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Diagnostic performance of circulating exosomes in human cancer: A meta-analysis

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Abstract

Background: Cancer has become a public health problem with high morbidity and mortality. Recent publications have shown that exosomes can be used as potential diagnostic biomarkers of cancer. However, the diagnostic accuracy and reliability of circulating exosomes remain unclear. The present meta-analysis was conducted to comprehensively summarize the overall diagnostic performance of circulating exosomes for cancer.

Methods: Eligible studies published up to June 27, 2019, on PubMed, Embase, and Cochrane Library were selected for the meta-analysis. All statistical analyses were performed by STATA 15.1 statistical software and Meta-DiSc 1.4. Quality Assessment for Studies of Diagnostic Accuracy 2 tool was used to access the quality of included studies. A bivariate mixed-effects model was applied to calculate the diagnostic indexes from included studies.

Results: A total of 5924 participants comprising 3161 cases and 2763 controls from 42 eligible studies were analyzed. The pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and the area under the curve with 95% confidence intervals (95% CI) were as follows: 0.79 (0.75-0.82), 0.81 (0.78-0.84), 4.1 (3.5-4.8), 0.26 (0.22-0.31), 16 (12-21), and 0.87 (0.84-0.89), respectively. Sensitivity analysis suggested no study exclusively contributed to the heterogeneity, and Deeks' funnel plot asymmetry test indicated no potential publication bias (P = .09).

Conclusions: The meta-analysis indicated that circulating exosomes could serve as effective and minimally invasive biomarkers for diagnosis of cancer, especially in patients with hepatocellular carcinoma or ovarian cancer, serum-based samples and exosomal proteins.

KEYWORDS

cancer, carcinoma, circulating, diagnosis, exosome, meta-analysis

Dongming Guo and Jinpeng Yuan equally contributed to this work.

1 | INTRODUCTION

Cancer is one of the most common diseases and has become a serious public health problem worldwide. In the United States, 1 735 350 new cancer cases and 609 640 cancer deaths are estimated to occur in 2018.[1] In China, it is estimated that there will be about 12 000 new cancer diagnoses and over 7500 cancer deaths on average each day in 2015.[2] One of the important reasons for high mortality and morbidity is the lack of effective screening and detection methods. Currently, traditional tumor markers such as carcinoembryonic antigen, carbohydrate antigen 199 and carbohydrate antigen 125 (CA125), are widely used in clinical practice, but their sensitivity (SEN) and specificity (SPE) are unsatisfied.[3-5] Therefore, identifying potential biomarkers for early detection and diagnosis of cancer is urgently needed.

Exosomes are small 40-100 nm vesicles delivered by many cells of the organism, including cancer cells.[6] They can be found in all body fluids and play a key role in intercellular communication, which provide information on various different cellular functions and disease states where they can constitute valuable biomarkers.[6,7] Tumor-derived exosomes transfer messages from tumor cells to tumor stroma, premetastatic niche, hematopoietic system, and non-cancer stem cells by cancer-initiating cells.[8] They contain abundant different types of proteins, nucleic acids, and lipids, which act important roles in tumorigenesis, growth, progression, metastasis, immune escape, and drug resistance as well as treatment of cancer.[9] Owing to their enriched contents and excellent stability, exosomes are suggested to be optimal noninvasive biomarkers for cancer diagnosis.[10] Increasing studies have shown that exosomes are considered to be a promising diagnostic biomarkers for various types of cancer.[11,12] However, due to small sample sizes and various exosomal marker types, there is still heterogeneity or inconsistency in the diagnostic accuracy of exosomes. Thus, we performed the meta-analysis to precisely assess the overall diagnostic accuracy of circulating exosomes in human cancer.

2 | MATERIALS AND METHODS

2.1 | Search strategy

A comprehensive and systematic search was conducted in PubMed, Embase, and Cochrane Library up to June 27, 2019. Search terms were as follows: (cancer OR carcinoma OR tumor OR tumour OR neoplasm) AND (circulating OR serum OR plasma OR blood) AND (exosome OR exosomes OR exosomal) AND (diagnosis OR diagnostic OR sensitivity OR specificity OR "receiver operating characteristic curve" OR ROC). The literature search was performed independently by two authors (DMG and JPY). Any disagreements between the two authors were resolved by discussion with a third author (JTC).

2.2 | Inclusion and exclusion criteria

The inclusion criteria for literature were as follows: (a) studies investigated diagnostic value of exosomal markers for any type of human cancers; (b) exosomes were isolated from serum or plasma; (c) studies included cancer cases and benign or healthy controls; and (d) studies provided sufficient data to construct a diagnostic 2 × 2 table. The exclusion criteria included the following: (a) studies that did not relate to exosomes or cancer; (b) studies that were duplicate articles, reviews, animal studies, editorials, case reports, comments, method articles, expert opinions, conference abstracts, and meta-analyses; (c) studies with at least 20 cases and 20 controls; (dd) studies without complete data; (e) studies with no difference in expression of markers; and (f) studies that were not published in English.

2.3 | Data extraction and quality assessment

Information from eligible literatures was independently extracted by two investigators (DMG and JPY). The following data from included studies were collected: first author, publication year, country, exosomal biomarker type, cancer type, sample type, isolation methods, number of case and control, and diagnostic value, including SEN, SPE, true-positive (TP), false-positive (FP), false-negative (FN), and true-negative (TN). The quality of each study was assessed independently by two authors (DMG and JPY) using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2),[13] which consists of four domains: patient selection, index text, reference standard, and flow and timing. Any discrepancies between the two authors were resolved by a third author (XXL).

2.4 | Statistical analysis

The meta-analysis was conducted by RevMan5.3, Meta-DiSc 1.4, and STATA 15.1 software. The heterogeneity of the study was estimated by the Cochran's Q test and I^2 index.[14] $P < .05$ for Q test or $I^2 > 50\%$ indicated the existence of heterogeneity. A bivariate mixed-effects model was used to calculate the pooled SEN, SPE, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR) with 95% confidence intervals (95% CI). Summary receiver operator characteristic (SROC) curve and forest plots of pooled SEN and SPE were applied to evaluate the diagnostic performance of circulating exosomes. Spearman's correlation coefficient and ROC plane were used to assess the heterogeneity generated by diagnostic threshold effect. Meta-regression and subgroup analysis were performed to investigate the heterogeneity generated by non-threshold effect. In addition, a bivariate box plot was used to evaluate the potential source of heterogeneity within the selected studies. The clinical practicality of circulating exosomes was examined by Fagan's nomogram. Moreover, sensitivity analysis and Deeks' funnel plot asymmetry test were constructed to test the stability of pooled HR and publication bias, respectively.

3 | RESULTS

3.1 | Search results

The flow diagram of article selection is presented in Figure 1A. A total of 3334 literatures were searched from PubMed, Embase, and Cochrane Library. After removing 865 duplicate publications, 2469 articles were included for further assessing. After screening of the title and abstract, 2342 articles were excluded and the remaining 127 literatures were further evaluated. After detailed evaluation of the full texts, 85 articles were excluded for the following reasons: (a) 34 studies not for diagnostic research; (b) 34 studies with insufficient data; (c) 7 studies based on combined diagnosis; (d) 1 study with no difference in expression; (e) 6 studies with sample size less than 20 in either case or control group; and (f) 3 studies with non-English full-text. Finally, a total of 70 studies from 42 publications[15-56] involving 3161 cases and 2763 controls were analyzed in the meta-analysis.

3.2 | Study characteristics and quality assessments

The main characteristics of included articles are provided in Table 1. All cancer cases were confirmed pathologically. There were fifteen cancer types: lung cancer (LC, n = 7),[15-21] esophageal cancer (EC, n = 1),[22] gastric cancer (GC, n = 5),[23-27] colorectal cancer (CRC, n = 5),[28-32] hepatocellular carcinoma (HCC, n = 4),[33-36] pancreatic cancer (PC, n = 3),[37-39] ovarian cancer (OC, n = 3),[40-42] glioma (n = 3),[43-45] clear cell renal cell carcinoma (ccRCC, n = 2),[50,51] bladder cancer (BC, n = 3),[46,48,49] prostate cancer (PCa, n = 2),[47,52] osteosarcoma (n = 1),[53] multiple myeloma (MM, n = 1),[54] melanoma (n = 1),[55] and laryngeal squamous cell carcinoma (LSCC, n = 1).[56] Publication years of all included researches range from 2013 to 2019. Fifty-nine studies were based on serum and eleven studies based on plasma. The sample sizes of the studies ranged from 40 to 468, and 35 studies included at least 110 participants. Of the seventy studies, thirty studies focused on microRNAs (miRNAs), twenty-two studies focused on long non-coding RNAs (lncRNAs), twelve studies focused on proteins, and six studies focused on other markers (circular RNA, messenger RNA, and small non-coding RNA). The results of study quality assessment were evaluated using QUADAS-2 (Figure 1B and Figure S1). Most studies had low or unclear risks of bias on patient selection, index text, reference standard, and flow and timing, indicating that the quality of included studies was medium.

3.3 | Diagnostic accuracy

Threshold and non-threshold effects are sources of heterogeneity on diagnostic tests. Heterogeneity caused by non-threshold effects was evaluated using Q tests and I-squared. The pooled SEN (I^2 = 86.81%, P < .01) and specificity (I^2 = 77.27%, P < .01) revealed significant heterogeneity (Figure 2). We conducted Spearman's correlation coefficient and ROC plane to identify heterogeneity

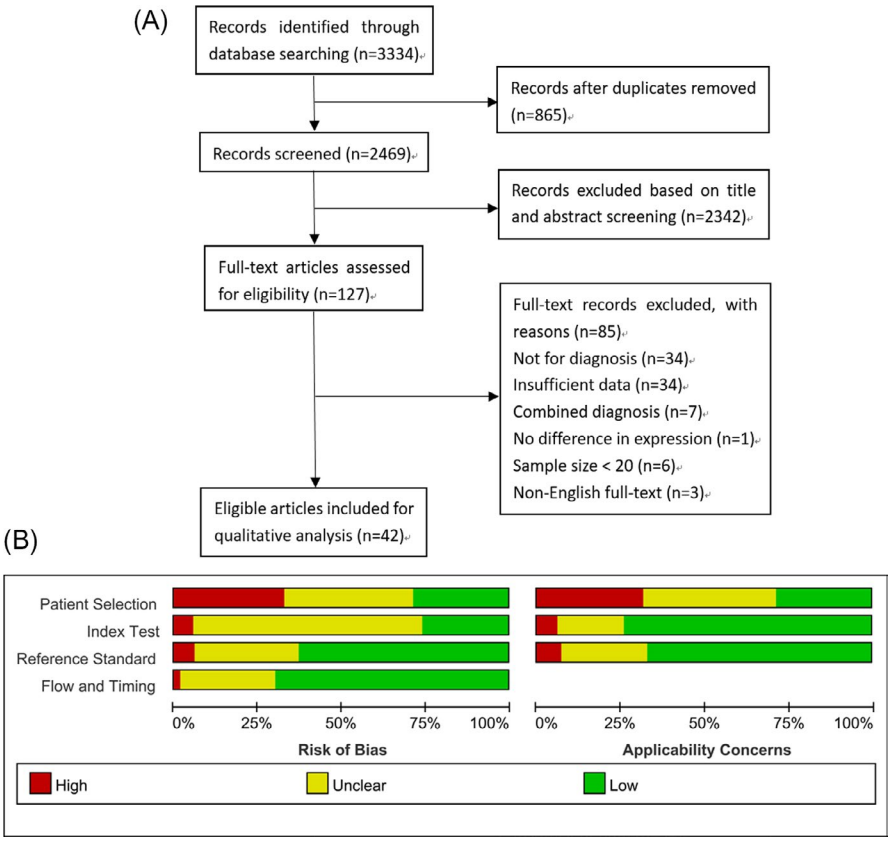

FIGURE 1 Flow diagram of studies' selection and quality assessment of the included articles

TABLE 1 Basic characteristics of the 42 eligible studies

Author	Year	Country	Exosomal markers	Cancer type	Specimen	Isolation method	Case	Control	TP	FP	FN	TN
Wang et al	2018	China	Protein	LC	Serum	Ultracentrifugation	183	90	119	22	64	68
Zhang et al	2019	China	miRNA	LC	Serum	Isolation kit	100	90	70	16	30	74
			miRNA	LC	Serum	Isolation kit	72	47	48	11	24	36
Sandfeld-Paulsen et al	2016	Denmark	Protein	LC	Plasma	Ultracentrifugation	57	126	34	31	23	95
Teng et al	2019	China	LncRNA	LC	Plasma	Ultracentrifugation	75	79	57	21	18	58
Zhang et al	2017	China	LncRNA	LC	Serum	Isolation kit	77	30	46	6	31	24
Li et al	2019	China	LncRNA	LC	Serum	Isolation kit	64	40	55	12	9	28
Niu et al	2019	China	Protein	LC	Serum	Ultracentrifugation	122	46	67	7	55	39
			Protein	LC	Serum	Ultracentrifugation	109	46	84	9	25	37
Zhao et al	2019	China	Protein	ESCC	Serum	Isolation kit	100	100	75	15	25	85
Yang et al	2018	China	miRNA	GC	Serum	Isolation kit	80	80	65	34	15	46
Zhao et al	2018	China	LncRNA	GC	Serum	Ultracentrifugation	126	120	88	18	38	102
Pan et al	2017	China	LncRNA	GC	Serum	Ultracentrifugation	40	37	32	9	8	28
Lin et al	2018	China	LncRNA	GC	Plasma	Ultracentrifugation	51	60	45	10	6	50
			LncRNA	GC	Plasma	Ultracentrifugation	51	60	46	26	5	34
Rahbari et al	2019	Germany	Protein	GC	Serum	Isolation kit	49	56	42	14	7	42
Barbagallo et al	2018	Italy	LncRNA	CRC	Serum	Isolation kit	20	20	20	11	0	9
			circRNA	CRC	Serum	Isolation kit	20	20	14	4	6	16
Liu et al	2016	China	LncRNA	CRC	Serum	Isolation kit	148	320	104	18	44	302
Liu et al	2018	China	miRNA	CRC	Plasma	Isolation kit	80	40	64	9	16	31
			miRNA	CRC	Plasma	Isolation kit	80	40	56	8	24	32
Liu et al	2018	China	miRNA	CRC	Plasma	Isolation kit	53	30	37	7	16	23
Sun et al	2019	China	Protein	CRC	Plasma	Ultracentrifugation	92	32	62	5	30	27
Abd El Gwad et al	2018	Egypt	LncRNA	HCC	Serum	Isolation kit	60	60	58	3	2	57
			miRNA	HCC	Serum	Isolation kit	60	60	57	12	3	48
			mRNA	HCC	Serum		60	60	45	16	15	44
Xu et al	2018	China	mRNA	HCC	Serum	Isolation kit	88	68	75	16	13	52
			mRNA	HCC	Serum	Isolation kit	88	67	76	16	12	36
Wang et al	2018	China	miRNA	HCC	Serum	Ultracentrifugation	50	50	50	4	0	46
Xu et al	2018	China	LncRNA	HCC	Serum	Isolation kit	60	96	43	16	17	80
			LncRNA	HCC	Serum	Isolation kit	55	60	40	12	15	48
			LncRNA	HCC	Serum	Isolation kit	60	96	46	21	14	75
			LncRNA	HCC	Serum	Isolation kit	55	60	44	15	11	45
Que et al	2013	China	miRNA	PC	Serum	Ultracentrifugation	22	27	16	2	6	25
			miRNA	PC	Serum	Ultracentrifugation	22	27	21	5	1	22

(Continues)

TABLE 1 (Continued)

Author	Year	Country	Exosomal markers	Cancer type	Specimen	Isolation method	Case	Control	TP	FP	FN	TN
Goto et al	2018	Japan	miRNA	PC	Serum	Isolation kit	32	22	23	3	9	19
			miRNA	PC	Serum	Isolation kit	32	22	26	4	6	18
			miRNA	PC	Serum	Isolation kit	32	22	21	3	11	19
Melo et al	2015	USA	Protein	PC	Serum	Ultracentrifugation	190	126	190	0	0	126
			Protein	PC	Serum	Ultracentrifugation	26	56	56	0	0	26
Meng et al	2016	Germany	miRNA	OC	Serum	Isolation kit	112	20	94	2	18	18
			miRNA	OC	Serum	Isolation kit	112	20	59	0	53	20
			miRNA	OC	Serum	Isolation kit	112	20	35	0	77	20
Kim et al	2019	Korea	miRNA	OC	Serum	Isolation kit	48	20	44	5	4	15
			miRNA	OC	Serum	Isolation kit	48	20	35	2	13	18
Su et al	2019	China	miRNA	OC	Serum	Isolation kit	50	65	31	8	19	57
			miRNA	OC	Serum	Isolation kit	50	65	17	4	33	61
Santangelo et al	2018	Italy	miRNA	Glioma	Serum	Isolation kit	60	30	49	7	11	23
			miRNA	Glioma	Serum	Isolation kit	60	30	36	1	24	29
			miRNA	Glioma	Serum	Isolation kit	60	30	50	11	10	19
Shao et al	2019	China	miRNA	Glioma	Serum	Isolation kit	24	24	19	2	5	22
Manterola et al	2014	Spain	sncRNA	Glioma	Serum	Isolation kit	50	30	33	10	17	20
			sncRNA	Glioma	Serum	Isolation kit	25	25	18	7	7	18
			miRNA	Glioma	Serum	Isolation kit	25	25	16	9	9	16
			miRNA	Glioma	Serum	Isolation kit	25	25	15	10	10	15
Wang et al	2018	China	LncRNA	BC	Serum	Isolation kit	52	104	39	23	13	81
Li et al	2018	China	Protein	PCa	Serum	Ultracentrifugation	50	21	44	4	6	17
Zheng et al	2018	China	LncRNA	BC	Plasma	Isolation kit	50	60	33	9	17	51
Xue et al	2017	China	LncRNA	BC	Serum	Isolation kit	30	30	24	5	6	25
Zhang et al	2018	China	miRNA	ccRCC	Serum	Isolation kit	82	80	57	30	25	50
			miRNA	ccRCC	Serum	Isolation kit	82	80	66	19	16	61
Wang et al	2019	China	miRNA	ccRCC	Serum	Isolation kit	40	30	33	6	7	24
Wang et al	2018	China	LncRNA	PCa	Plasma	Isolation kit	34	30	21	5	13	25
			LncRNA	PCa	Plasma	Isolation kit	34	30	30	7	4	23
Yuan et al	2019	China	LncRNA	Osteosarcoma	Serum	Isolation kit	46	45	40	15	6	30
Sedlarikova et al	2018	Czech	LncRNA	MM	Serum	Isolation kit	50	30	40	7	10	23
Alegre et al	2016	Spain	Protein	Melanoma	Serum	Isolation kit	53	25	42	5	11	20
			Protein	Melanoma	Serum	Isolation kit	53	25	42	5	11	20
Wang et al	2014	China	miRNA	LSCC	Serum	Isolation kit	52	49	36	10	10	40
			LncRNA	LSCC	Serum	Isolation kit	52	49	48	9	16	28

Abbreviations: BC, bladder cancer; CRC, colorectal cancer; ESCC, esophageal squamous cell carcinoma; FN, false negatives; FP, false positives; GC, gastric cancer; HCC, hepatocellular carcinoma; LC, lung cancer; LSCC, laryngeal squamous cell carcinoma; MM, multiple myeloma; OC, ovarian cancer; PC, pancreatic cancer; PCa, prostate cancer; TN, true negatives; TP, true positives.

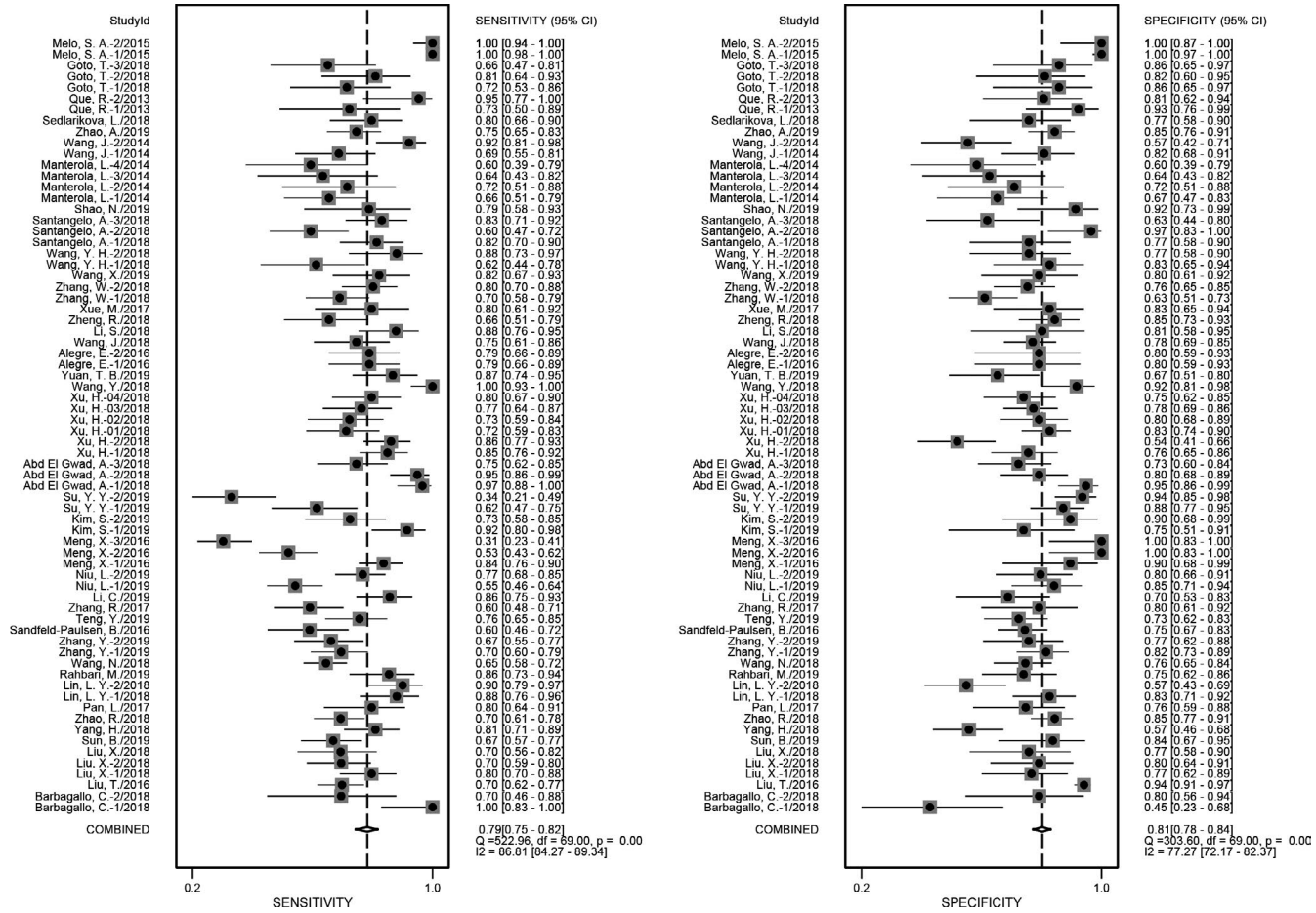

FIGURE 2 Forest plot of sensitivity and specificity of circulating exosomes for the diagnosis of cancer. CI, confidence interval; Q, Cochran's Q value; DF, degrees of freedom; I^2, inconsistency index

generated by threshold effects. Spearman's correlation coefficient was 0.200 (P = .097), and ROC plane did not show the typical shoulder arm (Figure 3A), suggesting that no threshold effects were found.

The forest plots showed that pooled SEN and SPE were 0.79 (95% CI: 0.75-0.82) and 0.81 (95% CI: 0.78-0.84), respectively. SROC curve exhibited that the overall AUC was 0.87 (95% CI: 0.84-0.89) (Figure 3B). In addition, the pooled PLR, NLR, and DOR were 4.1 (95% CI: 3.5-4.8), 0.26 (95% CI: 0.22-0.31), and 16 (95% CI: 12-21), respectively. Fagan's diagram was applied to assess the predictive value on clinical utility. With a pretest probability of 20%, Fagan's diagram exhibited that the positive posttest probability of accurately diagnosing cancer would increase to 51%, while the negative probability would drop to 6% (Figure 3C).

3.4 | Meta-regression and subgroup analysis

To investigate potential sources of heterogeneity, meta-regression and subgroup analysis were performed based on type of cancer (LC or not, CRC or not, GC or not, HCC or not, OC or not), sample type (serum or plasma), sample size (≥110 or <110), and exosomal markers (miRNA or not, lncRNA or not, protein or not) (Figure 3D). The exact results of meta-regression analysis are presented in Table 2. We found that research country, LC, CRC, HCC, OC, sample type, isolation method, sample size, exosomal miRNAs, exosomal lncRNAs, and exosomal proteins were likely the sources of heterogeneity in sensitivity. We also found that research country, LC, GC, CRC, HCC, sample type, isolation method, sample size, exosomal miRNAs, exosomal lncRNAs, and exosomal proteins might act as sources of heterogeneity in specificity. As shown in bivariate boxplot (Figure 3E), there were 19 studies not located in the boxplot. After removing these studies, the heterogeneity among studies decreased obviously (SEN: I^2 = 64.28%, P < .01 and SPE: I^2 = 36.52%, P = .01). The results of subgroup analysis are summarized in Table 3. Studies about HCC or OC exhibited larger AUC (0.90, 95% CI: 0.87-0.92 and 0.90, 95% CI: 0.87-0.93, respectively) compared with other cancer types. Studies involving serum presented higher SEN (0.79, 95% CI: 0.75-0.83), SPE (0.82, 95% CI: 0.78-0.83), PLR (4.3, 95% CI: 3.6-5.2), DOR (17, 95% CI: 12-24), and AUC (0.88, 95% CI: 0.84-0.90) than those involving plasma. In addition, exosomal proteins demonstrated superior SEN (0.86, 95% CI: 0.66-0.95), SPE (0.87, 95% CI: 0.78-0.93), and AUC (0.93, 95% CI: 0.90-0.95) compared to exosomal miRNAs or lncRNAs.

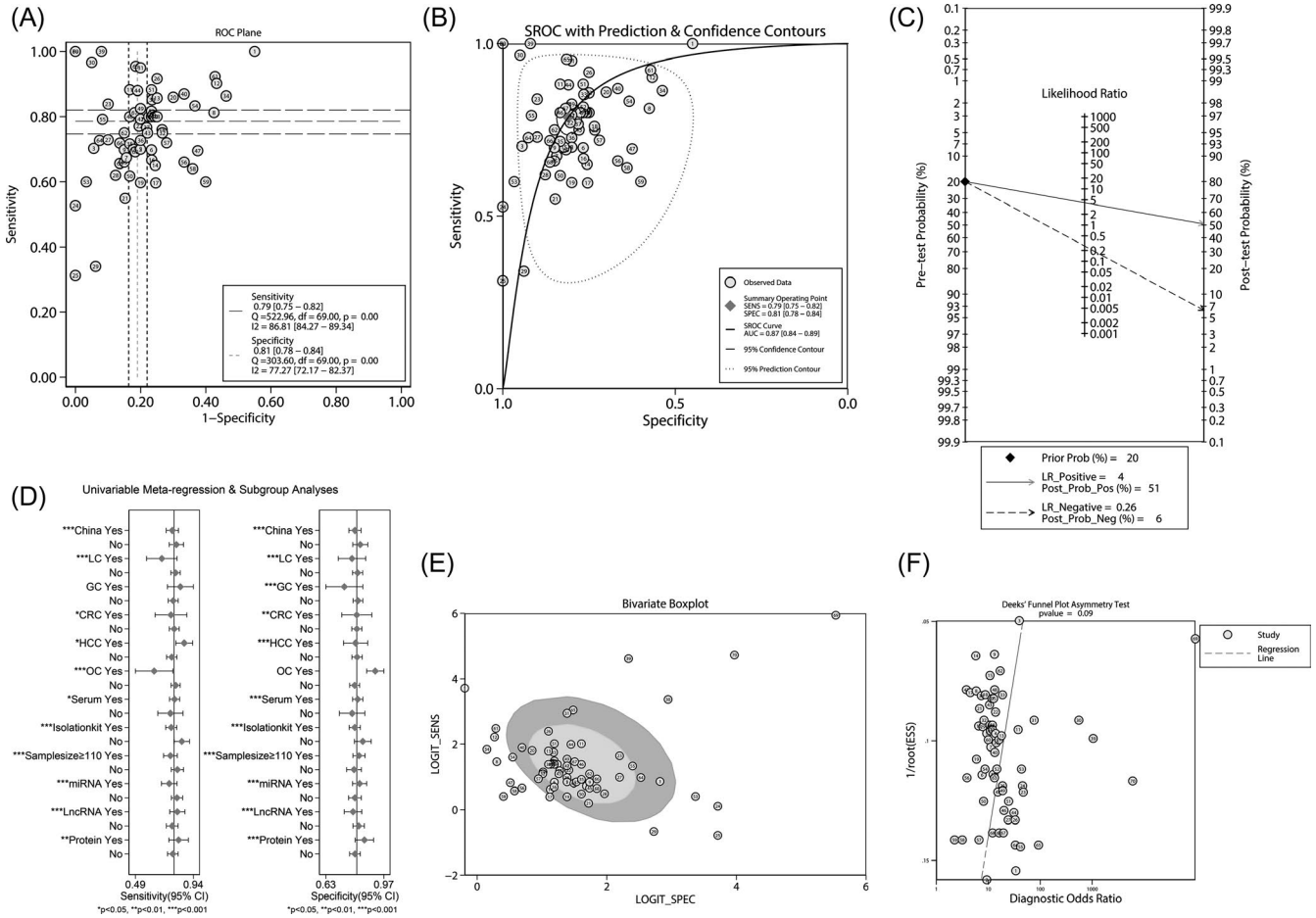

FIGURE 3 Diagnostic accuracy of included studies in our meta-analysis. (A) ROC plane. (B) SROC curve. (C) Fagan's nomogram. (D) Meta-regression plot. (E) Bivariate boxplot. (F) Deeks' funnel plot

3.5 | Sensitivity analysis and publication bias

To further explore the potential heterogeneity from any single study, sensitivity analysis was performed and showed that our results were not significantly affected by removing any study (Figure 4). Deeks' funnel plot asymmetry test was applied to examine publication bias for studies. As shown in Figure 2F, A *P* value of .093 (*P* > .05) suggested no obvious publication bias among these studies.

4 | DISCUSSION

In the last few years, the potential diagnostic significance of circulating exosomes has been intensively investigated in various diseases, especially in the field of cancer research. Several previous meta-analyses have published the diagnostic value of exosomes in cancer. However, Yang et al[11] focused only on exosomal miRNAs in their meta-analysis. Wong et al[12] did not conduct the overall diagnostic value in cancer, and the number of articles included in their meta-analysis was evidently less than ours. Our study, involving 5924 participants (3161 cases and 2763 controls), and 15 types of cancer, is the first study to comprehensively assess overall diagnostic value of circulating exosomes in human cancer through a meta-analysis. The quality assessment of the included studies was conducted, which exhibited moderate quality. The overall pooled SEN, SPE, and AUC were 0.79 (95% CI: 0.75-0.82), 0.81 (95% CI: 0.78-0.84), and 0.87 (95% CI: 0.83-0.89), respectively. These results indicated that circulating exosomes had relatively high diagnostic accuracy for cancer.

There was significant heterogeneity in the meta-analysis. Spearman's correlation coefficient was 0.200, and ROC plane showed the absence of typical shoulder arm, meaning heterogeneity was not from threshold effects. Meta-regression analysis was performed to identify heterogeneity caused by non-threshold effects. Our analysis showed that the heterogeneity resulted from research country, cancer type, specimen, isolation method, sample size, and type of exosomal marker. Moreover, there were 19 studies that did not locate in bivariate boxplot, suggesting that the results of these studies might be the main sources of heterogeneity.

According to subgroup analysis, HCC and OC demonstrated the largest AUC, implying that detection of circulating exosomes could be a promising approach for diagnosis of HCC and OC. Alpha-fetoprotein (AFP) is the most widely used tumor marker in diagnosis of liver cancer. The meta-analysis of Dai et al[57] reported that the

TABLE 2 The results of meta-regression analysis

Parameter	Category	N	SEN (95% CI)	*P*	SPE (95% CI)	*P*
China	Yes	43	0.77 (0.73-0.82)	<.001	0.80 (0.76-0.83)	<.001
	No	27	0.80 (0.75-0.86)		0.83 (0.79-0.87)	
LC	Yes	9	0.69 (0.58-0.81)	<.001	0.78 (0.70-0.86)	<.001
	No	61	0.80 (0.76-0.83)		0.81 (0.78-0.84)	
GC	Yes	6	0.84 (0.74-0.94)	.10	0.74 (0.63-0.85)	<.001
	No	64	0.78 (0.74-0.82)		0.82 (0.79-0.85)	
CRC	Yes	7	0.76 (0.64-0.89)	.01	0.81 (0.72-0.90)	<.001
	No	63	0.79 (0.75-0.83)		0.81 (0.78-0.84)	
HCC	Yes	10	0.87 (0.80-0.93)	.04	0.80 (0.73-0.87)	<.001
	No	60	0.77 (0.73-0.81)		0.81 (0.78-0.84)	
OC	Yes	7	0.63 (0.49-0.78)	<.001	0.92 (0.87-0.97)	.17
	No	63	0.80 (0.76-0.83)		0.80 (0.77-0.83)	
Serum	Yes	59	0.79 (0.75-0.83)	.01	0.82 (0.78-0.85)	<.001
	No	11	0.76 (0.66-0.86)		0.78 (0.71-0.86)	
Isolation Kit	Yes	54	0.77 (0.72-0.81)	<.001	0.80 (0.76-0.83)	<.001
	No	16	0.85 (0.79-0.91)		0.85 (0.80-0.89)	
Sample size ≥ 110	Yes	35	0.76 (0.71-0.81)	<.001	0.82 (0.79-0.86)	<.001
	No	35	0.81 (0.77-0.86)		0.79 (0.75-0.84)	
miRNA	Yes	30	0.75 (0.69-0.81)	<.001	0.83 (0.78-0.87)	<.001
	No	40	0.81 (0.77-0.85)		0.80 (0.76-0.84)	
LncRNA	Yes	22	0.81 (0.75-0.87)	<.001	0.79 (0.74-0.84)	<.001
	No	48	0.77 (0.73-0.82)		0.82 (0.79-0.85)	
Protein	Yes	12	0.82 (0.75-0.90)	<.01	0.85 (0.80-0.91)	<.001
	No	58	0.78 (0.74-0.82)		0.80 (0.77-0.83)	

Abbreviations: CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; LC, lung cancer; OC, ovarian cancer; SEN, sensitivity; SPE, specificity.

AUC of AFP for diagnosis HCC was 0.84. Our results showed that the AUC of circulating exosomes was 0.90, suggesting that the diagnostic value of exosomes was superior to AFP. In addition, Liao et al[58] concluded that the AUC of CA125 was 0.84 for diagnosis of OC after analyzing 19 literatures. In our meta-analysis, the AUC of blood-based exosomes was 0.90, which exhibited higher value than CA125 in distinguishing OC from non-OC. Additionally, among the included studies of HCC or OC, only one study by Wang et al exhibited high risk of bias on index text. Therefore, the results of these studies showing high efficacy for HCC and OC diagnosis are reliable.

The pooled SEN, SPE, PLR, DOR, and AUC of serum-based exosomes were significantly higher than plasma-based exosomes, meaning that serum seemed to be the better specimen for detection. Moreover, the proportion of low-risk bias in study using serum as a sample was higher than those using plasma, which suggested that studies based on serum specimen had superior quality and reliability. Currently, there is no consensus on sample selection for isolating blood exosomes. When preparing serum, additional extracellular vesicles are released by activated platelets during clot formation,[59] which cannot originally represent the pathophysiological status of the circulating blood in patients and may influent exosome isolation. On the contrary, experimental results of exosomes may be affected by anticoagulants when using plasma as sample. For example, heparin and ethylenediaminetetraacetic acid interfere with polymerase chain reaction.[60] Clearly, it is urgent to establish and validate guidelines for preparation of samples for exosome research.

The included studies used two different methods to isolate blood exosomes. The quality of studies with ultracentrifugation method was inferior to those with isolation kit because of the lower percentage of low-risk bias. Studies with ultracentrifugation method displayed higher diagnostic accuracy. Due to fewer included studies using this method in the meta-analysis, more large-sample studies are needed to confirm this finding. Purifying exosomes is a great challenge because their biophysical properties overlap with other secreted cell products. There are different methods of isolating exosomes, including ultracentrifugation, precipitation, immunoaffinity capturing, filtration techniques, and microfluidics,[61] which results in qualitative and quantitative variability in terms of extracting exosomes. Hence, exploring an

TABLE 3 The results of subgroup analysis for diagnostic value

Subgroup	N	SEN (95% CI)	SPE (95% CI)	PLR (95% CI)	NLR (95% CI)	DOR (95% CI)	AUC (95% CI)
Overall	70	0.79 (0.75-0.82)	0.81 (0.78-0.84)	4.1 (3.5-4.8)	0.26 (0.22-0.31)	16 (12-21)	0.87 (0.84-0.89)
Type of cancer							
Lung cancer	9	0.69 (0.62-0.75)	0.77 (0.73-0.81)	3.0 (2.6-3.5)	0.40 (0.33-0.49)	7 (6-10)	0.80 (0.76-0.83)
Colorectal cancer	7	0.75 (0.68-0.80)	0.81 (0.68-0.90)	4.0 (2.3-6.7)	0.31 (0.26-0.38)	13 (7-23)	0.81 (0.77-0.84)
Gastric cancer	6	0.82 (0.75-0.87)	0.73 (0.63-0.81)	3.1 (2.2-4.2)	0.24 (0.18-0.33)	13 (8-20)	0.85 (0.82-0.88)
Hepatocellular carcinoma	10	0.87 (0.78-0.93)	0.80 (0.73-0.86)	4.5 (3.0-6.7)	0.16 (0.09-0.30)	28 (11-73)	0.90 (0.87-0.92)
Ovarian cancer	7	0.64 (0.45-0.80)	0.91 (0.84-0.95)	7.1 (4.4-11.3)	0.39 (0.24-0.63)	18 (10-33)	0.90 (0.87-0.93)
Other cancers	31	0.81 (0.75-0.85)	0.81 (0.76-0.86)	4.3 (3.2-5.9)	0.24 (0.17-0.32)	18 (10-33)	0.88 (0.85-0.91)
Sample type							
Serum	59	0.79 (0.75-0.83)	0.82 (0.78-0.85)	4.3 (3.6-5.2)	0.25 (0.21-0.31)	17 (12-24)	0.88 (0.84-0.90)
Plasma	11	0.75 (0.68-0.81)	0.77 (0.72-0.82)	3.3 (2.7-4.0)	0.32 (0.26-0.41)	10 (7-14)	0.83 (0.79-0.86)
Isolation method							
Isolation kit	54	0.76 (0.73-0.80)	0.80 (0.76-0.83)	3.8 (3.3-4.4)	0.30 (0.26-0.34)	13 (10-16)	0.85 (0.82-0.88)
Ultracentrifugation	16	0.88 (0.74-0.95)	0.86 (0.78-0.92)	6.3 (3.6-11.2)	0.14 (0.06-0.33)	46 (11-187)	0.93 (0.90-0.95)
Sample size							
≥110	35	0.76 (0.70-0.81)	0.83 (0.78-0.86)	4.4 (3.4-5.6)	0.29 (0.23-0.37)	15 (10-23)	0.87 (0.83-0.89)
<110	35	0.81 (0.76-0.85)	0.79 (0.75-0.82)	3.8 (3.2-4.6)	0.24 (0.19-0.30)	16 (11-23)	0.86 (0.83-0.89)
Exosomal biomarkers							
miRNA	30	0.75 (0.68-0.80)	0.83 (0.78-0.87)	4.3 (3.4-5.5)	0.31 (0.24-0.38)	14 (10-20)	0.86 (0.83-0.89)
LncRNA	22	0.81 (0.76-0.85)	0.79 (0.73-0.83)	3.8 (3.1-4.7)	0.25 (0.20-0.31)	15 (11-21)	0.87 (0.83-0.89)
Protein	12	0.86 (0.66-0.95)	0.87 (0.78-0.93)	6.9 (3.2-14.6)	0.16 (0.05-0.46)	44 (7-263)	0.93 (0.90-0.95)
Other markers	6	0.78 (0.70-0.84)	0.70 (0.62-0.78)	2.6 (2.0-3.4)	0.32 (0.24-0.42)	8 (5-13)	0.80 (0.77-0.84)

Abbreviations: AUC, area under the curve; DOR, diagnostic odds ratio; NLR, negative likelihood ratio; PLR, positive likelihood ratio; SEN, sensitivity; SPE, specificity.

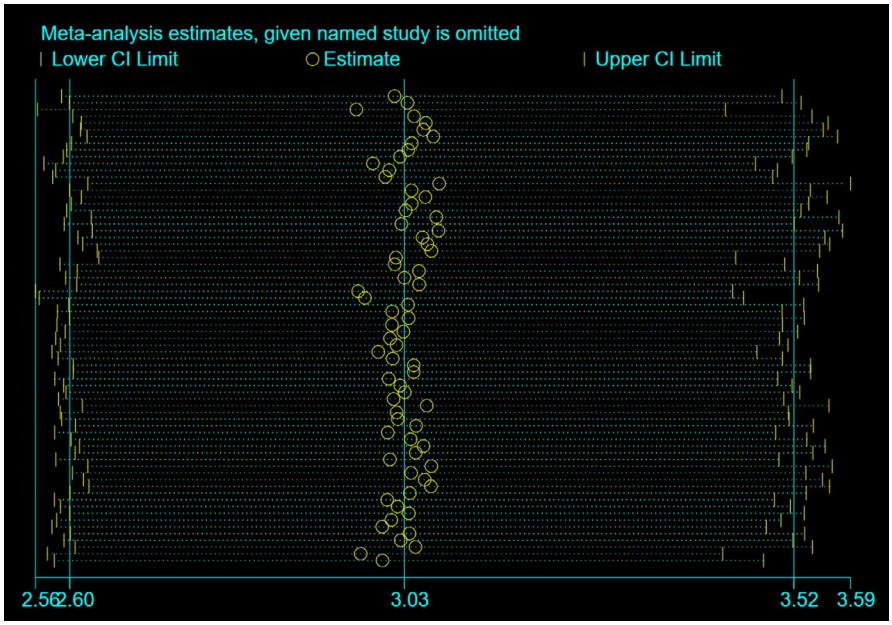

FIGURE 4 Sensitivity analysis of the overall pooled study

effective and standard technique of exosome isolation is urgently required. Suitable sample type and effective isolation method for exosomes detection may further improve the value of cancer diagnosis.

Among the various types of exosomal markers, superior SEN, SPE, and AUC were observed in exosomal protein, implying that exosomal proteins were probably the optimal biomarkers. In this subgroup analysis, the studies with other exosomal markers exhibited highest quality according to the QUADAS-2. Among other three types of exosomal biomarkers, the overall risks of bias were similar in each group. Owing to the variety of markers and cancer types, more large-scale studies are required to explore a specific type of exosomal biomarker with high diagnostic accuracy for a certain type of cancer.

We used Deeks' funnel plot to identify publication bias of enrolled studies, which did not show a very good symmetrical shape. Compared with other included studies, two studies deviated obviously from symmetry, suggesting a possible bias. These two studies were from the same article reported by Melo et al[39] After careful evaluation of this article, we believe that the possible bias was caused by statistical significance, because their studies revealed an AUC of 1.0 with a sensitivity and specificity of 100%. However, the *P*-value of funnel plot asymmetry test was .093, confirming that significant publication bias did not exist in general.

There were still some limitations that could not be neglected in this meta-analysis. First, most studies were from China, and the results might therefore not be universally applicable. Second, the inclusion of articles published only in English might result in publication bias. Third, there was significant heterogeneity among the included studies. Although we conducted subgroup analysis and meta-regression to explore the sources of heterogeneity, the results did not fully explain the potential heterogeneity. Thus, more well-designed and multicenter studies with larger sample size are needed to provide more valuable evidence.

In summary, the present meta-analysis indicated that circulating exosomes could be used as effective and minimally invasive biomarkers for distinguishing cancer patients from non-cancer individuals. Circulating exosomes showed higher diagnostic accuracy in patients with HCC or OC, serum-based samples, and exosomal proteins.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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