Extracellular vesicle biomarkers in circulation for the diagnosis of gastric cancer: A systematic review and meta-analysis

JINRU XUE[1*], SHAOYOU QIN[2*], NA REN[1], BO GUO[1], XIANQUAN SHI[3] and ERNA JIA[2]

Departments of [1]Thoracic Surgery and [2]Gastroenterology, China-Japan Union Hospital of Jilin University, Jilin, Changchun 130000; [3]Department of Ultrasound, Beijing Friendship Hospital of Capital Medical University, Beijing 100050, P.R. China

Received November 28, 2022; Accepted June 14, 2023

DOI: 10.3892/ol.2023.14009

Abstract. The prognosis of a gastric cancer (GC) diagnosis is poor due to the current lack of effective early diagnostic methods. Extracellular vesicle (EV) biomarkers have previously demonstrated strong diagnostic efficiency for certain types of cancer, including pancreatic and lung cancer. The present review aimed to summarize the diagnostic value of circulating EV biomarkers for early stage GC. The PubMed, Medline and Web of Science databases were searched from May 1983 to September 18, 2022. All studies that reported the diagnostic performance of EV biomarkers for GC were included for analysis. Overall, 27 studies were selected containing 2,831 patients with GC and 2,117 controls. A total of 58 EV RNAs were reported in 26 studies, including 39 microRNAs (miRNAs), 10 long non-coding RNAs (lncRNAs), five circular RNAs, three PIWI-interacting RNAs and one mRNA, in addition to one protein in the remaining study. Meta-analysis of the aforementioned studies demonstrated that the pooled sensitivity, specificity and AUC value of the total RNAs were 84, 67% and 0.822, respectively. The diagnostic values of miRNAs were consistent with the total RNA, as the pooled sensitivity, specificity and AUC value were 84, 67% and 0.808, respectively. The pooled sensitivity, specificity and AUC values of lncRNAs were 89, 69% and 0.872, respectively, markedly higher compared with that of miRNAs. A total of five studies reported the diagnostic performance of EV RNA panels for early stage GC and reported powerful diagnostic values with a pooled sensitivity, specificity and AUC value of 80, 77% and 0.879, respectively. Circulating EV RNAs could have the potential to be used in the future as effective, noninvasive biomarkers for early GC diagnosis. Further research in this field is necessary to translate these findings into clinical practice.

Correspondence to: Dr Erna Jia, Department of Gastroenterology, China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Jilin, Changchun 130000, P.R. China
E-mail: ernajia@jlu.edu.cn

*Contributed equally

Key words: extracellular vesicle, microRNA, long non-coding RNA, early diagnosis, gastric cancer

Introduction

Gastric cancer (GC) was the fifth most frequently diagnosed cancer and the third leading cause of cancer death worldwide in 2018 (1). The incidence and mortality of GC has decreased substantially in the United states and Western Europe over the past several decades; however, the number of new cases and current mortality rate contributes to ~50% of the global health problem, especially in East Asian countries (2). The 5-year survival rate of GC in Japan was ~50% in 2000, but in the United states, the 5-year survival rate ranges from 5-20%, as patients with GC are usually diagnosed at an advanced stage of disease with an increasing risk for tumor metastasis (3). Diagnosing GC at an early stage allows timely treatment interventions and can improve the overall prognosis for this type of malignancy (4).

The current recommended standard method for diagnosing GC is endoscopic biopsy (5). However, due to the discomfort caused, the invasive nature of the procedure and the high cost to the general public, the use of endoscopic biopsy for screening early stage GC is difficult in clinical practice (6). Serum biomarkers for GC, such as cancer antigen 724 and carcinoembryonic antigen, are associated with poor sensitivity and specificity for diagnosis (7,8). Furthermore, gastric precursor lesions, such as intestinal metaplasia and atypical hyperplasia, in addition to persistent *Helicobacter pylori* infection, increase the difficulty of the screening process for early GC (4). Thus, developing non-invasive and affordable screening approaches with a high specificity and sensitivity is important for clinical practice.

Extracellular vesicles (EVs) are secreted by numerous cell types and are nanostructured lipid bilayer membrane capsules (9). EVs contain numerous types of molecules, including nucleic acids such as DNA, mRNA and non-coding RNA, in addition to proteins, which enable communication from donor to recipient cells (9,10). EVs are present in certain biofluids, including plasma, serum, urine, gastric juice and saliva (10). Tumor-derived EVs modify tumor microenvironment, promote tumor progression, angiogenesis, metastasis

and immune evasion, and RNAs contained in tumor-derived EVs are associated with tumor progression, metastasis and aggressive tumor phenotypes (11,12). Previous studies reported that molecules contained in EVs, particularly exosome RNAs that can cause changes in gene expression, have the potential to serve as non-invasive, robust biomarkers for cancer screening (10,11,13). In the present study, the diagnostic performance of EV biomarkers for GC was summarized and analyzed, and subgroup analysis to determine the diagnostic accuracy of EV microRNAs (miRNAs/miRs) and long non-coding RNAs (lncRNAs) for GC was performed.

Materials and methods

Search strategy. The present review was performed according to the preferred reporting items for systematic reviews and meta-analysis (14). The PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (http://webofknowledge.com) and Medline (https://www.nlm.nih.gov/medline) online databases were searched (from May 1983 to September 18, 2022) for literature using the following key words: (Gastric OR stomach) AND (cancer OR carcinoma OR neoplasm OR tumor OR malignancy OR adenocarcinoma OR adenoma) AND (detection OR diagnosis OR biomarker OR marker OR sensitivity OR specificity OR area under the curve) AND (exosome OR extracellular vesicles OR exosomal OR membrane vesicles OR intracellular multivesicular endosomes). Duplicate studies were removed from the analysis.

Literature selection and data abstraction. Non-English language, non-human, non-original, non-related GC studies and articles not relevant to the research topic were excluded from the analysis. Subsequently, two authors independently screened all potential studies for inclusion into the meta-analysis. Inclusion criteria included: i) Studies that identified EV biomarkers for diagnosis of GC in plasma and serum; ii) patients with GC diagnosed according to histological examination; and iii) studies that reported the diagnostic value of EV biomarkers for GC, such as sensitivity, specificity, area under the curve (AUC) or receiver operator characteristic (ROC) curve. Any discrepancy surrounding study screening was resolved through discussion by the authors. Relevant information in the eligible studies was extracted using a pre-designed data collection table and the key information included was as follows: First author, year of publication, the country the study was performed in, study design, population characteristics (including sample size, mean age and sex distribution), type of blood-based specimen, GC stage, population composition of control group, names or panels of target biomarkers, detection method of target biomarkers, preparation approach of EVs, sensitivity, specificity and AUC value.

Quality assessment. The quality of each eligible study was evaluated using the diagnostic accuracy studies-2 checklist using Review Manager (v. 5.3; The Cochrane Collaboration) (15). The risk of bias and clinical application of eligible studies were assessed. Publication bias was assessed using Egger's test and the symmetry of the funnel plot was evaluated using R software (v. 3.5.3; R Foundation for Statistical Computing) (16).

Statistical analysis. If the values of sensitivity and specificity were not reported in the original study, the present study estimated these two diagnostic indicators based on ROC curves using OriginPro software (v. 9.0; OriginLab) according to the maximum Youden's index. The bivariate meta-analysis model was used to summarize the diagnostic value. The control groups contained healthy patients and/or those with benign diseases, and the present study analyzed the healthy patients; if the control groups contained healthy people and benign disease, they were analyzed as a whole. The sensitivity, specificity and AUC values of EV biomarkers were pooled for subgroup analysis using Meta-DiSc software (v.1.4) (17) using the random-effect model (18). Heterogeneity across studies was assessed using the c^2 and I^2 statistic. $P<0.05$ was considered to indicate a statistically significant difference and $I^2>50\%$ indicated a statistically significant heterogeneity.

Results

Database search results. A total of 1,045 studies were found as a result of the database searches and of these studies, 434 duplicates were detected and removed from the analysis (Fig. 1). After screening the title and abstracts of the remaining studies, 48 studies were selected for full review. Then, 21 studies were excluded due to the following criteria: i) Sample specimens used in 10 studies were not plasma or serum; ii) 8 studies reported no sensitivity, specificity or AUC value; and iii) 3 studies reported the EV biomarkers used to diagnose the recurrence of post-operation patients with GC. A total of 27 eligible studies were identified for further analysis.

Study characteristics. All eligible studies were performed in Asia and reported results from a total of 2,831 cases of GC and 2,117 controls (Tables I and II) (19-45). A singular study conducted prospective research (19), whereas the remaining studies were case control studies. The mean sample size of groups of patients with GC was 98 (range, 23-386 patients), whereas the mean sample size of the control groups was 62 (range, 12-151 patients). A total of 26 studies analyzed the diagnostic value of RNAs for GC: MiRNAs in 13 studies (22,24,25,28-33,37,40,42, 44,45), four of which performed validation tests (25,29,31,44); lncRNAs in nine studies (19,20,23,26,35,38,39,41,45); circular RNAs (circRNAs) in three studies (21,27,34); *P*-element induced wimpy testis-interacting RNAs (piRNAs) in one study (42); mRNA in one study (43); and a single study reported the diagnostic value of protein (36). A total of nine studies set a diagnostic cut-off value, which was determined using the Youden Index (19,23,27,31,33,34,39,41,45). A total of five studies reported the diagnostic value of RNA panels (25,27,29,31,40), two of which performed validation testing (25,29). A total of six studies reported the diagnostic performance of EV biomarkers for early stage GC (stage I/II) (31,33,34,36,38,41), of which one study performed validation testing (31).

With the development of EV extraction technologies, commercial exosome isolation kits were also used for the extraction of exosomes (46). From a total of 27 studies, 23 studies analyzed exosome biomarkers, and almost all extracted exosomes were reported to have a mean size of 30-200 nm and were positive for CD9, CD81, CD63 and/or TSG101 markers. The remaining four studies analyzed EV biomarkers.

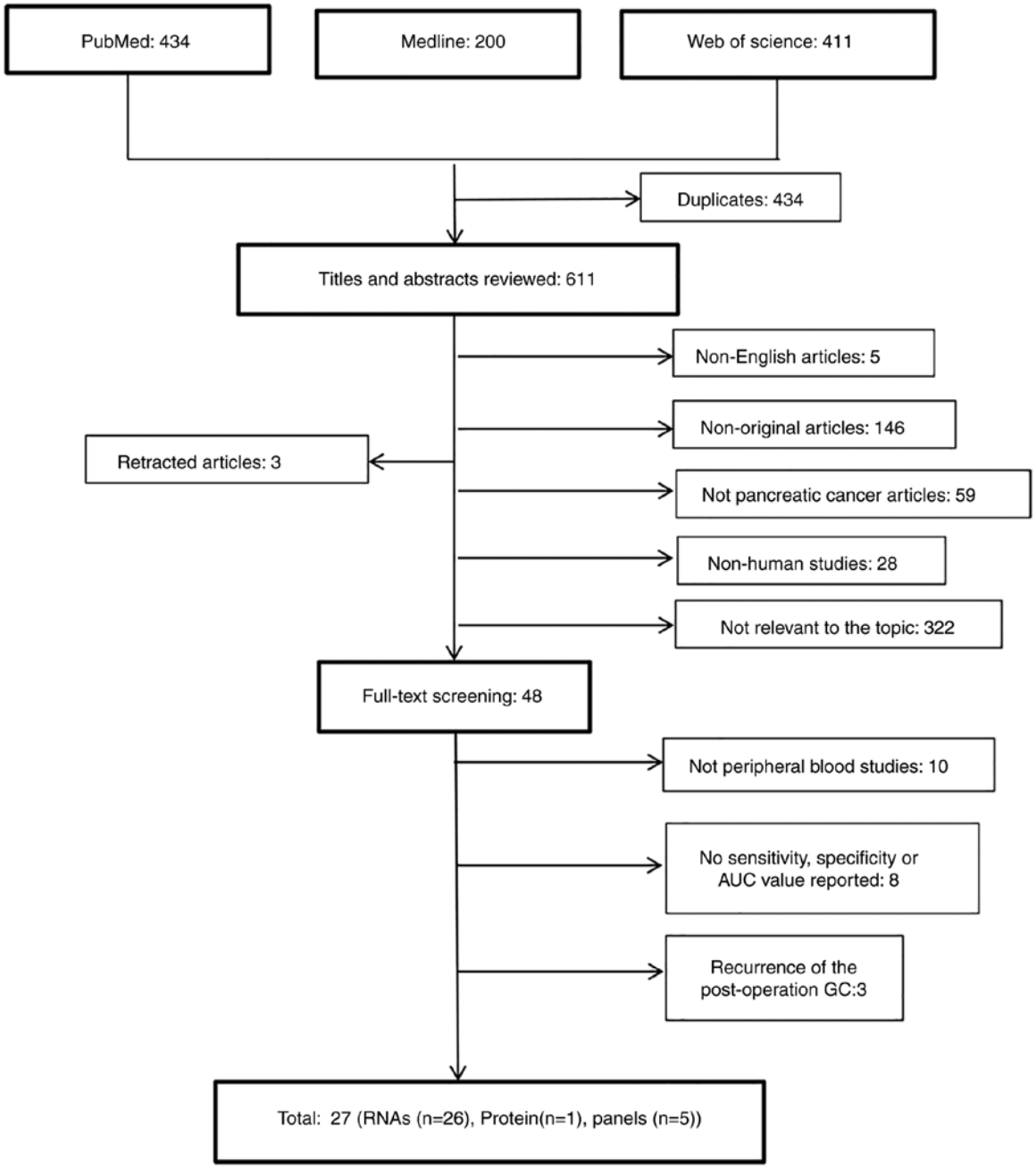

Figure 1. Flow chart of this study.

Quality assessment of included studies. Quality assessment of the analyzed studies was performed (Fig. S1). All 27 studies analyzed had a low risk of bias for the index test, four studies had unclear risk of reference standard, flow and timing. Quality assessment analysis also demonstrated that all studies had a low concern for application regarding the index test and reference standard. A total of four studies demonstrated an unclear risk of bias of patient selection and an unclear applicability concern of patient selection, due to non-random patient selection and a lack of basic patient information reported. Funnel plot analysis of the publication bias of studies demonstrated no statistically significant publication bias (Fig. S2).

Diagnostic performance. A total of 58 RNAs were reported in the 27 eligible studies. Of these RNAs, 39 were miRNAs, 10 were lncRNAs, five were circRNAs, three were piRNAs and one was mRNA. miR-19b-3p and miR-215-5p were reported in two studies and were consistently upregulated (Table III).

The median sensitivity, specificity and AUC value of the total RNAs were 74% (range, 43-100%), 86% (range, 51-99%) and 0.800 (range, 0.626-1.000), respectively. The median sensitivity, specificity and AUC value of miRNAs were 74% (range, 46-100%), 86% (range, 51-99%) and 0.783 (range, 0.540-1.000), respectively. A previous study by Tang *et al* (31) reported that miR-21-5p demonstrates diagnostic value for distinguishing patients with early stage GC from healthy controls with a sensitivity of 100% and a specificity of 91%. The median sensitivity, specificity and AUC value of lncRNAs were 82% (range, 43-97%), 84% (range, 34-97%) and 0.821 (range, 0.622-0.898), respectively. In the prospective study analyzed, the expression

Table I. Diagnostic performance of biomarkers in extracellular vesicles for gastric cancer.

A, miRNA

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
		Number	Mean age	Sex (% male)											
Wang *et al*, 2022	China	24/24		58/	Serum	I-IV	HC	RT-qPCR	miR-10401-3p	91	70	0.833	<0.001		(30)
									miR-1225-5p	83	86	0.832	<0.001		
									miR-6736-5p	95	63	0.814	<0.001		
Tang *et al*, 2022	China	120/60	49/		Plasma	I-IV	HC	RT-qPCR	miR-4741	77	87	0.855			(31)
									miR-32	79	99	0.946			
									miR-3149	51	93	0.768			
									miR-6727	66	96	0.892			
Kahroba *et al*, 2022	Iran	43/40		42/43	Serum		HC	RT-qPCR	miR-10a-5p	76	74	0.801			(40)
									miR-18a-5p	72	71	0.721			
									miR-19b-3p	74	69	0.780			
									miR-215-5p	68	67	0.736			
Zheng *et al*, 2021	China	168/50	61/		Serum	I-IV	HC	RT-qPCR	miR-590-5p	64	86	0.810		3.470	(22)
Zhang *et al*, 2021	China	118/70			Serum	I-IV	HC	RT-qPCR	miR-215-5p	69	97	0.866			(24)
		118/60					BGD			65	95	0.808			
Yang *et al*, 2021	China	108/108			Plasma	I-IV	HC		miR-195-5p	67	73	[a]0.762			(25)
									miR-211-5p	59	90	[a]0.798			
Lu *et al*, 2021	China	131/122		59/	Serum	I-IV	HC	RT-qPCR	miR-92a-3p	66	88	0.829	0.026		(37)
Wei *et al*, 2020	China	108/108	63/61	66/66	Serum		HC	RT-qPCR	miR-15b-3p	74	81	0.820			(28)
Ge *et al*, 2020	China	70/60	59/59	57/67	Serum	I-IV	HC	RT-qPCR	miR-1307-3p	81	77	0.845	<0.001		(42)
Tang *et al*, 2020	China	50/50	58/	76/	Serum	Ia-IIb	HC	RT-qPCR	Let-7g-5p	[b]54	[b]88	[a]0.756		<4.184	(31)
									miR-92b-3p	[b]58	[b]80	[a]0.714		<1.690	
									miR-146b-5p	[b]46	[b]83	[a]0.674		<0.674	
									miR-9-5p	[b]50	[b]84	[a]0.626		<0.626	
		36/12	57/	70/	Serum	Ia-IIb	HC	RT-qPCR	Let-7c-5p	80	91	0.886	0.0001		
									miR-101-3p	75	91	0.805	0.0057		
									miR-21-5p	100	91	1.000	0.0005		
									miR-26a-5p	86	91	0.961	0.0013		

Table I. Continued.

A, miRNA

First author, year	Country of study	GC cases/controls: Number	Mean age	Sex (% male)	Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
Chung *et al*, 2020	China	20/20		55/65	Serum	IIb-IIIb	HC	PBP	miR-423-5p			[a]0.780			(44)
									miR-484			[a]0.560			
									miR-186-5p			[a]0.540			
									miR-142-5p			[a]0.750			
									miR-320d			[a]0.740			
									miR-320a			[a]0.770			
									miR-320b			[a]0.720			
									miR-17-5p			[a]0.640			
		15/15		47/67		Ia-IIIb			miR-629-5p			0.750			
									miR-363-3p			0.780			
									miR-337-5p			0.750			
									miR-27a-3p			0.770			
Shi *et al*, 2019	China	85/50	60/58	66/68	Serum	I-IV	HC	RT-qPCR	miR-1246	82	86	0.911	<0.001	1.670	(33)
		28/50	/58	/68		I	HC			86	74	0.843	<0.001		
		28/30	/58	/67			BD			79	80	0.811			
Wang *et al*, 2017	China	110/110		55/	Serum		HC	RT-qPCR	miR-106a -5p	[c]63	[c]89	[a]0.786	<0.0001		(29)
									miR-19b-3p	[c]84	[c]51	[a]0.769	<0.0001		

B, lncRNA

First author, year	Country of study	GC cases/controls: Number	Mean age	Sex (% male)	Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
[b]Zhou *et al*, 2020	China	81/78	64/60	63/	Serum	I-IV	HC	RT-qPCR	LncRNA H19	74	84	0.849	<0.01	1.770	(19)
Zheng *et al*, 2020	China	60/60		63/	Plasma	I-IV	HC	RT-qPCR	Lnc-SLC2A12-10:1	69	75	0.776	<0.001		(20)
Xu *et al*, 2020	China	109/50		74/	Serum	I-IV	HC	RT-qPCR	LncRNA	[c]82	[c]94	0.892			(26)
		109/48		74/			GA		MIAT	[c]65	[c]89	0.787			

Table I. Continued.

B, lncRNA

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
		Number	Mean age	Sex (% male)											
Guo *et al*, 2020	China	386/151	61/	59/62	Serum	I-IV	HC	RT-qPCR	LncRNA-GC1	85	85	0.898		5.200	(41)
		386/37	61/54	59/70			CAG			89	88	0.842			
		386/48	61/55	59/65			IM			90	81	0.860			
		179/151		/62		I-II	HC			89	80	0.861			
		179/ 37	/54	/70			CAG			92	82	0.884			
		179/48	/55	/65			IM			81	88	0.885			
Piao *et al*, 2020	China	281/80			Plasma	I-IV	HC	RT-qPCR	CEBPA-ASI	[c]72	[c]87	0.824			(35)
Li *et al*, 2020	China	43/27		74/	Plasma	I-IV	HC	RT-qPCR	Lnc-GNAQ-6:1	84	56	0.736	<0.001	1.855	(39)
Cai *et al*, 2019	China	63/29		71/	Serum	I-IV	HC	RT-PqCR	Lnc RNA PCSK1-2:1	84	87	0.896		2.390	(45)
Zhao *et al*, 2018	China	126/120		55/	Serum	I-IV	HC		Lnc RNA HOTTIP	70	85	0.827		1.720	(23)
Lin *et al*, 2018	China	51/60	61/58	61/63	Plasma	Ia-IIb	HC	RT-qPCR	LncUEGC1	[c]97	[c]96	0.876	<0.0001		(38)
									lncUEGC2	[c]89	[c]58	0.758	<0.0001		
		23/60				I	HC		LncUEGC1	[c]96	[c]73	0.850	<0.0001		
									LncUEGC2	[c]74	[c]71	0.749	0.0456		
		23/18					CAG		LncUEGC1	[c]74	[c]88	0.841	0.0002		

C, circRNA

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
		Number	Mean age	Sex (% male)											
Zheng *et al*, 2022	China	60/60	63.7/	53/	Plasma	I-IV	CG, HC	RT-qPCR	circ_0015286	82	66	0.778	<0.001		(21)
Xiao *et al*, 2022	China	112/120			Serum		CG, TH, HC	RT-qPCR	circRNA Chr10q11	77	66	0.726		1.330	(27)
									circRNA Chr1p11	82	77	0.822		2.000	
									circRNA Chr7q11	80	59	0.749		1.070	
Shao *et al*, 2020	China	41/39			Plasma	I-II	HC	RT-qPCR	circ-0065149	49	90	0.640	0.031	6.430	(34)

Table I. Continued.

D, piRNA

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
		Number	Mean age	Sex (% male)											
Ge *et al*, 2020	China	70/60	59/59	57/67	Serum	I-IV	HC	RT-qPCR	piR-018569	44	97	0.732	<0.001		(42)
									piR-004918	43	95	0.754	<0.001		
									piR-019308	57	92	0.820	<0.001		

E, mRNA

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
		Number	Mean age	Sex (% male)											
Dong *et al*, 2019	China	119/31		75/	Serum	I-IV	HC	RT-qPCR	MT1-MMP mRNA	64	87	0.788			(43)

F, Protein

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection method	Marker	Sensitivity (%)	Specificity (%)	AUC	P-value	Cut-off value	(Refs.)
		Number	Mean age	Sex (% male)											
Okuda *et al*, 2021	Japan	93/90	72/72	69/67	Serum	I-IV	HC	ELISA	Dicer	[c]93	[c]34	0.622			(36)
		63/90	/72	/67		I				[c]92	[c]37	0.623			

[a]Results generated using the validation set; [b]prospective study; [c]estimated sensitivity or specificity values. EV, extracellular vesicle; AUC, area under the curve; HC, healthy control; BGD, benign gastric disease; BD, benign disease; GA, gastric adenoma; CAG, chronic atrophic gastritis; IM; intestinal metaplasia; CG, chronic gastritis; TH, typical hyperplasia; PBP, polymer-based precipitation.

Table II. Diagnostic performance of biomarker panels in extracellular vesicles for gastric cancer.

First author, year	Country of study	GC cases/controls			Sample type	GC stage	Health status of controls	Detection Method	Marker panel	Sensitivity (%)	Specificity (%)	AUC	P-value	(Refs.)
		Number	Mean age	Sex (% male)										
Kahroba *et al*, 2022	Iran	43/40		42/43	Serum		HC	RT-qPCR	A	73	72	0.813		(40)
Yang *et al*, 2021	China	108/108			Plasma	I-IV	HC		B	[b]68	[b]89	[a]0.820		(25)
Tang *et al*, 2020	China	50/50	58/	76/	Serum	Ia-IIb	HC	RT-qPCR	C	64	78	0.775	<0.001	(31)
									D	60	82	0.736	<0.001	
									E	44	88	0.705	0.0004	
									F	58	86	0.774	<0.001	
									G	60	82	0.774	<0.001	
									H	68	74	0.750	<0.001	
									I	60	84	0.773	<0.001	
Wang *et al*, 2017	China	110/110		55/	Serum		HC	RT-qPCR	J	[b]84	[b]51	[a]0.814	<0.0001	(29)
Xiao *et al*, 2022	China	112/120			Serum		CG/TH/HC	RT-qPCR	K	73	84	0.839		(27)

[a]Results from the validation dataset; [b]estimated sensitivity or specificity. Panel A, miR-10a-5p/miR-18a-5p/miR-19b-3p/miR-215-5p; Panel B, miR-195-5p/miR-211-5p; Panel C, miR-92b-3p/Let-7g-5p; Panel D, miR-92b-3p/miR-146b-5p; Panel E, miR-146b-5p/miR-9-5p; Panel F, miR-92b-3p/Let-7g-5p/miR-146b-5p; Panel G, miR-92b-3p/Let-7g-5p/miR-9-5p; Panel H, miR-92b-3p/miR-146b-5p/miR-9-5p; Panel I, miR-92b-3p/Let-7g-5p/miR-146b-5p/miR-9-5p; Panel J, miR-106a-5p/miR-19b-3p; Panel K, circRNA Chr10q11/circRNA Chr1p11/circRNA Chr7q11. AUC, area under the curve; HC, healthy control; CG, chronic gastritis; TH, typical hyperplasia.

Table III. Summary of studies reporting a significant association of miRNA with development of gastric cancer.

	First author, year (refs.)													
miRNA	Chung *et al*, 2020 (44)	Ge *et al*, 2020 (42)	Kahroba *et al*, 2022 (40)	Lu *et al*, 2021 (37)	Shi *et al*, 2019 (33)	Tang *et al*, 2022 (32)	Tang *et al*, 2020 (31)	Wang *et al*, 2022 (30)	Wang *et al*, 2017 (29)	Wei *et al*, 2020 (28)	Yang *et al*, 2021 (25)	Zhang *et al*, *2021* (24)	Zheng *et al*, 2021 (22)	Sum of studies reporting the mi RNA
miR-19b-3p			[a]Upregulated						[a]Upregulated					2
miR-215-5p			[a]Upregulated									[b]Upregulated		2
Let-7c-5p							[a]							1
Let-7g-5p							[a]Upregulated							1
miR-101-3p							[a]							1
miR-10401-3p								[b]Downregulated						1
miR-106a-5p									[a]Upregulated					1
miR-10a-5p			[a]Upregulated											1
miR-1225-5p								[b]Downregulated						1
miR-1246					[b]Upregulated									1
miR-1307-3p		[b]Upregulated												1
miR-142-5p	[b]													1
miR-146b-5p							[a]Upregulated							1
miR-15b-3p										[b]Upregulated				1
miR-17-5p	[b]													1
miR-186-5p	[b]													1
miR-18a-5p			[a]Upregulated											1
miR-195-5p											[b]			1
miR-211-5p											[b]			1
miR-21-5p							[a]							1
miR-26a-5p							[a]							1
miR-27a-3p	[b]													1
miR-3149						[b]Downregulated								1
miR-32						[b]Downregulated								1
miR-320a	[b]													1
miR-320b	[b]													1
miR-320d	[b]													1
miR-337-5p	[b]													1.

Table III. Continued.

	First author, year (refs.)													
miRNA	Chung *et al*, 2020 (44)	Ge *et al*, 2020 (42)	Kahroba *et al*, 2022 (40)	Lu *et al*, 2021 (37)	Shi *et al*, 2019 (33)	Tang *et al*, 2022 (32)	Tang *et al*, 2020 (31)	Wang *et al*, 2022 (30)	Wang *et al*, 2017 (29)	Wei *et al*, 2020 (28)	Yang *et al*, 2021 (25)	Zhang *et al*, *2021* (24)	Zheng *et al*, 2021 (22)	Sum of studies reporting the mi RNA
miR-363-3p	[b]													1
miR-423-5p	[b]													1
miR-4741						[b]Upregulated								1
miR-484	[b]													1
miR-590-5p													[b]Downregulated	1
miR-629-5p	[b]													1
miR-6727						[b]Downregulated								1
miR-6736-5p								[b]Downregulated						1
miR-92a-3p				[b]Downregulated										1
miR-92b-3p							[a]Upregulated							1
miR-9-5p							[a]Upregulated							1

[a]RNAs analyzed as part of a panel; [b]RNAs analyzed individually

.

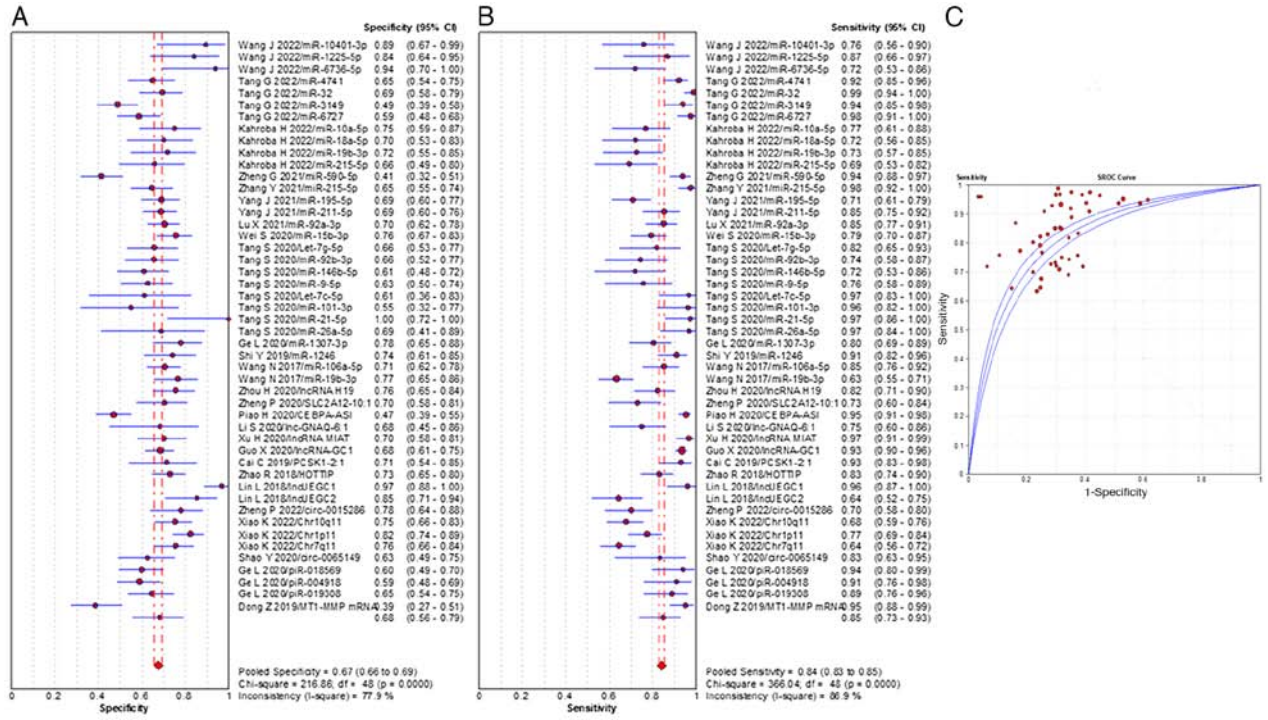

Figure 2. Summary of the diagnostic performance of extracellular vesicle RNAs for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

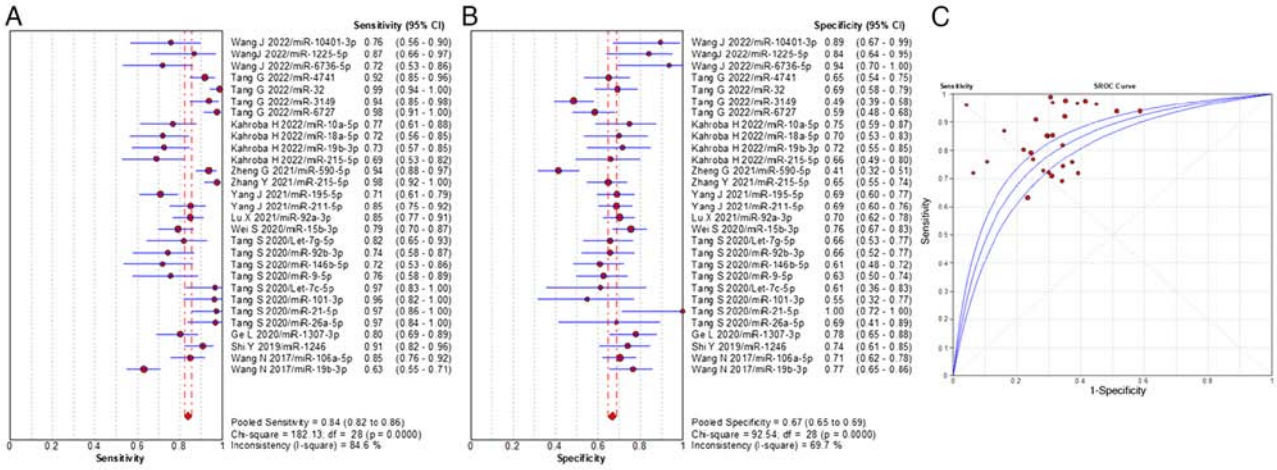

Figure 3. Summary of the diagnostic performance of extracellular vesicle microRNAs for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

level of exosome lncRNA H19 in serum was significantly upregulated in patients with GC and the AUC value was 0.849 (19). The optimal cut-off value was 1.770, with a sensitivity of 74% and a specificity of 84%. The median sensitivity, specificity and AUC value of circRNAs were 78% (range, 49-82%), 72% (range, 59-90%) and 0.774 (range, 0.640-0.893), respectively. The median sensitivity, specificity and AUC value of EV biomarker panels were 64% (range, 44-84%), 82% (range, 51-89%) and 0.774 (range, 0.705-0.839).

Meta-analysis. Meta-analysis was performed according to the type of molecule reported in the study. The diagnostic values of all EV total RNAs were summarized and the meta-analysis demonstrated that the pooled sensitivity, specificity and the AUC value were 84% (range, 95% CI 83-85%), 67% (range, 95% CI 66-69%) and 0.822, respectively (Fig. 2). The pooled sensitivity, specificity and AUC value of miRNAs were 84% (range, 95% CI 82-86%), 67% (range, 95% CI 65-69%) and 0.808, respectively (Fig. 3), which demonstrated consistent diagnostic accuracy with the EV total RNAs. The pooled sensitivity, specificity and AUC value of EV miRNA panels were 74% (range, 95% CI 70-78%), 69% (range, 95% CI 66-73%) and 0.784, respectively (Fig. 4). The miRNA panels demonstrated a lower diagnostic efficiency compared with the individual miRNAs. The pooled sensitivity, specificity and AUC value of EV lncRNAs were 89% (range, 95% CI 81-91%), 69% (range, 95% CI 66-72%) and 0.872, respectively (Fig. 5). The diagnostic efficiency of EV lncRNAs was higher

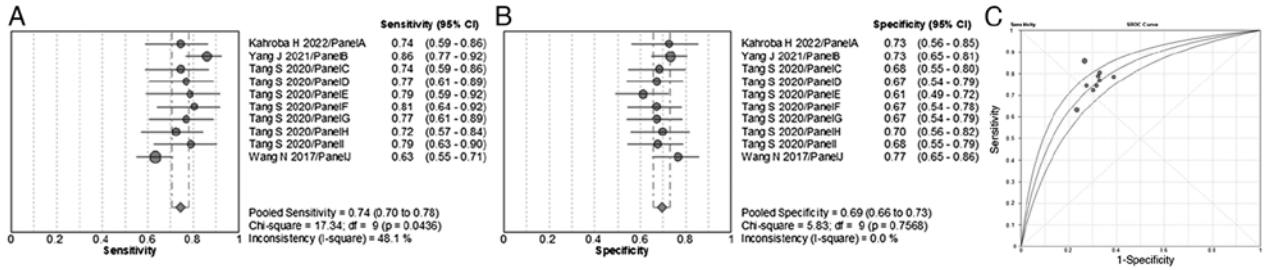

Figure 4. Summary of the diagnostic performance of extracellular vesicle micro RNA panels for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

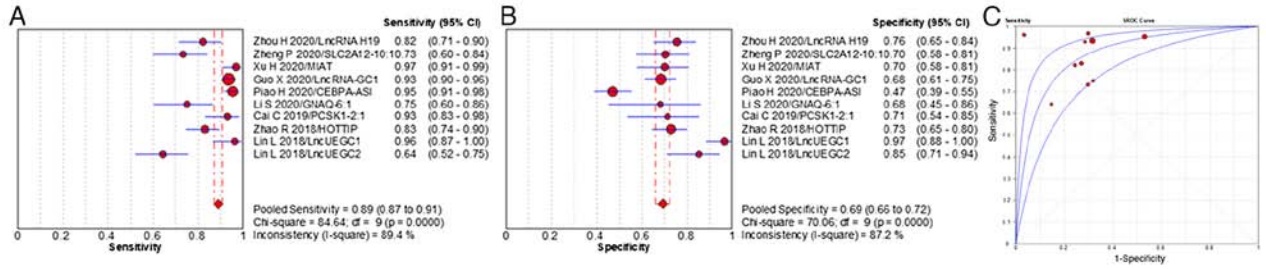

Figure 5. Summary of the diagnostic performance of extracellular vesicle long non-coding RNAs for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

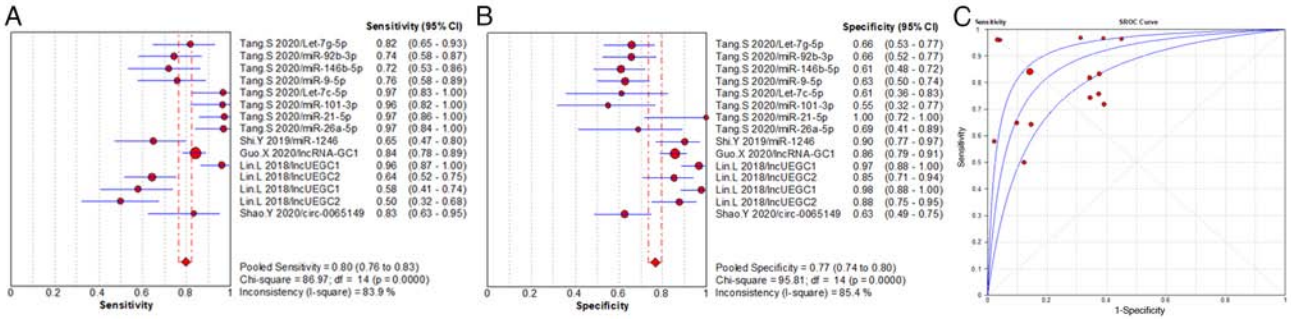

Figure 6. Summary of the diagnostic performance of extracellular vesicle RNAs for the detection of stage I/II gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

compared with that of EV miRNAs. A meta-analysis of early stage GC cases with 13 individual EV RNAs was performed. The pooled sensitivity, specificity and AUC value of individual EV RNAs were 80% (range, 95% CI 76-83%), 77% (range, 95% CI 74-80%) and 0.879, respectively (Fig. 6). Therefore, EV RNAs demonstrated a promising diagnostic efficiency for cases of early stage GC.

Discussion

In the present study, the diagnostic performance of EV biomarkers in plasma and serum for GC was analyzed. A total of 27 studies that assessed 58 EV RNAs and one EV protein for the diagnosis of GC were selected for meta-analysis. These studies reported results from 2,831 patients with GC and 2,117 healthy controls from 2017-2022. The meta-analysis demonstrated that out of the total number of miRNAs reported, miR-19b-3p and miR-215-5p were the only two miRNAs reported twice in the literature, therefore further studies validating the diagnostic value of these miRNAs are required. The diagnostic efficiency of EV miRNAs and lncRNAs were analyzed and EV lncRNAs demonstrated a higher diagnostic performance compared with EV miRNAs. When compared with the EV total RNAs, EV miRNAs demonstrated a similar diagnostic performance. Analysis of the studies that reported the diagnostic efficiency of EV biomarkers for early stage GC demonstrated that EV biomarkers showed promise for the diagnosis of early stage GC. However, in the present review, the majority of the studies analyzed were case controls; therefore, well-designed prospective studies are needed to improve the diagnostic accuracy of EV biomarkers for GC.

Late diagnosis is a major reason for the poor survival rate of GC patients (2). In China, the proportion of GC patients diagnosed at an early stage of disease was 9% in 2008 (47). The survival rate of patients with early stage GC ranges from 60-80% compared with 15-24% of patients with advanced stage GC (48). Therefore, it is crucial to find a novel, non-invasive and efficient diagnostic strategy of screening for early stage GC. In

the present study, the diagnostic performance of EV RNAs for early stage GC was analyzed and it was demonstrated that EV RNAs demonstrated an AUC value of 0.879 and showed a high diagnostic efficiency for early stage GC. Lin *et al* (38) reported that in patients with stage I GC, EV lncUEGC1 effectively distinguished 23 patients with GC from 60 healthy controls with an AUC value of 0.850. In a Chinese population, the presence of EV lncRNA-GC1 is reported to be sufficient for discriminating between patients with early stage GC and healthy controls, with a sensitivity of 89% and a specificity of 80% (41). Moreover, detection of lncRNA-GC1 is sufficient for discriminating patients with early stage GC from those with precancerous lesions, with a sensitivity of 92% and a specificity of 82% (41). Nevertheless, as there was no repetition study to report the same EV RNAs for early stage GC, it is essential to perform repetitive researches on the same RNAs for early stage GC.

In previous years, EV-derived RNAs as novel, effective, non-invasive biomarkers for the diagnosis of GC have attracted increasing attention (49). RNAs are one of the most abundant types of molecule present in EVs (50). EV RNAs are reported to have a high stability in the blood due to the ability of EVs to protect RNA from degradation by RNases (51). EV RNAs can regulate gene expression at post-transcriptional, transcriptional and translational levels by modulating relevant signaling pathways in the tumor microenvironment, effecting both angiogenesis and metastasis (52). Previous studies have reported that EV-derived RNAs serve critical roles in the tumorigenesis and metastasis of GC (53), and the most promising EV RNAs used as diagnostic biomarkers are miRNAs, lncRNAs and circRNAs (52). In the present study, EV miRNAs and lncRNAs were the most frequently reported type of biomarker, and the diagnostic performance of lncRNAs was higher compared with the diagnostic performance of miRNAs. All lncRNAs were reported once in the literature and no replicated studies were found; therefore, further studies demonstrating the diagnostic value of these lncRNAs are needed to verify these results. In the present study, miR-19b-3p and miR-215-5p were reported twice in the literature, were both consistently upregulated and miR-19b-3p was also tested in a validation study. This result suggested that miRNAs are more promising diagnostic biomarkers for GC, comparing to lncRNAs. A total of three studies reported five EV circRNAs that had a powerful diagnostic efficiency for GC (20,27,34). circRNAs are a class of RNA with a unique closed loop-structure structure without 5' and 3' ends, which increases RNase R resistance compared with other non-coding RNAs (ncRNAs) (54,55). Based on the unique structure of circRNAs, EV circRNA could be a more efficient non-invasive diagnostic marker for GC compared with other EV ncRNAs. circRNAs are an endogenous RNAs with a covalently closed cyclic structure, and owing to this structure, circRNAs are more resistant to RNA exonuclease than linear RNAs (56). However, as the research on the use of EV circRNAs as a biomarker for GC tumors is currently limited, further studies are needed to validate this hypothesis.

In previous studies, compared with individual EV biomarkers, EV biomarker panels have been reported to show a greater efficacy for the diagnosis of lung and pancreatic cancer (57,58). Previous studies reported that EV miRNA panels demonstrate a higher efficiency for distinguishing patients with GC from healthy controls, with an AUC value of >0.800, while the AUC value is <0.800 for the corresponding individual EV miRNAs (25,29). By contrast, previous studies reported that the diagnostic value of EV miRNA panels are similar to the corresponding individual EV miRNAs (31,40). In present study, EV miRNA panels did not demonstrate a higher diagnostic value compared with individual miRNAs, consistent with the previous reports, which could be due to fewer studies focused on EV miRNA panels being included in the meta-analysis. In the present study, two miRNAs (miR-19b-3p and miR-215-5p) were reported twice in the literature and were both included in panels A and J. miR-19b-3p inhibits GC cell proliferation, migration and invasion by negatively regulating neuropilin-1 (NRP1), and the miR-19b-3p/NRP1 axis can regulate the epithelial-to-mesenchymal transition and focal adhesions that occur in GC, which could contribute to the development and progression of GC (59). Previous studies reported that miR-215-5p expression is significantly upregulated in GC tissues and cell lines, and that the aberrant expression of miR-215-5p promotes the malignancy of GC cells, which results in enhanced carcinogenesis (60,61). Overexpression of miR-215-5p stimulates the migration and invasion of cancer cells via the degradation of Forkhead Box Protein O1 (62). Therefore, miRNAs that have been repeatedly verified were deemed more suitable than other RNAs to construct a biomarker panel to improve the robustness and diagnostic accuracy of these panels. Previous studies reported that both EV proteins alone and EV proteins combined with miRNA demonstrate a powerful diagnostic efficiency for certain types of lung and pancreatic cancer (58,63). In the present study, only one EV protein was reported, for which the diagnostic performance was not promising; however, the protein demonstrated a high sensitivity for the diagnosis of GC (36). Therefore, EV proteins should be studied to further analyze the diagnostic efficiency of EV biomarker panels for GC.

Currently, circulating tumor DNAs (ctDNAs), circulating tumor cells (CTCs) and EVs, particularly exosomes, are the main components that have been mostly analyzed in liquid biopsy samples (64,65). A previous study reported that 10^9 exosome particles can be detected in 1 ml of blood, while only a few CTCs are detected in the same sample volume (66). The expression level of exosomes in biofluids is higher compared with that of CTCs or ctDNAs and exosomes are more stable than CTCs and ctDNAs due to the presence of lipid bilayers (66,67). Therefore, compared with CTCs and ctDNAs, exosomes may potentially be a more promising non-invasive biomarker tested for in liquid biopsy.

Currently, ultralcentrifugation (UC) is the recommended and most widely used extraction method for EV isolation and separation (68). However, there is presently no standardized protocol for the centrifugation time, centrifugal force, or rotor type, which can influence the purity and yield of isolated EVs (69,70). Of the studies included for meta-analysis in the present study, one study reported the use of UC to isolate EVs and no uniform centrifugal time or number of centrifugations were reported, which could affect purity and concentration of the target EVs isolated. Furthermore, due to the high time consumed, high cost, potential for structural damage of EVs, aggregation into blocks and lipoprotein co-separation associated with UC, this EV isolation method is not conducive to clinical applications (71,72). With the advent of advanced sequencing techniques, the development

of commercial exosome isolation kits occurred, which can be used in the extraction of exosomes from plasma and serum (46). EV isolation methods in the majority of studies included in the present meta-analysis used commercial exosome isolation kits, with transmission electron microscopy and western blotting used to further verify exosome identity (42,43,45). These results suggest that commercial exosome isolation kits can be used to efficiently extract exosomes from both plasma and serum samples. Additional techniques used to isolate EVs from human bodily fluids include size-based isolation techniques, immunoaffinity chromatography and other new isolation techniques (such as immunomagnetic beads conjugated with combined antibodies) can also be used for the extraction of EVs, which might be suitable for extractions from plasma and serum; however, there are currently a limited number of studies that report using these techniques (73-75). Thus, it is necessary to develop a unified, convenient and effective method for the extraction of EVs from plasma and serum samples.

There were a number of limitations in the present study. Firstly, all studies selected for meta-analysis performed analysis on samples obtained from Asian populations, therefore, there was an absence of samples taken from other ethnicities. Secondly, plasma and serum were both used as potential sources of circulating EVs; however, further verification is required to determine if one is a more suitable source of EVs compared with the other. There was no standardized method reported for EV extraction and the cost related to EV detection was also not reported. Thus, further research is required to determine an effective standard method for extraction and detection of EVs. Thirdly, from a total of 27 studies selected for meta-analysis, just nine studies reported the cut-off values used, no studies reported the cut-off value of the same biomarker, thus there was no uniform cut-off value used as a standard reference. Finally, all studies selected for meta-analysis were case studies, with the exception of a single prospective study. Therefore, further prospective research should be conducted to analyze the diagnostic efficiency of EV biomarkers for GC.

The detection of EV RNAs in plasma and serum demonstrated promise for use as novel noninvasive biomarkers in the early diagnosis of GC in Asian populations. Future studies are required to further research the diagnostic efficacy of EV RNAs and EV RNA panels.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors read and approved the final version of the manuscript. EJ contributed to the conception and design of the study. JX and SQ analyzed data, performed the statistical analysis and drafted the manuscript. NR, BG and XS acquired data and revised the manuscript critically for important intellectual content. JX, SQ, NR, BG, XS and EJ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC and Lordick F: Gastric cancer. Lancet 396: 635-648, 2020.
3. Hochwald SN, Kim S, Klimstra DS, Brennan MF and Karpeh MS: Analysis of 154 actual five-year survivors of gastric cancer. J Gastrointest Surg 4: 520-525, 2000.
4. Karimi P, Islami F, Anandasabapathy S, Freedman ND and Kamangar F: Gastric cancer: Descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiol Biomarkers Prev 23: 700-713, 2014.
5. Young E, Philpott H and Singh R: Endoscopic diagnosis and treatment of gastric dysplasia and early cancer: Current evidence and what the future may hold. World J Gastroenterol 27: 5126-5151, 2021.
6. Mabe K, Inoue K, Kamada T, Kato K, Kato M and Haruma K: Endoscopic screening for gastric cancer in Japan: Current status and future perspectives. Dig Endosc 34: 412-419, 2022.
7. Chen C, Chen Q, Zhao Q, Liu M and Guo J: Value of combined detection of serum CEA, CA72-4, CA19-9, CA15-3 and CA12-5 in the diagnosis of gastric cancer. Ann Clin Lab Sci 47: 260-263, 2017.
8. Jelski W and Mroczko B: Molecular and circulating biomarkers of gastric cancer. Int J Mol Sci 23: 7588, 2022.
9. Kalluri R and LeBleu VS: The biology, function, and biomedical applications of exosomes. Science 367: eaau6977, 2020.
10. Xu R, Rai A, Chen M, Suwakulsiri W, Greening DW and Simpson RJ: Extracellular vesicles in cancer-implications for future improvements in cancer care. Nat Rev Clin Oncol 15: 617-638, 2018.
11. Kinoshita T, Yip KW, Spence T and Liu FF: MicroRNAs in extracellular vesicles: potential cancer biomarkers. J Hum Genet 62: 67-74, 2017.
12. Qian Z, Shen Q, Yang X, Qiu Y and Zhang W: The role of extracellular vesicles: An epigenetic view of the cancer microenvironment. Biomed Res Int 2015: 649161, 2015.
13. Xu YX, Pu SD, Li X, Yu ZW, Zhang YT, Tong XW, Shan YY and Gao XY: Exosomal ncRNAs: Novel therapeutic target and biomarker for diabetic complications. Pharmacol Res 178: 106135, 2022.
14. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J and Moher D: The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. PLoS Med 6: e1000100, 2009.
15. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB,Leeflang MM, Sterne JA and Bossuyt PM; QUADAS-2 Group: QUADAS-2: A revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med 155: 529-536, 2011.
16. Egger M, Davey Smith G, Schneider M and Minder C: Bias in meta-analysis detected by a simple, graphical test. BMJ 315: 629-634, 1997.
17. Zamora J, Abraira V, Muriel A, Khan KS and Coomarasamy A: Meta-DiSc: A software for meta-analysis of test accuracy data. BMC Med Res Methodol 6: 31, 2006.

18. Dias S, Welton NJ, Sutton AJ and Ades AE: NICE DSU technical support document 2: A generalised linear modelling framework for pairwise and network meta-analysis of randomised controlled trials [Internet]. NICE Decision Support Unit Technical Support Documents. London: National Institute for Health and Care Excellence (NICE), 2014.
19. Zhou H, Shen W, Zou H, Lv Q and Shao P: Circulating exosomal long non-coding RNA H19 as a potential novel diagnostic and prognostic biomarker for gastric cancer. J Int Med Res 48: 300060520934297, 2020.
20. Zheng P, Zhang H, Gao H, Sun J, Li J, Zhang X, Gao L, Ma P and Li S: Plasma exosomal long noncoding RNA lnc-SLC2A12-10:1 as a novel diagnostic biomarker for gastric cancer. Onco Targets Ther 13: 4009-4018, 2020.
21. Zheng P, Gao H, Xie X and Lu P: Plasma exosomal hsa_circ_0015286 as a potential diagnostic and prognostic biomarker for gastric cancer. Pathol Oncol Res 28: 1610446, 2022.
22. Zheng GD, Xu ZY, Hu C, Lv H, Xie HX, Huang T, Zhang YQ, Chen GP, Fu YF and Cheng XD: Exosomal miR-590-5p in serum as a biomarker for the diagnosis and prognosis of gastric cancer. Front Mol Biosci 8: 636566, 2021.
23. Zhao R, Zhang Y, Zhang X, Yang Y, Zheng X, Li X, Liu Y and Zhang Y: Exosomal long noncoding RNA HOTTIP as potential novel diagnostic and prognostic biomarker test for gastric cancer. Mol Cancer 17: 68, 2018.
24. Zhang Y, Huang F, Xu N, Wang J, Li D and Yin L: Overexpression of serum extracellular vesicle microRNA-215-5p is associated with early tumor recurrence and poor prognosis of gastric cancer. Clinics (Sao Paulo) 76: e2081, 2021.
25. Yang J, Li X, Wei S, Peng L, Sang H, Jin D, Chen M, Dang Y and Zhang G: Evaluation of the diagnostic potential of a plasma exosomal miRNAs panel for gastric cancer. Front Oncol 11: 683465, 2021.
26. Xu H, Zhou J, Tang J, Min X, Yi T, Zhao J and Ren Y: Identification of serum exosomal lncRNA MIAT as a novel diagnostic and prognostic biomarker for gastric cancer. J Clin Lab Anal 34: e23323, 2020.
27. Xiao K, Li S, Ding J, Wang Z, Wang D, Cao X, Zhang Y and Dong Z: Expression and clinical value of circRNAs in serum extracellular vesicles for gastric cancer. Front Oncol 12: 962831, 2022.
28. Wei S, Peng L, Yang J, Sang H, Jin D, Li X, Chen M, Zhang W, Dang Y and Zhang G: Exosomal transfer of miR-15b-3p enhances tumorigenesis and malignant transformation through the DYNLT1/Caspase-3/Caspase-9 signaling pathway in gastric cancer. J Exp Clin Cancer Res 39: 32, 2020.
29. Wang N, Wang L, Yang Y, Gong L, Xiao B and Liu X: A serum exosomal microRNA panel as a potential biomarker test for gastric cancer. Biochem Biophys Res Commun 493: 1322-1328, 2017.
30. Wang JF, Jiang YM, Zhan WH, Ye SP, Li TY and Zhang JN: Screening of serum exosomal miRNAs as diagnostic biomarkers for gastric cancer using small RNA sequencing. J Oncol 2022: 5346563, 2022.
31. Tang S, Cheng J, Yao Y, Lou C, Wang L, Huang X and Zhang Y: Combination of four serum exosomal MiRNAs as novel diagnostic biomarkers for early-stage gastric cancer. Front Genet 11: 237, 2022.
32. Tang G, Wang J, Dong W, Dai K and Du J: Exosomal miRNA expression profiling and the roles of exosomal miR-4741, miR-32, miR-3149, and miR-6727 on gastric cancer progression. Biomed Res Int 2022: 1263812, 2022.
33. Shi Y, Wang Z, Zhu X, Chen L, Ma Y, Wang J, Yang X and Liu Z: Exosomal miR-1246 in serum as a potential biomarker for early diagnosis of gastric cancer. Int J Clin Oncol 25: 89-99, 2020.
34. Shao Y, Tao X, Lu R, Zhang H, Ge J, Xiao B, Ye G and Guo J: Hsa_circ_0065149 is an indicator for early gastric cancer screening and prognosis prediction. Pathol Oncol Res 26: 1475-1482, 2020.
35. Piao HY, Guo S, Wang Y and Zhang J: Exosomal long non-coding RNA CEBPA-AS1 inhibits tumor apoptosis and functions as a non-invasive biomarker for diagnosis of gastric cancer. Onco Targets Ther 13: 1365-1374, 2020.
36. Okuda Y, Shimura T, Iwasaki H, Katano T, Kitagawa M, Nishigaki R, Fukusada S, Natsume M, Tanaka M, Nishie H, *et al*: Serum exosomal dicer is a useful biomarker for early detection of differentiated gastric adenocarcinoma. Digestion 102: 640-649, 2021.
37. Lu X, Lu J, Wang S, Zhang Y, Ding Y, Shen X, Jing R, Ju S, Chen H and Cong H: Circulating serum exosomal miR-92a-3p as a novel biomarker for early diagnosis of gastric cancer. Future Oncol 17: 907-919, 2021.
38. Lin LY, Yang L, Zeng Q, Wang L, Chen ML, Zhao ZH, Ye GD, Luo QC, Lv PY, Guo QW, *et al*: Tumor-originated exosomal lncUEGC1 as a circulating biomarker for early-stage gastric cancer. Mol Cancer 17: 84, 2018.
39. Li S, Zhang M, Zhang H, Hu K, Cai C, Wang J, Shi L, Ma P, Xu Y and Zheng P: Exosomal long noncoding RNA lnc-GNAQ-6:1 may serve as a diagnostic marker for gastric cancer. Clin Chim Acta 501: 252-257, 2020.
40. Kahroba H, Samadi N, Mostafazadeh M, Hejazi MS, Sadeghi MR, Hashemzadeh S, Eftekhar Sadat AT and Karimi A: Evaluating the presence of deregulated tumoral onco-microRNAs in serum-derived exosomes of gastric cancer patients as noninvasive diagnostic biomarkers. Bioimpacts 12: 127-138, 2022.
41. Guo X, Lv X, Ru Y, Zhou F, Wang N, Xi H, Zhang K, Li J, Chang R, Xie T, *et al*: Circulating exosomal gastric cancer-associated long noncoding RNA1 as a biomarker for early detection and monitoring progression of gastric cancer: A multiphase study. JAMA Surg 155: 572-579, 2020.
42. Ge L, Zhang N, Li D, Wu Y, Wang H and Wang J: Circulating exosomal small RNAs are promising non-invasive diagnostic biomarkers for gastric cancer. J Cell Mol Med 24: 14502-14513, 2020.
43. Dong Z, Sun X, Xu J, Han X, Xing Z, Wang D, Ge J, Meng L and Xu X: Serum membrane type 1-matrix metalloproteinase (MT1-MMP) mRNA protected by exosomes as a potential biomarker for gastric cancer. Med Sci Monit 25: 7770-7783, 2019.
44. Chung KY, Quek JM, Neo SH and Too HP: Polymer-based precipitation of extracellular vesicular miRNAs from serum improve gastric cancer miRNA biomarker performance. J Mol Diagn 22: 610-618, 2020.
45. Cai C, Zhang H, Zhu Y, Zheng P, Xu Y, Sun J, Zhang M, Lan T, Gu B, Li S and Ma P: Serum exosomal long noncoding RNA pcsk2-2:1 as a potential novel diagnostic biomarker for gastric cancer. Onco Targets Ther 12: 10035-10041, 2019.
46. Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, Khaled ML, Seremwe M, Dismuke WM, Bieberich E, Stamer WD, *et al*: A Comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS One 12: e0170628, 2017.
47. Hu Y, Fang JY and Xiao SD: Can the incidence of gastric cancer be reduced in the new century? J Dig Dis 14: 11-15, 2013.
48. Wang J, Yu JC, Kang WM, Ma ZQ: Treatment strategy for early gastric cancer. Surg Oncol 21: 119-123, 2012.
49. Tang XH, Guo T, Gao XY, Wu XL, Xing XF, Ji JF and Li ZY: Exosome-derived noncoding RNAs in gastric cancer: Functions and clinical applications. Mol Cancer 20: 99, 2021.
50. Zhang J, Li S, Li L, Li M, Guo C, Yao J and Mi S: Exosome and exosomal microRNA: Trafficking, sorting, and function. Genomics Proteomics Bioinformatics 13: 17-24, 2015.
51. Zhu L, Li J, Gong Y, Wu Q, Tan S, Sun D, Xu X, Zuo Y, Zhao Y, Wei YQ, *et al*: Exosomal tRNA-derived small RNA as a promising biomarker for cancer diagnosis. Mol Cancer 18: 74, 2019.
52. Li C, Ni YQ, Xu H, Xiang QY, Zhao Y, Zhan JK, He JY, Li S and Liu YS: Roles and mechanisms of exosomal non-coding RNAs in human health and diseases. Signal Transduct Target Ther 6: 383, 2021.
53. Anastasiadou E, Jacob LS and Slack FJ: Non-coding RNA networks in cancer. Nat Rev Cancer 18: 5-18, 2018.
54. Saaoud F, Drummer I V C, Shao Y, Sun Y, Lu Y, Xu K, Ni D, Jiang X, Wang H and Yang X: Circular RNAs are a novel type of non-coding RNAs in ROS regulation, cardiovascular metabolic inflammations and cancers. Pharmacol Ther 220: 107715, 2021.
55. Long F, Lin Z, Li L, Ma M, Lu Z, Jing L, Li X and Lin C: Comprehensive landscape and future perspectives of circular RNAs in colorectal cancer. Mol Cancer 20: 26, 2021.
56. Zhang Y, Liang W, Zhang P, Chen J, Qian H, Zhang X and Xu W: Circular RNAs: Emerging cancer biomarkers and targets. J Exp Clin Cancer Res 36: 152, 2017.
57. Yu H, Guan Z, Cuk K, Brenner H and Zhang Y: Circulating microRNA biomarkers for lung cancer detection in Western populations. Cancer Med 7: 4849-4862, 2018.
58. Jia E, Ren N, Shi X, Zhang R, Yu H, Yu F, Qin S and Xue J: Extracellular vesicle biomarkers for pancreatic cancer diagnosis: A systematic review and meta-analysis. BMC Cancer 22: 573, 2022.
59. Wei Y, Guo S, Tang J, Wen J, Wang H, Hu X and Gu Q: MicroRNA-19b-3p suppresses gastric cancer development by negatively regulating neuropilin-1. Cancer Cell Int 20: 193, 2020.
60. Chen Z, Liu K, Li L, Chen Y and Du S: miR-215 promotes cell migration and invasion of gastric cancer by targeting Retinoblastoma tumor suppressor gene 1. Pathol Res Pract 213: 889-894, 2017.

61. Li N, Zhang QY, Zou JL, Li ZW, Tian TT, Dong B, Liu XJ, Ge S, Zhu Y, Gao J and Shen L: miR-215 promotes malignant progression of gastric cancer by targeting RUNX1. Oncotarget 7: 4817-4828, 2016.
62. Zang Y, Wang T, Pan J and Gao F: miR-215 promotes cell migration and invasion of gastric cancer cell lines by targeting FOXO1. Neoplasma 64: 579-587, 2017.
63. Melo SA, Luecke LB, Kahlert C, Fernandez AF, Gammon ST, Kaye J, LeBleu VS, Mittendorf EA, Weitz J, Rahbari N, *et al*: Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature 523: 177-182, 2015.
64. Vaidyanathan R, Soon RH, Zhang P, Jiang K and Lim CT: Cancer diagnosis: From tumor to liquid biopsy and beyond. Lab Chip 19: 11-34, 2018.
65. Ye Q, Ling S, Zheng S and Xu X: Liquid biopsy in hepatocellular carcinoma: Circulating tumor cells and circulating tumor DNA. Mol Cancer 18: 114, 2019.
66. Cai X, Janku F, Zhan Q and Fan JB: Accessing genetic information with liquid biopsies. Trends Genet 31: 564-575, 2015.
67. Yu W, Hurley J, Roberts D, Chakrabortty SK, Enderle D, Noerholm M, Breakefield XO and Skog JK: Exosome-based liquid biopsies in cancer: Opportunities and challenges. Ann Oncol 32: 466-477, 2021.
68. Sidhom K, Obi PO and Saleem A: A review of exosomal isolation methods: Is size exclusion chromatography the best option? Int J Mol Sci 21: 6466, 2020.
69. Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV and Govorun VM: Corrigendum: Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Sci Rep 6: 21447, 2016.
70. Cvjetkovic A, Lötvall J and Lässer C: The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. J Extracell Vesicles 3, 2014.
71. Yang XX, Sun C, Wang L and Guo XL: New insight into isolation, identification techniques and medical applications of exosomes. J Control Release 308: 119-129, 2019.
72. Böing AN, van der Pol E, Grootemaat AE, Coumans FA, Sturk A and Nieuwland R: Single-step isolation of extracellular vesicles by size-exclusion chromatography. J Extracell Vesicles 3, 2014.
73. Cha BS, Park KS and Park JS: Signature mRNA markers in extracellular vesicles for the accurate diagnosis of colorectal cancer. J Biol Eng 14: 4, 2020.
74. Fu F, Jiang W, Zhou L and Chen Z: Circulating exosomal miR-17-5p and miR-92a-3p predict pathologic stage and grade of colorectal cancer. Transl Oncol 11: 221-232, 2018.
75. Nazarova I, Slyusarenko M, Sidina E, Nikiforova N, Semiglazov V, Semiglazova T, Aigner A, Rybakov E and Malek A: Evaluation of colon-specific plasma nanovesicles as new markers of colorectal cancer. Cancers (Basel) 13: 3905, 2021.