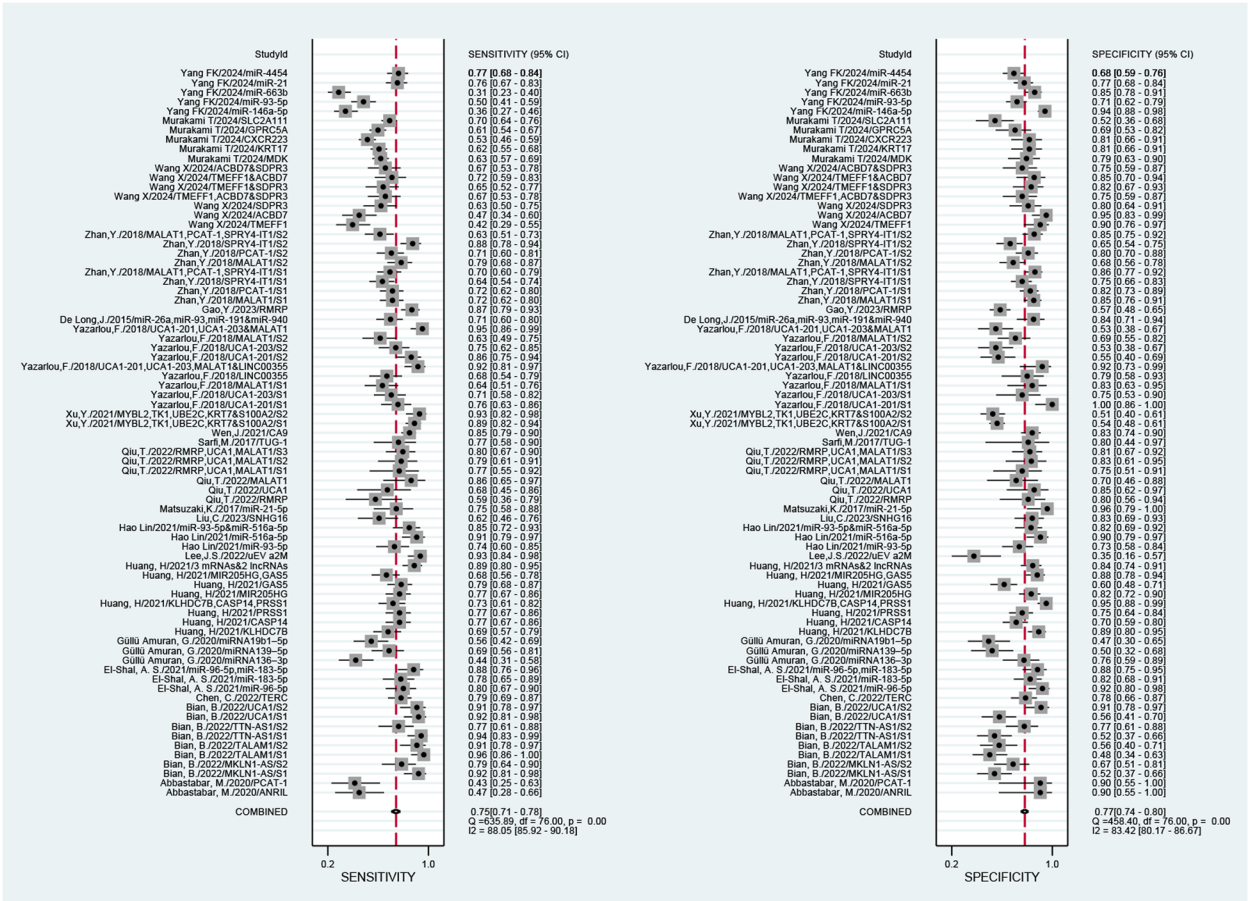

Fig. 3 The Forest plot shows the sensitivity and specificity of total exosomes in differentiating BC patients from non-tumor controls

CI = 0.80—0.86, 95% CI = 0.79—0.86). The AUC of exosome mRNAs was slightly lower than the former two, at 0.81 (95% CI = 0.77—0.84).

The summary results of the diagnostic effects of different subgroups are presented in Table 4. It can be observed that the combined AUC for exosome detection of the yellow race was 0.83 (95%CI = 0.79—0.86), and the diagnostic efficacy was superior to that of the white race (AUC = 0.82 (95%CI = 0.78—0.85)). Based on the total sample size of the study, the AUC of the group with a total sample size > 100 (AUC = 0.82 (95%CI = 0.78—0.85)) was marginally smaller than that of the group with a total sample size ≤ 100 (AUC = 0.84 (95%CI = 0.80—0.87)). Regarding the methods utilized for extracting total exosomes from urine, the combined AUC of the exosome extraction group employing the kit (AUC = 0.84 (95%CI = 0.81—0.87)) was superior to that of the exosome extraction group using ultracentrifugation (AUC = 0.82 (95%CI = 0.78—0.85)). Additionally, the combined AUC of biomarker panels with multiple exosomes for combined diagnosis was 0.87 (95% CI = 0.84—0.90), significantly surpassing the combined AUC of single exosomes (AUC = 0.81 (95% CI = 0.78—0.84))

Publication bias

The Deeks funnel plot was utilized to assess the presence of publication bias within this study. The results demonstrated that the graph was largely symmetrical, and no publication bias was identified in this meta-analysis ($P = 0.19$, Fig. 5a). In the subgroup analysis, Deeks funnel plots for various exosomes RNAs were constructed. The findings indicated that the graphs of the exosomes lncRNA ($P = 0.32$, Fig. 5b), exosomes miRNA ($P = 0.15$, Fig. 5c), and exosomes mRNA ($P = 0.28$, Fig. 5d) groups were also essentially symmetrical, with no publication bias being detected.

Discussion

BC is a highly aggressive malignancy of the genitourinary system. In the United States, 2023 estimates indicate approximately 16,710 deaths from BC, with the death rate among men being three times higher than among women

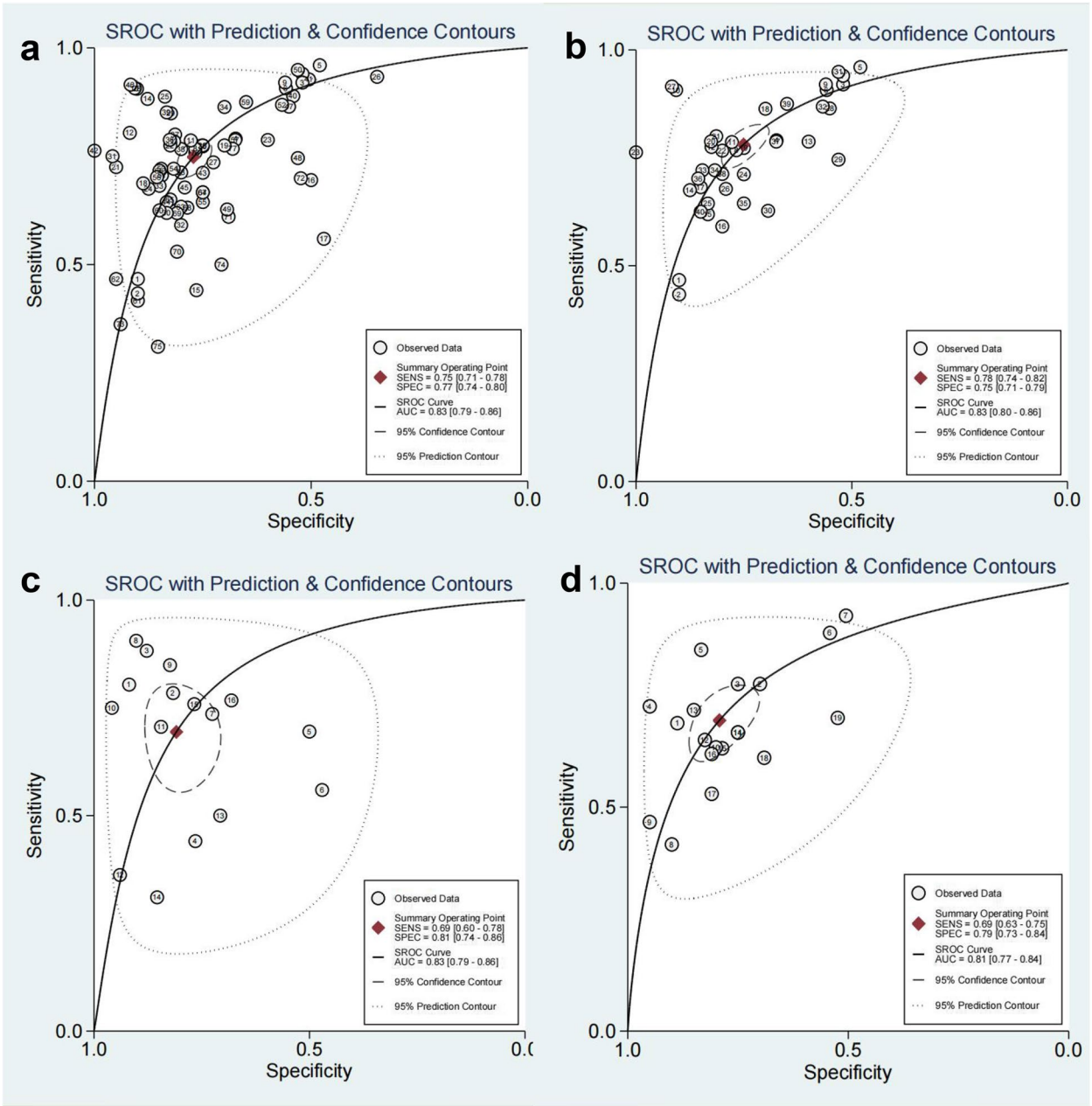

Fig. 4 Summary receiver operating characteristic curves of studied biomarkers. a) total exosomes, b) exosomes lncRNAs, c) exosomes miRNAs, d) exosomes mRNAs

[44]. The poor prognosis of BC is partly due to the lack of effective early diagnostic methods. Currently, cystoscopy remains the primary method for diagnosing and detecting BC recurrence. However, it is an invasive and costly procedure, limiting its use as a routine screening tool for BC.Non-invasive tests, such as urine cytology, are available but have proven ineffective for detecting low-grade malignancies [45]. Biomarkers like BTA and NMP22 are not only less sensitive but also prone to false positives in benign urinary conditions, which significantly hinders early diagnosis and treatment of BC [11, 12].

Traditional cancer diagnostic methods have seen limited progress, resulting in many cases being detected too late for effective treatment. This underscores the urgent need for novel, faster, and more accurate diagnostic techniques to combat cancer [46]. The domain of exosome diagnosis is rapidly growing in biomedical science. Our understanding of exosomes has significantly

Table 4 Summary estimates of diagnostic efcacy for exosomal profling in bladder cancer detection

Analysis		SEN(95% CI)	SPE(95% CI)	PLR(95% CI)	NLR(95% CI)	DOR(95% CI)	AUC(95% CI)
Exosomal ncRNA types	lncRNA	0.78	0.75	3.13	0.29	10.72	0.83
	miRNA	0.69	0.81	3.63	0.38	9.6	0.83
	mRNA	0.69	0.79	3.34	0.39	8.63	0.81
Ethnicity	Asian	0.75	0.77	3.28	0.32	10.24	0.83
	Caucasian	0.73	0.78	3.29	0.35	9.50	0.82
Sample size	＞100	0.74	0.76	3.14	0.34	9.35	0.82
	≤100	0.75	0.78	3.51	0.31	11.22	0.84
Exosome extraction methods	Exosome isolation kit	0.78	0.76	3.29	0.29	11.50	0.84
	ultracentrifugation	0.70	0.79	3.33	0.37	8.89	0.82
Exosomal panels	Single exosomes	0.73	0.76	3.04	0.35	8.59	0.81
	Multiple exosomes	0.79	0.80	4.04	0.26	15.80	0.87
Overall	/	0.75	0.77	3.28	0.33	10.04	0.83

CI confdence interval, *SEN* sensitivity, *SPE* specifcity, *PLR* positive likelihood ratio, *NLR* negative likelihood ratio, *DOR* diagnostic odds ratio, *AUC* area under the curve

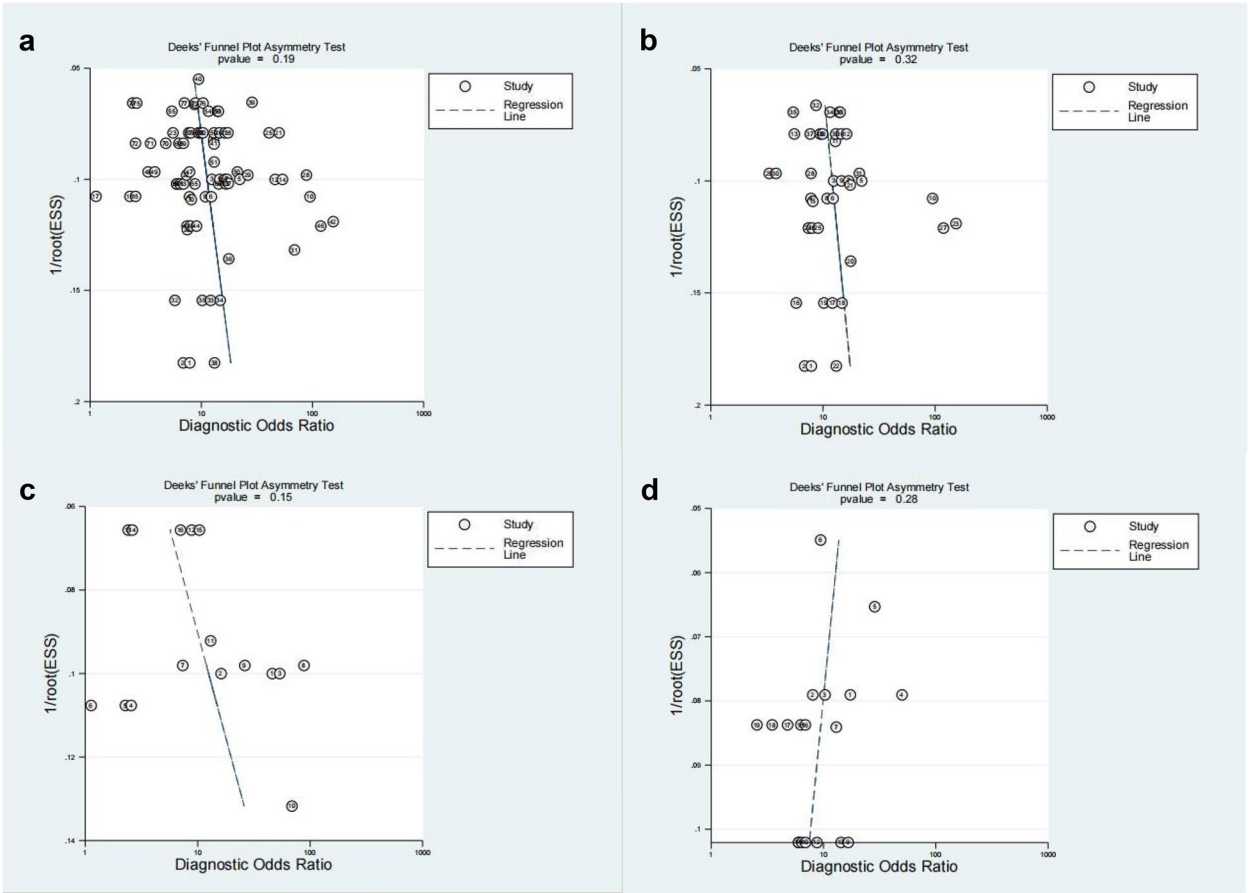

Fig. 5 Deek's funnel plot and asymmetry test for assessing the possibility of publication bias for included studies in meta-analysis. a) total exosomes, b) exosomes lncRNAs, c) exosomes miRNAs, d) exosomes mRNAs

advanced, and numerous studies have highlighted their role in the early detection and prognosis of various malignant tumors [47–49]. A recent meta-analysis and systematic review investigated the diagnostic value of exosomal circRNAs in six types of solid tumors, including lung and liver cancer. It reported a combined

sensitivity of 0.74 (95% CI = 0.70—0.78) and a combined specificity of 0.81 (95% CI = 0.78—0.83) across 21 studies [50]. Exosomes secreted by bladder cancer cells can be directly released into the urine and remain stable, making it feasible to detect bladder cancer through urinary exosome analysis. Currently, numerous studies, both domestic and international, have examined the differential expression of various exosomes in bladder cancer patients. Our study aims to assess the diagnostic value of urine-derived exosomes in bladder cancer patients through meta-analysis.

This study aimed to evaluate the accuracy of urine-derived exosomes as a non-invasive diagnostic tool for bladder cancer. To achieve this, we undertook a systematic review and meta-analysis, conducting a comprehensive search across various databases to identify all relevant studies on BC-related exosomal biomarkers, including lncRNAs, miRNAs, mRNAs, and proteins. Strict inclusion and exclusion criteria were applied, resulting in the incorporation of 21 articles involving a total of 3,348 subjects. The extracted data revealed that urine-derived exosomes had a combined Area Under the Curve (AUC) of 0.83 for diagnosing bladder cancer, with a sensitivity of 75% and a specificity of 77%. The AUC is a crucial metric for assessing diagnostic accuracy; a value closer to 1 indicates higher diagnostic value. AUC values exceeding 0.75 are generally considered satisfactory [51], and in this study, the AUC was 0.84, suggesting that urine-derived exosomes exhibit a high level of diagnostic accuracy for bladder cancer. Additionally, the combined diagnostic odds ratio (DOR) was computed to be 11. The DOR measures the effectiveness of a diagnostic test, with higher values indicating better diagnostic performance [52]. In conclusion, urine-derived exosomes show significant potential as a diagnostic tool for bladder cancer.

Due to the considerable heterogeneity in this study, a subgroup analysis was conducted. We found that exosome types (lncRNA, miRNA, or mRNA), total sample size, subject ethnicity, and exosome extraction methods (using a kit or ultracentrifugation) are potential sources of heterogeneity in detecting bladder cancer exosomes. In the meta-analysis grouped by exosome type, no significant difference in diagnostic efficacy was observed between lncRNAs and miRNAs, both showing an AUC of 0.83. The diagnostic efficacy for the mRNA group was slightly lower with an AUC of 0.81. However, it's important to note that the number of studies on miRNAs and mRNAs included in this research was significantly smaller than that on lncRNAs, advising caution in interpreting these results. Moreover, the combined AUC for multiple exosome panels was 0.87, in contrast to 0.81 for single-exosome panels. This indicates that diagnostic performance for bladder cancer is significantly enhanced when using multiple exosome combined panels compared to a single panel. Additionally, diagnostic efficacy appears slightly higher in individuals of Asian descent compared to those of European descent. This could be attributed to varying genetic polymorphisms, environmental factors, and lifestyle habits across different regions. The heterogeneity in exosome content might also be influenced by the diverse extraction and purification methods used [53]. To explore this, we categorized the included studies based on the methods of exosome extraction: ultracentrifugation or commercial extraction kits. The findings showed that the AUC for the kit group (AUC = 0.84) was marginally higher than that of the ultracentrifugation group (AUC = 0.82). However, further research is needed to confirm the stability and efficacy of the kit method in exosome extraction.

In [54], Su et al. assessed the diagnostic significance of exosome-derived lncRNAs for bladder cancer based on 23 studies across 10 articles (6 focused on urine and 4 on blood), demonstrating an overall AUC of 0.74 [54]. In [55], Zhao L et al. evaluated the diagnostic efficacy of exosome-derived ncRNAs (lncRNAs and miRNAs) for bladder cancer, analyzing 46 studies within 15 articles (11 on urine and 4 on blood), and reported a total AUC of 0.84 [55]. Both studies included exosomes derived from urine, plasma, and serum. Our report, however, exclusively evaluated the diagnostic significance of exosomes from urine, encompassing exosome-derived miRNAs, lncRNAs, mRNA, and proteins, which distinguishes it from the studies by Su and Zhao L. While Su and Zhao L conducted subgroup analyses based on sample sources, their studies incorporated a limited number of articles and did not cover the variety of urine exosomes as comprehensively as ours. Moreover, they did not perform subgroup analyses based on exosome extraction methods or sample sizes, making our report more extensive in these aspects. Additionally, we conducted a meticulous subgroup analysis of urine-derived exosomes. Our findings indicated that urinary exosome lncRNA and miRNA exhibited the same diagnostic value, with a combined AUC of 0.83, which was higher than that of urinary exosome mRNA. Furthermore, we demonstrated that panels combining multiple exosomes had higher diagnostic efficacy than single exosomal markers, consistent with the findings of Su and Zhao L. Finally, we evaluated the diagnostic efficiency of two distinct exosome extraction methods. Our analysis revealed that commercial kits provided superior diagnostic efficiency compared to ultrafast centrifugation. These comprehensive evaluations suggest that our study offers a more extensive and detailed analysis of urine-derived exosomes for diagnosing bladder cancer compared to previous reports.

Although we conducted a comprehensive systematic review and meta-analysis in line with the latest diagnostic guidelines, this study has some limitations. Firstly, despite a meticulous search, the current literature on exosomal diagnosis of bladder cancer (BC) is scarce. The number of included studies and subjects is relatively small, indicating a need for more research to verify the role of exosomes in BC diagnosis. Secondly, most of the studies in our analysis were conducted in Asia, potentially limiting the generalizability of our findings to other populations. Thirdly, the heterogeneity of biomarkers presents a significant constraint. The statistical heterogeneity of exosome types, races, sample sizes, and extraction methods inevitably affects our results. Additionally, although the control group included patients with benign diseases exhibiting similar symptoms to bladder cancer, the small sample size limits our ability to differentiate cancer from other diseases with similar symptoms. Despite these limitations, our study found that urine-derived exosome detection has a high predictive power for BC. Increasing evidence suggests that exosome biomarkers can be effectively utilized for cancer diagnosis. We hope that future studies by other scholars will further verify these results.

Conclusions

After screening 675 major research papers on exosomes and bladder cancer (BC), we included 77 studies from 21 articles in our meta-analysis. Our findings revealed that urine-derived exosomes, as novel biomarkers, exhibit high sensitivity and specificity in diagnosing BC. In conclusion, urine-derived exosomes have significant diagnostic potential for BC. However, their clinical feasibility and applicability still require validation through extensive clinical trials.

Abbreviations

BC	Bladder cancer
ROC	Receiver operating characteristic
TP	True positive
FP	False positive
TN	True negative
FN	False negative
PLR	Positive likelihood ratio
NLR	Negative likelihood ratio
DOR	Diagnostic odds ratio
SROC	Corresponding summary receiver operating characteristics
AUC	Area under the curve

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12957-024-03569-1.

Supplementary Material 1: Figure S1. Forest plots show pooled estimates of positive and negative likelihood ratios of studied biomarkers for differentiating of BC patients from nontumor controls.

Supplementary Material 2: Figure S2.Forest plots show pooled estimate of diagnostic odds ratio of studied biomarkers for differentiating of BC patients from nontumor controls. a) total exosomes, b) exosomes lncRNAs, c) exosomes miRNAs, d) exosomes mRNAs.

Supplementary Material 3: Figure S3.The Forest plot shows the sensitivity and specificity of exosomes lncRNAs in differentiating BC patients from non-tumor controls.

Supplementary Material 4: Figure S4.The Forest plot shows the sensitivity and specificity of exosomes miRNAs in differentiating BC patients from non-tumor controls.

Supplementary Material 5: Figure S5.The Forest plot shows the sensitivity and specificity of exosomes mRNAs in differentiating BC patients from non-tumor controls.

Supplementary Material 6. PRIMSA 2009 Checklist of items to include when reporting a systematic review or meta-analysis.

Supplementary Material 7. Table 1. Search Strategy of five databases.

Supplementary Material 8.

Acknowledgements

Not applicable.

Authors' contributions

Study concept and design: CL, HS; Acquisition of data: CL, HS; Analysis and interpretation: JL, CL, HS; Draft the manuscript and preliminary revise: LC, ML, WT; Analyses and reviewed the manuscript: HW, Study supervision and final approval: YX. All authors read and approved the final manuscript.

Funding

This work was supported by grants from National Natural Science Foundation of China (grant No. 82260609),Yunnan Fundamental Research Projects (grant No. 202201AS070085), Scientific Research Fund Project of Education Department of Yunnan Province (grant No. 2024Y231), and Kunming Medical University graduate Student Innovation Fund (grant No. 2024B025).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All analyses were based on previous published studies thus no ethical approval and patient consent are required.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Clinical Laboratory, the Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650000, China. [2]Department of Urology, the Second Affiliated Hospital of Kunming Medical University, Kunming, Yunnan 650000, China. [3]Department of Clinical Laboratory, Fuqing People's Hospital, Fuqing, Fujian 350300, China.

Received: 8 August 2024 Accepted: 19 October 2024
Published online: 29 October 2024

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: Cancer J Clin. 2024;74(3):229–63.

2. Witjes JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, et al. European association of urology guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2020 guidelines. Eur Urol. 2021;79(1):82–104.
3. Zhao M, He XL, Teng XD. Understanding the molecular pathogenesis and prognostics of bladder cancer: an overview. Chin J Cancer Res = Chung-kuo yen cheng yen chiu. 2016;28(1):92–8.
4. Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015;15(1):25–41.
5. Jocham D, Stepp H, Waidelich R. Photodynamic diagnosis in urology: state-of-the-art. Eur Urol. 2008;53(6):1138–48.
6. Burke DM, Shackley DC, O'Reilly PH. The community-based morbidity of flexible cystoscopy. BJU Int. 2002;89(4):347–9.
7. Johnson MI, Merrilees D, Robson WA, Lennon T, Masters J, Orr KE, et al. Oral ciprofloxacin or trimethoprim reduces bacteriuria after flexible cystoscopy. BJU Int. 2007;100(4):826–9.
8. Sun S, Lee D, Ho AS, Pu JK, Zhang XQ, Lee NP, et al. Inhibition of prolyl 4-hydroxylase, beta polypeptide (P4HB) attenuates temozolomide resistance in malignant glioma via the endoplasmic reticulum stress response (ERSR) pathways. Neuro Oncol. 2013;15(5):562–77.
9. Zhu Z, He A, Lv T, Xu C, Lin L, Lin J. Overexpression of P4HB is correlated with poor prognosis in human clear cell renal cell carcinoma. Cancer Biomarkers. 2019;26(4):431–9.
10. Fu L, Zhang J, Li L, Yang Y, Yuan Y. Diagnostic accuracy of urinary survivin mRNA expression detected by RT-PCR compared with urine cytology in the detection of bladder cancer: a meta-analysis of diagnostic test accuracy in head-to-head studies. Oncol Lett. 2020;19(2):1165–74.
11. Bhat A, Ritch CR. Urinary biomarkers in bladder cancer: where do we stand? Curr Opin Urol. 2019;29(3):203–9.
12. Tan WS, Tan WP, Tan MY, Khetrapal P, Dong L, deWinter P, et al. Novel urinary biomarkers for the detection of bladder cancer: A systematic review. Cancer Treat Rev. 2018;69:39–52.
13. Laukhtina E, Shim SR, Mori K, D'Andrea D, Soria F, Rajwa P, et al. Diagnostic accuracy of novel urinary biomarker tests in non-muscle-invasive bladder cancer: a systematic review and network meta-analysis. Eur Urol Oncol. 2021;4(6):927–42.
14. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, et al. Reassessment of Exosome Composition. Cell. 2019;177(2):428–45.e18.
15. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020;367(6478):eaau6977. https://doi.org/10.1126/science.aau6977.
16. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373–83.
17. Zhang X, Yuan X, Shi H, Wu L, Qian H, Xu W. Exosomes in cancer: small particle, big player. J Hematol Oncol. 2015;8:83.
18. Kalluri R. The biology and function of exosomes in cancer. J Clin Investig. 2016;126(4):1208–15.
19. Beckham CJ, Olsen J, Yin PN, Wu CH, Ting HJ, Hagen FK, et al. Bladder cancer exosomes contain EDIL-3/Del1 and facilitate cancer progression. J Urol. 2014;192(2):583–92.
20. McInnes MDF, Moher D, Thombs BD, McGrath TA, Bossuyt PM, Clifford T, et al. Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies: The PRISMA-DTA Statement. JAMA. 2018;319(4):388–96.
21. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ (Clin Research ed). 2003;327(7414):557–60.
22. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol. 2005;58(9):882–93.
23. Abbastabar M, Sarfi M, Golestani A, Karimi A, Pourmand G, Khalili E. Tumor-derived urinary exosomal long non-coding rnas as diagnostic biomarkers for bladder cancer. EXCLI J. 2020;19:301–10.
24. Bian B, Li L, Ke X, Chen H, Liu Y, Zheng N, et al. Urinary exosomal long non-coding RNAs as noninvasive biomarkers for diagnosis of bladder cancer by RNA sequencing. Front Oncol. 2022;12:976329.
25. Chen C, Shang A, Sun Z, Gao Y, Huang J, Ping Y, et al. Urinary exosomal long noncoding RNA TERC as a noninvasive diagnostic and prognostic biomarker for bladder urothelial carcinoma. J Immunol Res. 2022;2022:9038808.
26. El-Shal AS, Shalaby SM, Abouhashem SE, Elbary EHA, Azazy S, Rashad NM, et al. Urinary exosomal microRNA-96-5p and microRNA-183-5p expression as potential biomarkers of bladder cancer. Mol Biol Rep. 2021;48(5):4361–71.
27. Güllü Amuran G, Tinay I, Filinte D, Ilgin C, Peker Eyüboğlu I, Akkiprik M. Urinary micro-RNA expressions and protein concentrations may differentiate bladder cancer patients from healthy controls. Int Urol Nephrol. 2020;52(3):461–8.
28. Huang H, Du J, Jin B, Pang L, Duan N, Huang C, et al. Combination of urine exosomal mRNAs and lncRNAs as novel diagnostic biomarkers for bladder cancer. Front Oncol. 2021;11:667212.
29. Lee JS, Park HS, Han SR, Kang YH, Mun JY, Shin DW, et al. Alpha-2-macroglobulin as a novel diagnostic biomarker for human bladder cancer in urinary extracellular vesicles. Front Oncol. 2022;12:976407.
30. Lin H, Shi X, Li H, Hui J, Liu R, Chen Z, et al. Urinary exosomal miRNAs as biomarkers of bladder cancer and experimental verification of mechanism of miR-93-5p in bladder cancer. BMC Cancer. 2021;21(1):1293.
31. Liu C, Xu P, Shao S, Wang F, Zheng Z, Li S, et al. The value of urinary exosomal lncRNA SNHG16 as a diagnostic biomarker for bladder cancer. Mol Biol Rep. 2023;50(10):8297–304.
32. Matsuzaki K, Fujita K, Jingushi K, Kawashima A, Ujike T, Nagahara A, et al. MiR-21-5p in urinary extracellular vesicles is a novel biomarker of urothelial carcinoma. Oncotarget. 2017;8(15):24668–78.
33. Qiu T, Xue M, Li X, Li F, Liu S, Yao C, et al. Comparative evaluation of long non-coding RNA-based biomarkers in the urinary sediment and urinary exosomes for non-invasive diagnosis of bladder cancer. Mol Omics. 2022;10:938–47.
34. Sarfi M, Abbastabar M, Khalili E. Increased expression of urinary exosomal LnCRNA TUG-1 in early bladder cancer. Gene Reports. 2021;22.
35. Wen J, Yang T, Mallouk N, Zhang Y, Li H, Lambert C, et al. Urinary Exosomal CA9 mRNA as a Novel Liquid Biopsy for Molecular Diagnosis of Bladder Cancer. Int J Nanomed. 2021;16:4805–11.
36. Xu Y, Zhang P, Tan YZ, Jia Z, Chen GF, Niu YN, et al. A potential panel of five mRNAs in urinary extracellular vesicles for the detection of bladder cancer. Transl Androl Urol. 2021;10(2):809-+.
37. Yazarlou F, Modarressi MH, Mowla SJ, Oskooei VK, Motevaseli E, Tooli LF, et al. Urinary exosomal expression of long non-coding RNAs as diagnostic marker in bladder cancer. Cancer Manag Res. 2018;10:6357–65.
38. De Long J, Sullivan TB, Humphrey J, Logvinenko T, Summerhayes KA, Kozinn S, et al. A non-invasive miRNA based assay to detect bladder cancer in cell-free urine. Am J Trans Res. 2015;7(11):2500–9.
39. Gao Y, Wang X, Luo H, et al. Exosomal Long Non-Coding Ribonucleic Acid Ribonuclease Component of Mitochondrial Ribonucleic Acid Processing Endoribonuclease Is Defined as a Potential Non-Invasive Diagnostic Biomarker for Bladder Cancer and Facilitates Tumorigenesis via the miR-206/G6PD Axis. Cancers (Basel). 2023;15(21):5305. https://doi.org/10.3390/cancers15215305.
40. Zhan Y, Du L, Wang L, Jiang X, Zhang S, Li J, et al. Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer. Mol Cancer. 2018;17(1):142.
41. Wang X, Song D, Zhu B, Jin Y, Cai C, Wang Z. Urinary exosomal mRNA as a biomarker for the diagnosis of bladder cancer. Anticancer Drugs. 2024;35(4):362–70.
42. Murakami T, Minami K, Harabayashi T, Maruyama S, Takada N, Kashiwagi A, et al. Cross-sectional and longitudinal analyses of urinary extracellular vesicle mRNA markers in urothelial bladder cancer patients. Sci Rep. 2024;14(1):6801.
43. Yang FK, Tian C, Zhou LX, Guan TY, Chen GL, Zheng YY, et al. The value of urinary exosomal microRNA-21 in the early diagnosis and prognosis of bladder cancer. Kaohsiung J Med Sci. 2024;40(7):660–70.
44. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA: Cancer J Clin. 2023;73(1):17–48.
45. Grégoire M, Fradet Y, Meyer F, Têtu B, Bois R, Bédard G, et al. Diagnostic accuracy of urinary cytology, and deoxyribonucleic acid flow cytometry and cytology on bladder washings during followup for bladder tumors. J Urol. 1997;157(5):1660–4.
46. Sharma A, Dulta K, Nagraik R, Dua K, Singh S, Chellappan D, et al. Potentialities of aptasensors in cancer diagnosis. Mater etters. 2022;308(null): 131240.

47. Song X, Duan L, Dong Y. Diagnostic accuracy of exosomal long non-coding RNAs in diagnosis of NSCLC: a meta-analysis. Mol Diagn Ther. 2024;28(4):455–68.
48. Qiao Z, Wang E, Bao B, et al. Diagnostic and prognostic value of circulating exosomal glypican-1 in pancreatic cancer: a meta-analysis. Lab Med. 2024;55(5):543–52. https://doi.org/10.1093/labmed/lmae013.
49. Tiyuri A, Baghermanesh SS, Davatgaran-Taghipour Y, Eslami SS, Shaygan N, Parsaie H, et al. Diagnostic accuracy of serum derived exosomes for hepatocellular carcinoma: a systematic review and meta-analysis. Expert Rev Mol Diagn. 2023;23(11):971–83.
50. Yuan X, Mao Y, Ou S. Diagnostic accuracy of circulating exosomal circRNAs in malignances: A meta-analysis and systematic review. Medicine. 2023;102(21): e33872.
51. Jones CM, Athanasiou T. Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. Ann Thorac Surg. 2005;79(1):16–20.
52. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol. 2003;56(11):1129–35.
53. Tauro BJ, Greening DW, Mathias RA, Ji H, Mathivanan S, Scott AM, et al. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods (San Diego, Calif). 2012;56(2):293–304.
54. Su Q, Wu H, Zhang Z, Lu C, Zhang L, Zuo L. Exosome-derived long non-coding RNAs as non-invasive biomarkers of bladder cancer. Front Oncol. 2021;11: 719863.
55. Zhao L, Li J, Xue Z, Wang J. Exosomal noncoding RNAs as noninvasive biomarkers in bladder cancer: a diagnostic meta-analysis. Clin Transl Oncol. 2024;26(6):1497–507.

Publisher's Note