

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

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**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.

## 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

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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  $\chi^2$  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.

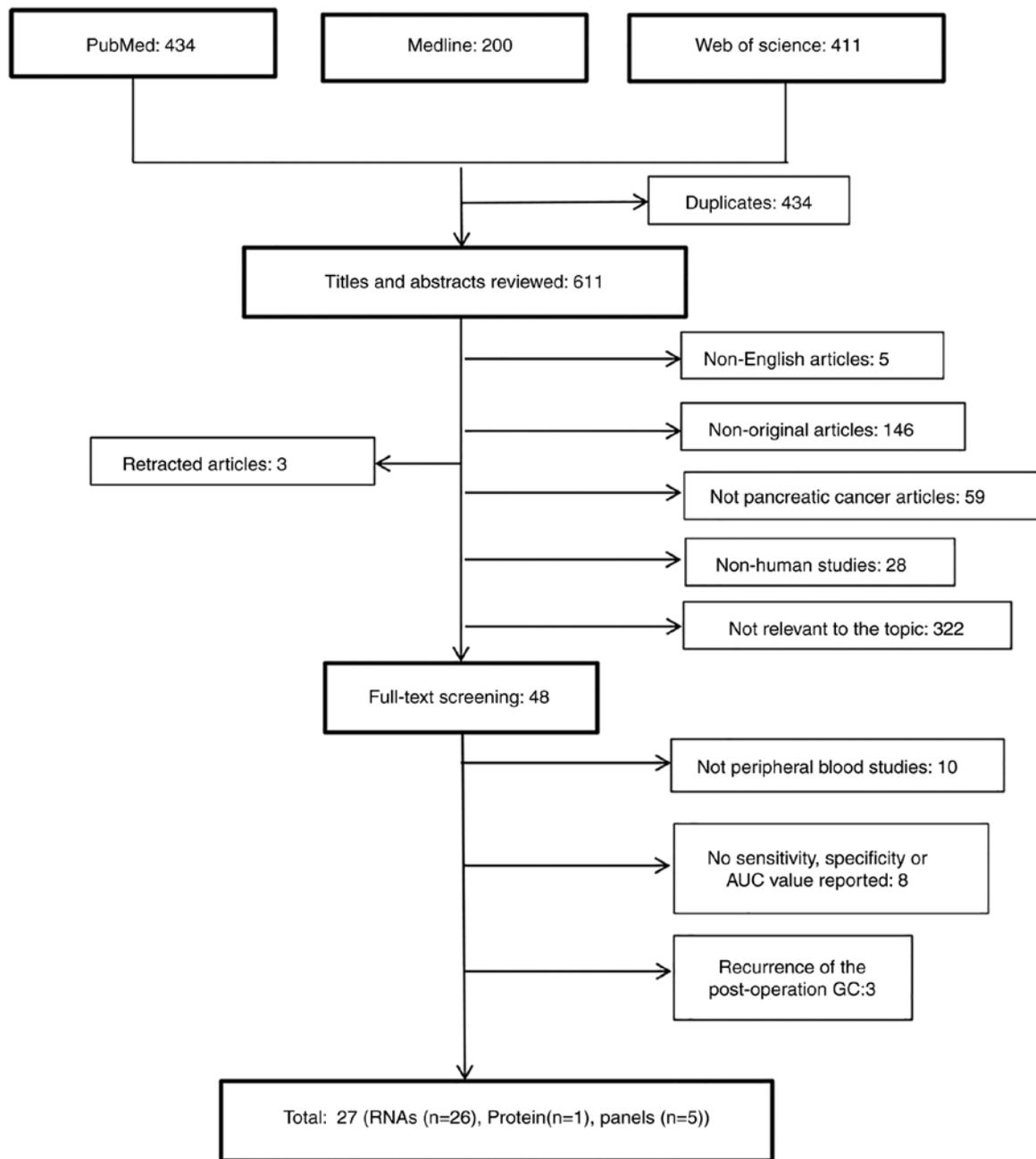


Figure 1. Flow chart of this study.

**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.

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

Table I. Continued.

GC cases/controls																	
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)			GC	status of									controls
<b>A, miRNA</b>																	
Chung <i>et al.</i> , 2020	China	20/20		55/65	Serum	IIb-IIIb	HC	HC	PBP	miR-423-5p miR-484 miR-186-5p miR-142-5p miR-320d miR-320a miR-320b miR-17-5p miR-629-5p miR-363-3p miR-337-5p miR-27a-3p miR-1246			<sup>a</sup> 0.780 <sup>a</sup> 0.560 <sup>a</sup> 0.540 <sup>a</sup> 0.750 <sup>a</sup> 0.740 <sup>a</sup> 0.770 <sup>a</sup> 0.720 <sup>a</sup> 0.640 0.750 0.780 0.750 0.770				(44)
Shi <i>et al.</i> , 2019	China	85/50 28/50 28/30	60/58 /58 /58	66/68 /68 /67	Serum	I-IV I	HC HC BD	HC	RT-qPCR		82 86 79	86 74 80	0.911 0.843 0.811	<0.001 <0.001	1.670	(33)	
Wang <i>et al.</i> , 2017	China	110/110		55/	Serum	Ia-IIIb	HC	HC	RT-qPCR	miR-106a-5p miR-19b-3p	<sup>e</sup> 63 <sup>e</sup> 84	<sup>e</sup> 89 <sup>e</sup> 51	<sup>a</sup> 0.786 <sup>a</sup> 0.769	<0.0001 <0.0001		(29)	
<b>B, lncRNA</b>																	
GC cases/controls																	
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)			GC	status of									controls
<sup>b</sup> Zhou <i>et al.</i> , 2020	China	81/78	64/60	63/	Serum	I-IV	HC	HC	RT-qPCR	LncRNA H19	74	84	0.849	<0.01	1.770	(19)	
Zheng <i>et al.</i> , 2020	China	60/60		63/	Plasma	I-IV	HC	HC	RT-qPCR	Lnc-SLC2A12-10:1 LncRNA MIAT	69	75	0.776	<0.001		(20)	
Xu <i>et al.</i> , 2020	China	109/50 109/48		74/ 74/	Serum	I-IV	HC GA	HC	RT-qPCR		<sup>e</sup> 82 <sup>e</sup> 65	<sup>e</sup> 94 <sup>e</sup> 89	0.892 0.787			(26)	

Table I. Continued.

GC cases/controls																				
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 <i>et al.</i> , 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	89	88	0.842		
		386/48	61/55												59/65	90	81	0.860		
		179/151	/62													89	80	0.861		
		179/37	/54												/70	92	82	0.884		
		179/48	/55												/65	81	88	0.885		
Piao <i>et al.</i> , 2020	China	281/80		Plasma	I-IV	HC	RT-qPCR	CEBPA-ASI	72	87	0.824			(35)						
Li <i>et al.</i> , 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 <i>et al.</i> , 2019	China	63/29	71/	Serum	I-IV	HC	RT-PqCR	Lnc RNA	84	87	0.896		2.390	(45)						
Zhao <i>et al.</i> , 2018	China	126/120	55/	Serum	I-IV	HC		Lnc RNA	70	85	0.827		1.720	(23)						
Lin <i>et al.</i> , 2018	China	51/60	61/58	61/63	Ia-IIb	HC	RT-qPCR	LncUEGC1	97	96	0.876			(38)						
C, circRNA	China	23/60		Plasma	I	HC	RT-qPCR	LncUEGC1	96	73	0.850									
Zheng <i>et al.</i> , 2022	China	60/60	63.7/	53/	I-IV	CG, HC	RT-qPCR	circ_0015286	82	66	0.778			(21)						
Xiao <i>et al.</i> , 2022	China	112/120		Serum		CG, TH, HC	RT-qPCR	circRNA	77	66	0.726		1.330	(27)						
Shao <i>et al.</i> , 2020	China	41/39		Plasma	I-II	HC	RT-qPCR	circ-0065149	49	90	0.640		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 <i>et al</i> , 2020	China	70/60	59/59	57/67	Serum	I-IV	HC	RT-qPCR	piR-018569 piR-004918 piR-019308	44 43 57	97 95 92	0.732 0.754 0.820	<0.001 <0.001 <0.001	(42)
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 <i>et al</i> , 2019	China	119/31	75/	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 <i>et al</i> , 2021	Japan	93/90 63/90	72/72 /72	69/67 /67	Serum	I-IV I	HC	ELISA	Dicer	°93 °92	°34 °37	0.622 0.623		(36)

<sup>a</sup>Results generated using the validation set; <sup>b</sup>prospective study; <sup>c</sup>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 III. Continued.

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

<sup>a</sup>RNAs analyzed as part of a panel; <sup>b</sup>RNAs analyzed individually

<sup>b</sup>Upregulated

<sup>b</sup>Downregulated

<sup>b</sup>Downregulated

<sup>b</sup>Downregulated

<sup>a</sup>Upregulated

<sup>a</sup>Upregulated

<sup>b</sup>Downregulated

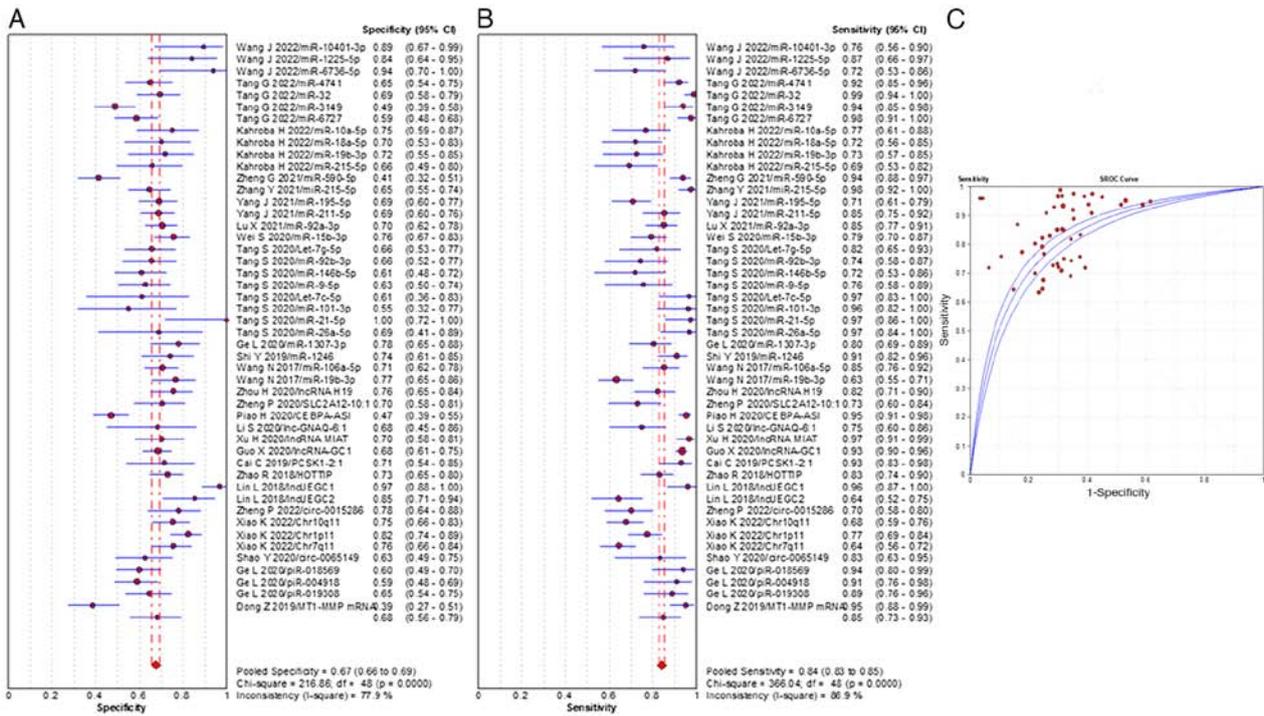


Figure 2. Summary of the diagnostic performance of extracellular vesicle RNAs for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

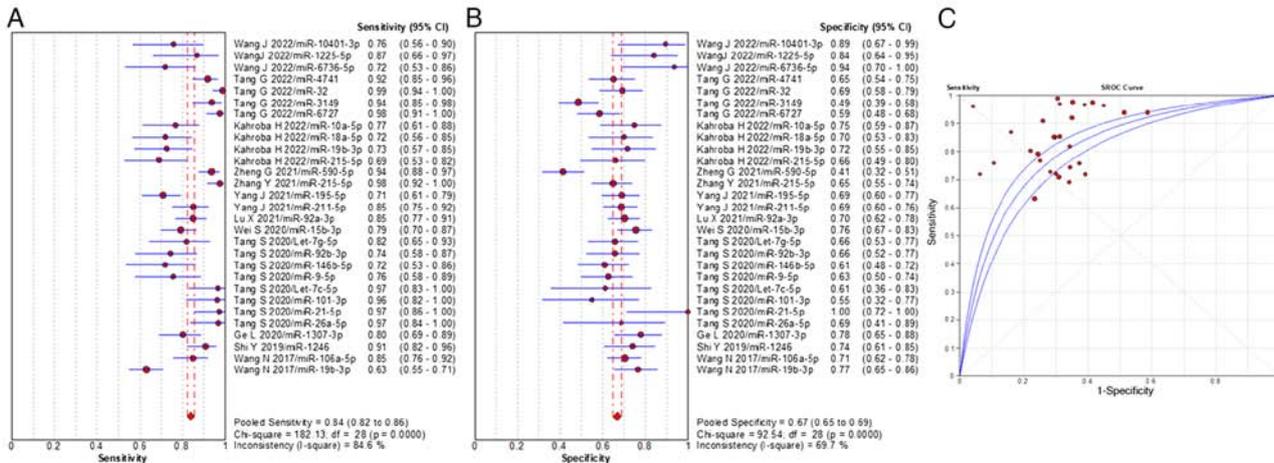


Figure 3. Summary of the diagnostic performance of extracellular vesicle microRNAs for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

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

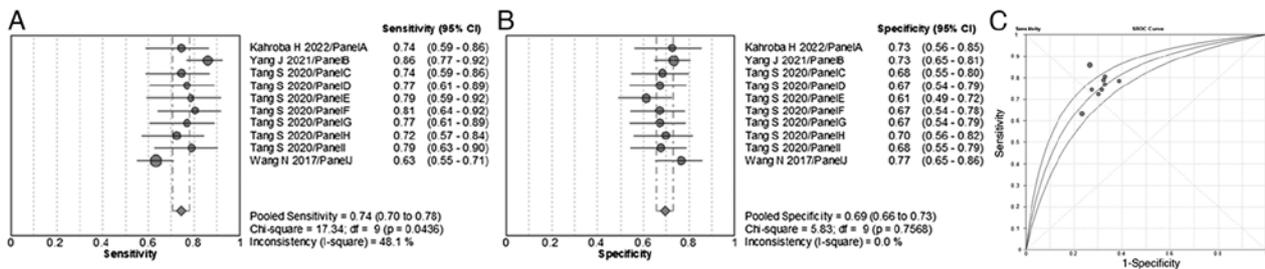


Figure 4. Summary of the diagnostic performance of extracellular vesicle micro RNA panels for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

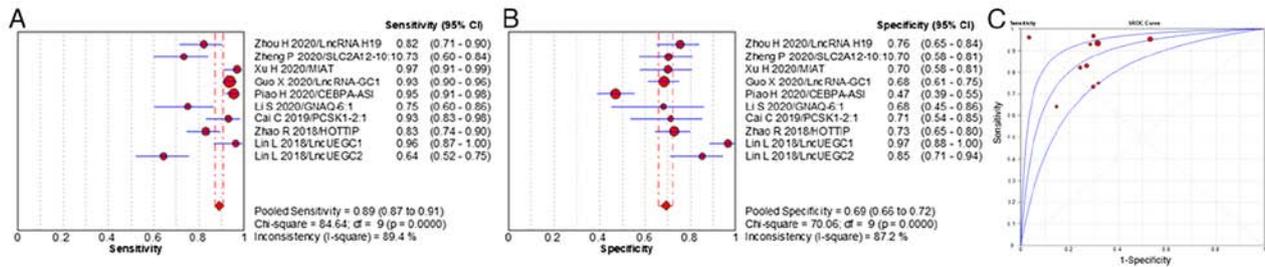


Figure 5. Summary of the diagnostic performance of extracellular vesicle long non-coding RNAs for the detection of gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

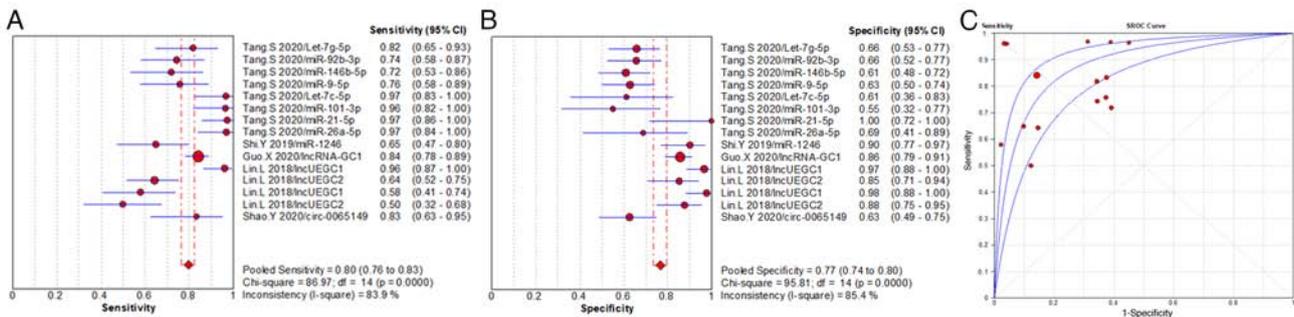


Figure 6. Summary of the diagnostic performance of extracellular vesicle RNAs for the detection of stage I/II gastric cancer. (A) Forest plot of sensitivity values, (B) forest plot of specificity values, (C) receiver operator characteristic curve.

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 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, ultracentrifugation (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.

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### 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.

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