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Original Paper

A Meta-Analysis of the Diagnostic Accuracy of Circular RNAs in Digestive System Malignancy

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Key Words
Circular RNA • Digestive System Neoplasm • Meta-analysis • Diagnosis • Biomarker

Abstract
Background/Aims: Circular RNAs (circRNAs), a novel class of noncoding RNAs, have been found to be dysregulated in various cancers. However, the clinical application value of these circRNAs in digestive system cancers remains to be clarified. We aimed to comprehensively explore the potential role of circRNAs as diagnostic indicators in digestive system malignancies. ***Methods:*** Relevant studies were systematically retrieved from PubMed, Web of Science and the Cochrane Library. The data that were required to complete 2 × 2 contingency tables were obtained from the included studies. Stratified analyses by cancer type, sample size and publication year were performed. ***Results:*** Thirteen studies with 2,276 individuals were included in the meta-analysis. The pooled sensitivity and specificity of circRNAs in the diagnosis of digestive system malignancy were 0.72 [95% confidence interval (CI): 0.65-0.77] and 0.77 (95% CI: 0.72-0.81), respectively. The overall positive likelihood ratio was 3.09 (95% CI: 2.64-3.62), and the overall negative likelihood ratio was 0.37 (95% CI: 0.31-0.44). The pooled diagnostic odds ratio was 8.38 (95% CI: 6.86-10.25), and the overall area under the curve was 0.81 (95% CI: 0.77-0.84), indicating good discriminative ability of circRNAs as biomarkers for digestive system malignancy. ***Conclusion:*** circRNAs distinguish patients with digestive system cancer from controls with relatively high diagnostic accuracy. circRNAs may be used as potential biomarkers for the diagnosis of digestive system malignancy.

Introduction

Digestive system cancer, with approximately 3.4 million new diagnosed cases annually, is one of the most common malignancies around the world [1]. Despite recent advances in clinical and experimental oncology, a large number of patients are diagnosed in late stages.

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Many deaths could be avoided if early-stage tumors were identified and treated prior to the development of more advanced malignancies. Thus, there is a need for clinically applicable biomarkers with high sensitivity and specificity to permit early detection and intervention.

Circular RNAs (circRNAs), a novel class of endogenous noncoding RNA, are characterized by a covalently closed continuous loop with neither 5'-3' polarity nor a polyadenylated tail [2-4]. First found in RNA viruses more than four decades ago, circRNAs were once considered abnormal splicing products of RNAs due to their low expression levels [5, 6]. With the development of high-throughput sequencing and the improvement of bioinformatics technologies, recent studies have revealed that circRNAs are abundant, stable, conserved and involved in various physiological and physiopathological processes [7-12]. circRNAs can act as competitive endogenous RNAs (ceRNAs) to compete for microRNA (miRNA)-binding sites, thereby affecting miRNA function [13, 14]. Some circRNAs can also regulate parental gene expression and modulate alternative splicing and transcription [15, 16]. In recent years, emerging evidence has indicated the crucial role of circRNAs in human digestive system malignancy [17]. The overall levels of circRNAs are globally decreased in colorectal cancer (CRC) and are negatively correlated with CRC proliferation and disease status [18]. Aberrant circRNA expression patterns have also been observed in tumor tissues compared to normal tissue in several kinds of digestive system tumors, including gastric cancer (GC), hepatocellular carcinoma (HCC) and pancreatic ductal adenocarcinoma [19-23]. Differentially expressed circRNAs provide a novel molecular focus in the study of tumor pathogenesis. The wide existence, high stability and variety of regulatory functions of circRNAs undoubtedly signal a new area of interest in the early diagnosis of cancer [24, 25].

The aim of this meta-analysis is to summarize all of the circRNAs that have been investigated in the context of digestive system cancer and to investigate the potential role of circRNAs as novel diagnostic biomarkers in human digestive system malignancy. We utilized a comprehensive approach to systematically identify and assess all published studies addressing the diagnostic significance of circRNAs in digestive system neoplasm.

Materials and Methods

This report was conducted according to the recommendations of the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines [26].

Search strategy and study selection

An electronic search of PubMed, Web of Science and the Cochrane Library was performed for relevant articles using the following terms: (circRNA OR "circular RNA") AND (cancer OR carcinoma OR tumor OR neoplas* OR tumour OR malignan*) AND (diagnosis OR diagnostic OR sensitivity OR specificity OR "receiver operating characteristic curve"). The search was last updated to include articles published through July 2017. No language restrictions were imposed. Based on the title and abstract, manuscripts of interest were obtained for full-text review. The references of relevant review papers and original articles were manually searched to identify potentially eligible studies.

Studies were included in the meta-analysis if the following inclusion criteria were met: (1) the studies assessed the diagnostic accuracy of circRNAs in digestive system malignancy; (2) the diagnosis of digestive system malignancy was confirmed by histological examination; and (3) the studies should contain true-positive (TP), false-positive (FP), false-negative (FN) and true-negative (TN) values (or the possibility of deriving such statistics from the manuscript). The exclusion criteria included (1) duplicate publications; (2) reviews, letters, meeting abstracts and case reports; and (3) insufficient or unqualified data. Each study was assessed by two independent investigators (Zhiqiang Chen and Long Zhang). Disagreements were resolved by full discussion with a third investigator (Jindao Wu) to reach a consensus.

Data extraction and quality assessment

Two investigators (Zhiqiang Chen and Guoyong Han) perused the full text of each manuscript and independently extracted the following data: the first author, publication year, cancer type, expression level

of the circRNA, specimen source, area under the curve (AUC), sensitivity and specificity, sample size, as well as the cut-off value. For each included study, TP, FP, FN and TN values were obtained and 2 × 2 contingency tables were constructed.

The Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) was adopted to evaluate the methodological quality of included studies [27]. The QUADAS-2 checklist consists of four primary domains: patient selection, index test, reference standard, flow and timing. Each domain is assessed on the basis of risk of bias, and the first three domains are evaluated according to applicability. The quality assessment of each included study was carried out by two independent researchers (Xueliang Zuo and Yao Zhang).

Statistical analysis

All statistical analyses were performed with STATA version 12.0 (Stata Corporation, College Station, TX, USA) and Meta-DiSc version 1.4 (Universidad Complutense, Madrid, Spain). Pooled sensitivity, specificity, positive likelihood ratio (LR) and negative LR were calculated to assess the ability of circRNAs to distinguish digestive system cancer patients from the controls. The diagnostic odds ratio (DOR) and AUC of the summary receiver operator characteristic (sROC) curve were used to evaluate the overall performance of the diagnostic test. The heterogeneity of the included studies caused by the threshold effect was quantified by Spearman's correlation analysis. The non-threshold effect was assessed using a chi-squared test and I^2 statistics. A chi-squared test of $P < 0.10$ or $I^2 > 50\%$ indicated the existence of heterogeneity caused by a non-threshold effect. Subgroup analyses by cancer type, sample size and publication year were conducted. In addition, meta-regression was used to find the possible sources of heterogeneity caused by the non-threshold effect. Fagan's nomogram was employed to calculate the post-test probabilities. Potential publication bias was evaluated by Deeks' funnel plot asymmetry test. $P < 0.05$ was considered statistically significant.

Results

Study selection and study characteristics

The search strategy identified 161 potentially relevant records, of which 55 were excluded as they were duplicates. The remaining 106 manuscripts were subject to title and abstract screening. We further removed 78 publications because 40 of them were reviews, letters or conference abstracts and because 38 were unrelated studies. Therefore, 28 articles were eligible for full-text review and data assessment. Finally, 15 articles were excluded due to unavailable data for constructing a 2 × 2 contingency table, and the remaining 13 studies were enrolled in the meta-analysis [28-40]. A flowchart demonstrating the study selection process is illustrated in Fig. 1.

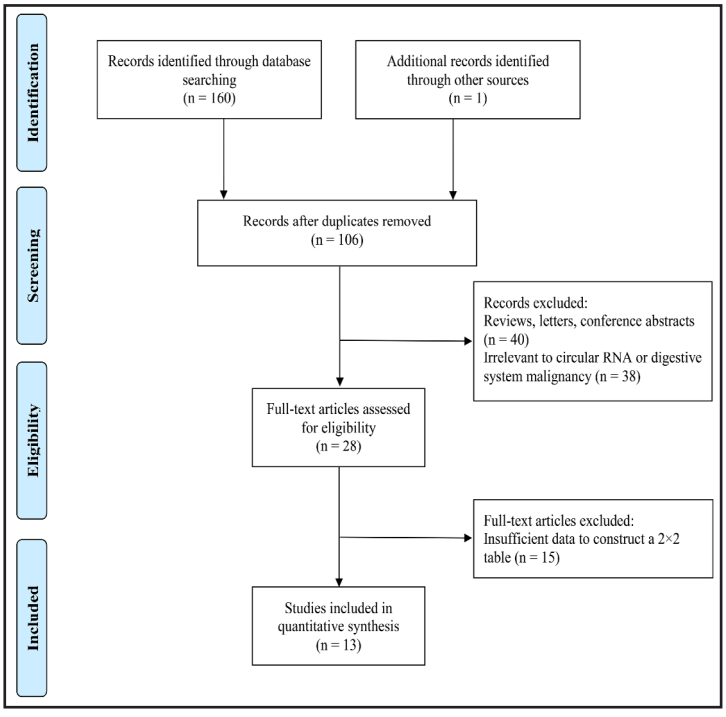

Fig. 1. A flow diagram demonstrating the study selection process.

All of the included studies were performed between 2015 and 2017 and included a total of 2, 276 individuals. Among

these studies, seven were on GC, five were on HCC, and one was on CRC. The diagnostic significance of 12 different circRNAs was assessed. Almost all of the investigated circRNAs were down-regulated in digestive system malignancies, whereas the expression level of hsa_circ_0005075 was increased in HCC [31]. The specimen source of most studies was from a tissue sample, and only one study combined the use of tissue and plasma samples to examine the diagnostic accuracy of hsa_circ_0000190 in GC [32]. The sample size of the studies ranged from 62 to 259, with a median of 192. Study characteristics of the included studies are summarized in Table 1.

Table 1. Characteristics of the thirteen studies included in the meta-analysis. Abbreviations: circRNA, circular RNA; AUC, area under the curve; TP, true positive; FP, false positive; FN, false negative; TN, true negative; GC, gastric cancer; CRC, colorectal cancer; HCC, hepatocellular carcinoma; D, down-regulated; U, up-regulated; NA, not available

Author	Year	circRNA	Cancer type	Expression level	Specimen source	AUC	Sensitivity	Specificity	TP	FP	FN	TN	No. of patients	No. of controls	Sample size	Cut-off value
Li	2015	hsa_circ_002059	GC	D	Tissue	0.73	0.81	0.62	82	38	19	63	101	101	202	12.9
Wang	2015	hsa_circ_001988	CRC	D	Tissue	0.788	0.68	0.73	21	8	10	23	31	31	62	6.04
Qin	2016	hsa_circ_0001649	HCC	D	Tissue	0.63	0.81	0.69	72	28	17	61	89	89	178	0.0007855
Shang	2016	hsa_circ_0005075	HCC	U	Tissue	0.94	0.833	0.900	27	3	6	30	33	33	66	0.000586
Chen	2017	hsa_circ_0000190	GC	D	Tissue and plasma combined	0.78	0.712	0.750	74	26	30	78	104	104	208	NA
Fu (1)	2017	hsa_circ_0004018	HCC	D	Tissue	0.848	0.716	0.815	73	29	29	128	102	157	259	NA
Fu (2)	2017	hsa_circ_0003570	HCC	D	Tissue	0.70	0.449	0.868	48	14	59	93	107	107	214	12.24
Li	2017	hsa_circ_0001649	GC	D	Tissue	0.834	0.711	0.816	54	14	22	62	76	76	152	0.2269225
Lu	2017	hsa_circ_0006633	GC	D	Tissue	0.741	0.60	0.81	57	18	39	78	96	96	192	8.17
Shao (1)	2017	hsa_circ_0014717	GC	D	Tissue	0.696	0.5938	0.8125	57	18	39	78	96	96	192	12.14
Shao (2)	2017	hsa_circ_0001895	GC	D	Tissue	0.792	0.678	0.857	65	5	31	30	96	35	131	9.53
Tian	2017	hsa_circ_0003159	GC	D	Tissue	0.75	0.852	0.565	92	47	16	61	108	108	216	12.31
Yao	2017	circZKSCAN1	HCC	D	Tissue	0.834	0.822	0.724	84	28	18	74	102	102	204	NA

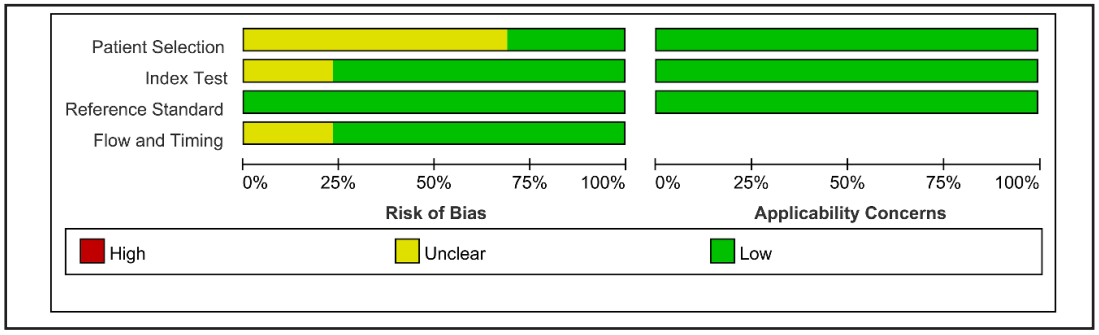

Fig. 2. Methodological quality graph.

Table 2. Assessment of diagnostic accuracy and heterogeneity in subgroup analysis. Abbreviations: CI, confidence interval; LR, likelihood ratio; DOR, diagnostic odds ratio; AUC, area under the curve; HCC, hepatocellular carcinoma; GC, gastric cancer

Subgroups	No. of studies	Pooled sensitivity (95% CI)	I^2 (%) sensitivity	Pooled specificity (95% CI)	I^2 (%) specificity	Pooled positive LR (95% CI)	I^2 (%) positive LR	Pooled negative LR (95% CI)	I^2 (%) negative LR	Pooled DOR	I^2 (%) DOR	AUC
All studies	13	0.72 (0.65-0.77)	84.33	0.77 (0.72-0.81)	78.41	3.09 (2.64-3.62)	28.38	0.37 (0.31-0.44)	75.57	8.38 (6.86-10.25)	95.82	0.81 (0.77-0.84)
Cancer type												
HCC	5	0.73 (0.59-0.83)	91.76	0.79 (0.72-0.85)	75.96	3.51 (2.83-4.37)	0.00	0.34 (0.24-0.50)	90.70	10.21 (6.91-15.10)	95.02	0.83 (0.80-0.86)
GC	7	0.72 (0.63-0.78)	79.15	0.75 (0.67-0.82)	81.99	2.86 (2.28-3.57)	23.12	0.38 (0.31-0.46)	45.24	7.51 (5.79-9.74)	58.60	0.80 (0.76-0.83)
Sample size												
≤192	7	0.69 (0.62-0.75)	63.97	0.79 (0.75-0.84)	45.82	3.37 (2.76-4.11)	0.00	0.39 (0.32-0.47)	50.90	8.67 (6.36-11.82)	96.27	0.82 (0.78-0.85)
>192	6	0.74 (0.63-0.83)	89.82	0.74 (0.64-0.82)	83.94	2.86 (2.26-3.63)	30.15	0.35 (0.26-0.47)	84.28	8.24 (6.28-10.80)	74.76	0.81 (0.77-0.84)
Publication year												
Prior to 2017	4	0.79 (0.73-0.84)	1.29	0.73 (0.61-0.83)	69.92	2.98 (1.97-4.51)	10.52	0.28 (0.21-0.38)	3.31	10.53 (5.67-19.55)	96.85	0.81 (0.77-0.84)
2017	9	0.69 (0.60-0.77)	86.39	0.79 (0.72-0.84)	79.81	3.21 (2.69-3.85)	23.57	0.39 (0.32-0.49)	77.95	8.17 (6.46-10.33)	72.06	0.81 (0.77-0.84)

Quality assessment

Quality assessment results of the studies are shown in Fig. 2 and Fig. 3 using the QUADAS-2 evaluation tool. The quality of the included studies varied from moderate to high.

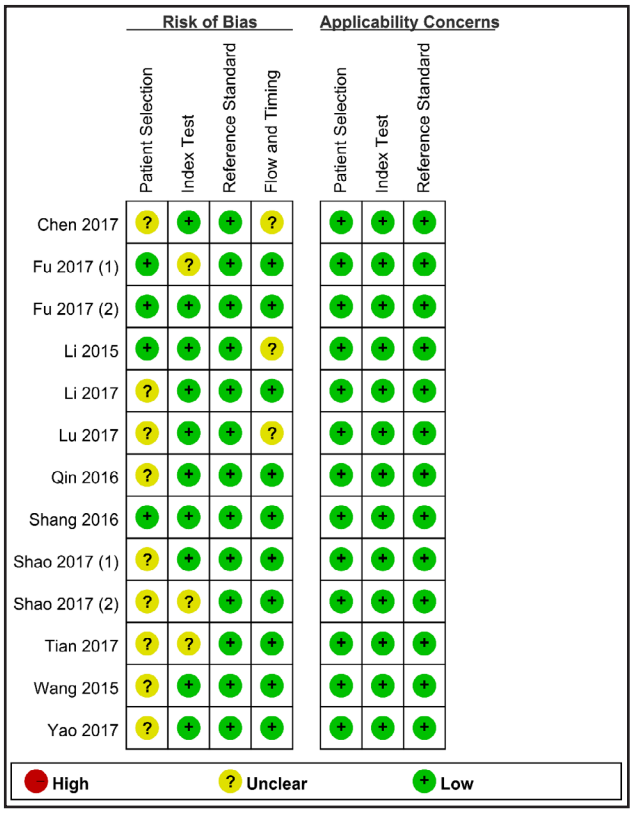

Fig. 3. Methodological quality summary.

Diagnostic accuracy analysis

The pooled sensitivity for the studies included in the final meta-analysis was 0.72 [95% confidence interval (CI): 0.65-0.77], and the pooled specificity was 0.77 (95% CI: 0.72-0.81) (Fig. 4A and B). In addition, the overall positive LR and negative LR were 3.09 (95% CI: 2.64-3.62) and 0.37 (95% CI: 0.31-0.44), respectively (Fig. 4C and D). The overall diagnostic accuracy was assessed by pooled DOR (8.38, 95% CI: 6.86-10.25) and by AUC of the sROC curve (0.81, 95% CI: 0.77-0.84) (Fig. 5A and B). Significant heterogeneity across the studies was detected according to the I^2 value of DOR (95.82%) (Table 2). To find the origin of the heterogeneity, we further performed a series of analyses, including threshold effect, subgroup analysis and meta-regression.

Diagnostic threshold effect

Threshold effect is a pivotal source of heterogeneity in diagnostic tests. It is caused by the differences in sensitivity and specificity. One good way to assess the threshold effect is by using Spearman's correlation coefficient of sensitivity and specificity. Our analysis showed that the Spearman correlation coefficient in total across the 13 studies was 0.515 (P = 0.072), indicating the absence of a threshold effect.

Subgroup analyses

We carried out stratified analyses according to cancer type, sample size and publication year. The heterogeneity of the DOR was lower in studies on GC than studies on HCC (I^2, 58.60% vs. 95.02%). Moreover, less heterogeneity was observed in studies with a larger sample size (I^2, 74.76% vs. 96.27%) and in studies performed in the year 2017 (I^2, 72.06% vs. 96.85%). A higher diagnostic value was found in patients with HCC (DOR = 10.21, 95% CI: 6.91-15.10) than in GC patients (DOR = 7.51, 95% CI: 5.79-9.74). There was little difference in DOR among different subgroups according to sample size and publication year (Table 2).

Meta-regression analysis

Subsequently, meta-regression analysis was performed. There was no significant correlation between cancer type (P = 0.2011), sample size (P = 0.6975), and publication year (P = 0.9719) and DOR in the univariate meta-regression analysis.

Publication bias

The publication bias of the included studies was checked by Deeks' funnel plot asymmetry test, and the result is presented in Fig. 6. A statistically non-significant value (P = 0.122) in the funnel plot indicated no potential publication bias.

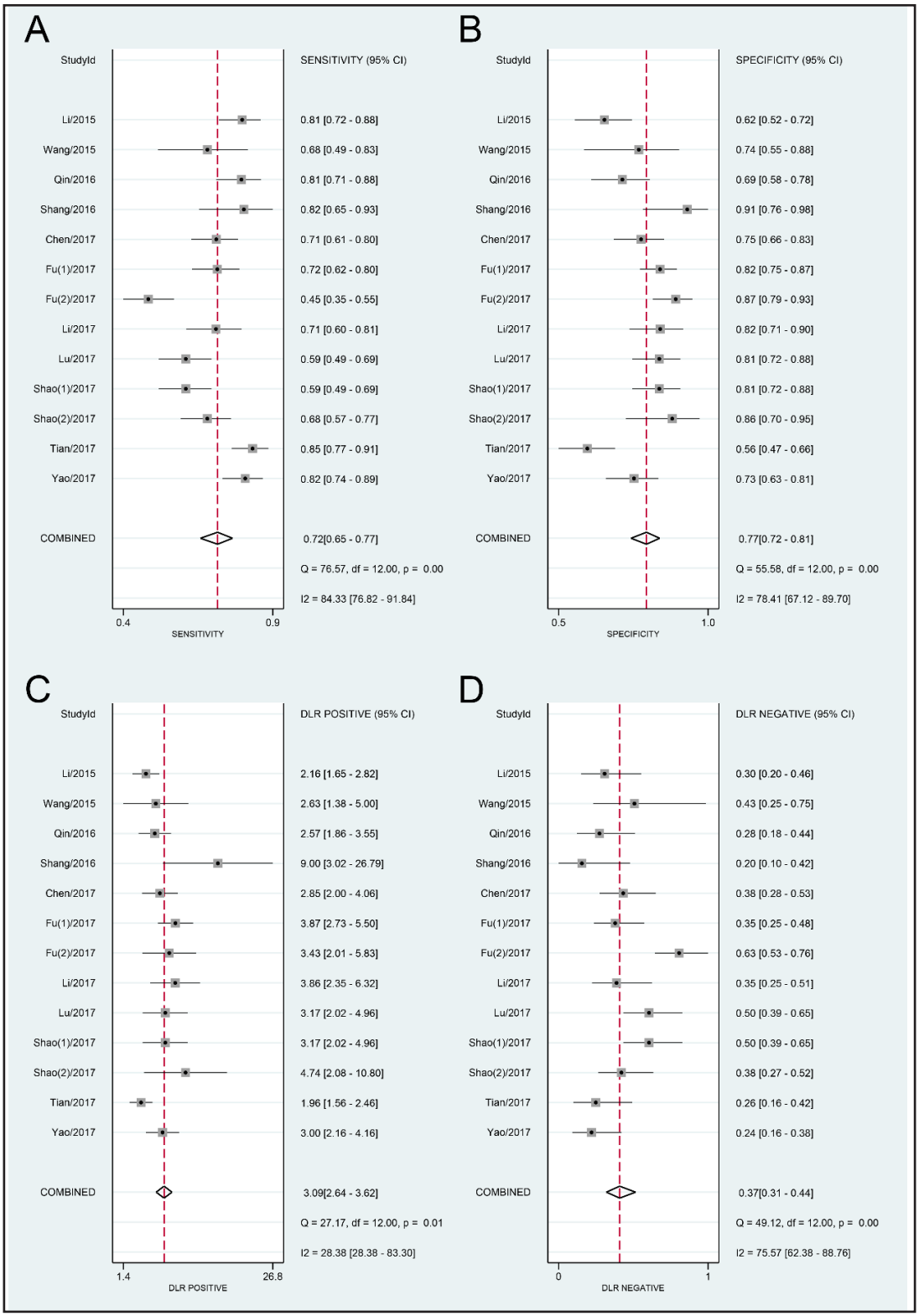

Fig. 4. Forest plot of diagnostic accuracy index of circular RNAs in digestive system malignancy. (A) Sensitivity of circular RNAs in diagnosis of digestive system malignancy. (B) Specificity of circular RNAs in diagnosis of digestive system malignancy. (C) Positive likelihood ratio of circular RNAs in diagnosis of digestive system malignancy. (D) Negative likelihood ratio of circular RNAs in diagnosis of digestive system malignancy.

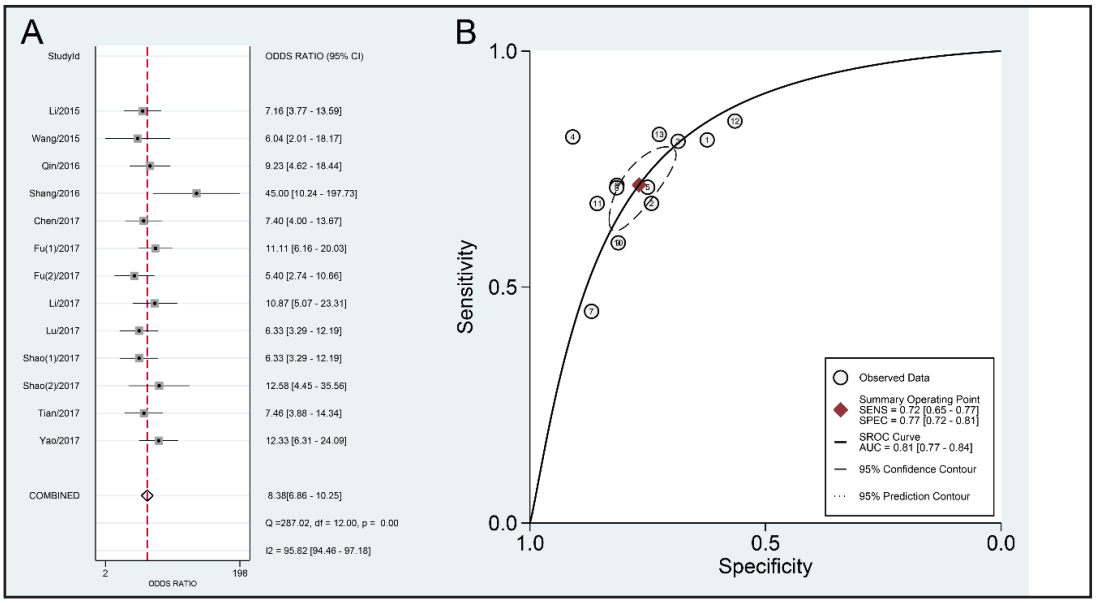

Fig. 5. Overall performance of circular RNAs in diagnosis of digestive system malignancy. (A) Diagnostic odds ratio of circular RNAs in diagnosis of digestive system malignancy. (B) Summary receiver operator characteristic curve of circular RNAs in diagnosis of digestive system malignancy.

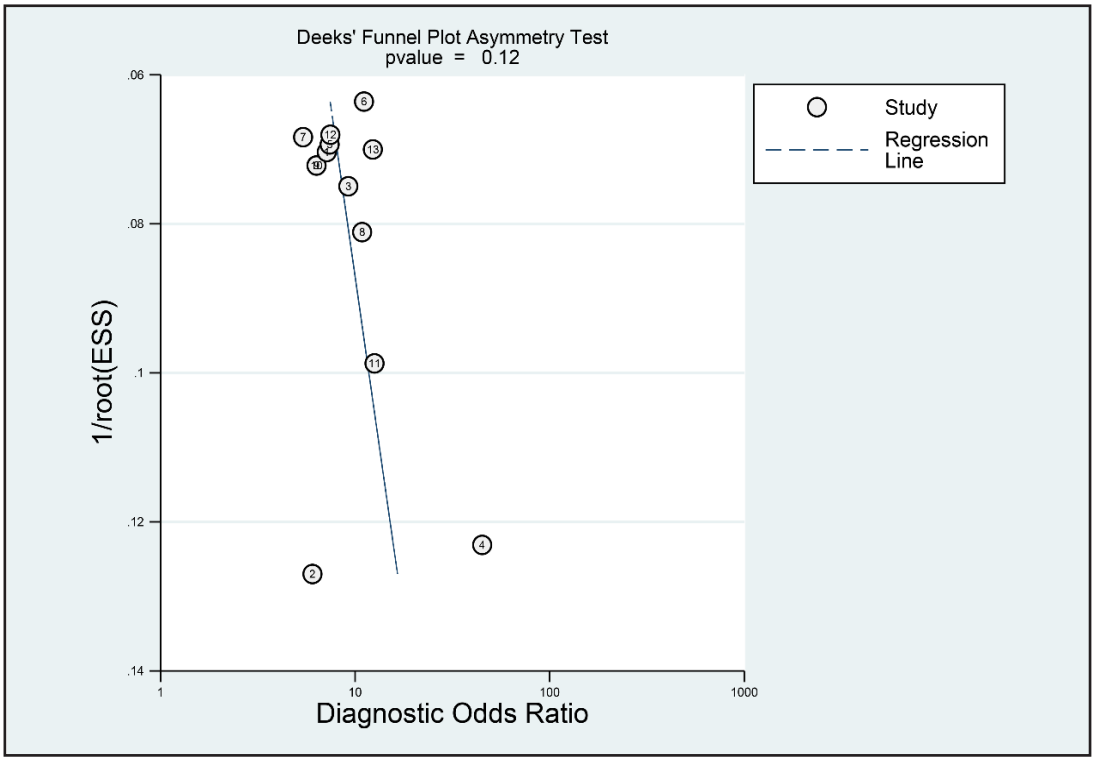

Fig. 6. Deeks' funnel plot evaluating the potential publication bias of the included studies.

Clinical utility of index test

Fagan's nomogram is a graphical tool for calculating post-test probabilities. As presented in Fig. 7, we found that when the pre-test probability was set at 20%, the post-test probability rose to 44% with a positive LR of 3, and the post-test probability decreased to 8% with a negative LR of 0.37.

Discussion

As a special type of noncoding RNAs, circRNAs have been confirmed to be far more ubiquitous than previously thought [4]. The closed loop structure of circRNAs grants them high resistance to nucleases, thus making circRNAs more stable than their linear counterparts. It has been reported that the half-life of circRNAs is longer than 48 hours, whereas the average half-life of mRNAs is only 10 hours [41]. The stability of circRNA partly contributes to their abundant expression. It is indicated that the expression level of some circRNAs is higher than that of their corresponding linear mRNAs [42]. Therefore, circRNAs seem to possess distinct advantages as candidate biomarkers for cancer diagnosis.

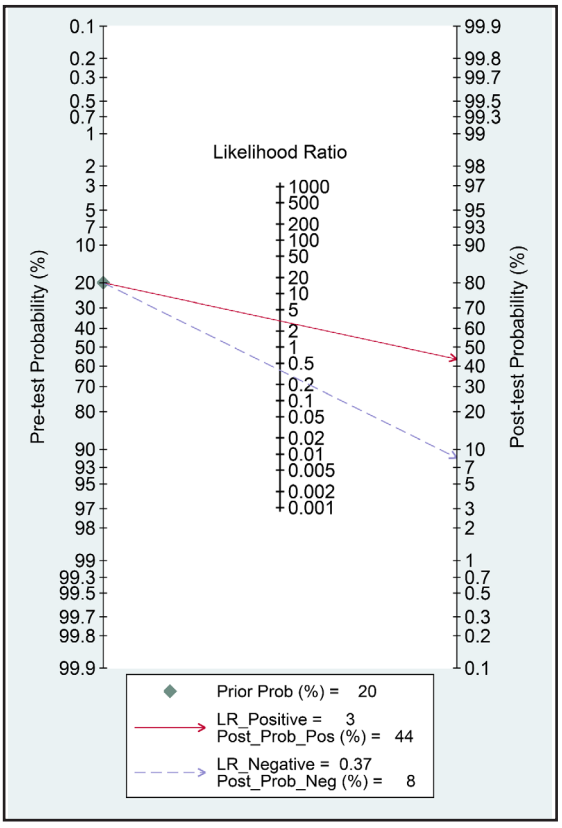

Fig. 7. Fagan's nomogram evaluating the overall value of circular RNA for diagnosis of digestive system malignancy.

In this study, we thoroughly searched multiple databases and retrieved thirteen studies pertaining to the diagnostic value of circRNAs for human digestive system malignancy. As far as we know, the present study is the first meta-analysis specifically directed at the diagnostic value of circRNAs in digestive system cancer. Based on the pooled results, the overall sensitivity and specificity were 0.72 (95% CI:0.65-0.77) and 0.77 (95% CI: 0.72-0.81), respectively. The DOR combines the strengths of sensitivity and specificity, and expresses the diagnostic performance as a single term. A higher value of DOR represents a better discriminatory test performance [43]. The pooled DOR of 8.38 (95% CI: 6.86-10.25) showed the diagnostic significance of circRNAs for patients with digestive system cancer. In addition, the AUC of sROC was used to assess the overall test performance. An AUC with a value ranging between 0.93 and 0.96 is recognized to be excellent, and a value from 0.75 to 0.92 is acceptable [44, 45]. Our results showed that circRNAs have good diagnostic accuracy in digestive system malignancy, with an AUC of 0.81 (95% CI: 0.77-0.84). A great number of researchers have devoted themselves to defining signatures to predict digestive system malignancies, but there are still no practical diagnostic biomarkers with satisfactory sensitivity and specificity. A meta-analysis by Liu *et al.* showed that the pooled sensitivity and specificity of carcinoembryonic antigen (CEA) for CRC are 0.46 (95% CI: 0.45–0.47) and 0.89 (95% CI: 0.88-0.90), respectively [46]. In addition, the overall sensitivity ranged from 0.18 to 0.65 for carbohydrate antigen 19-9 (CA19-9) and from 0.30 to 0.55 for carbohydrate antigen 242 (CA242) [47, 48]. The pooled diagnostic sensitivity and specificity were 0.61 (95% CI: 0.58-0.63) and 0.92 (95% CI: 0.91-0.94) for α-fetoprotein in HCC [49]. Although the specificity of CEA and α-fetoprotein is relatively high, the sensitivity is not satisfactory. With a pooled sensitivity of 0.72, circRNAs may be a promising biomarker for diagnosing potential patients with digestive system malignancy. Moreover, it might be of greater diagnostic value if circRNAs are employed in combination with other biomarkers such as CEA, CA19-9, CA242 or α-fetoprotein.

The considerable amount of heterogeneity across the studies represents a potential source of bias. We calculated the Spearman correlation coefficient to detect the existence

of a potential threshold effect, which is a non-negligible cause of heterogeneity. A Spearman correlation coefficient of 0.515 ($P = 0.072$) suggested that the threshold effect was not the cause of heterogeneity in the meta-analysis. Furthermore, we used a stratified analysis to examine the heterogeneity in prespecified subgroups. According to our data, the heterogeneity was decreased in some subgroups. Unsurprisingly, less heterogeneity was detected in studies with a larger sample size and in studies conducted in the most recent year of publication. In addition, a decreased amount of heterogeneity was observed in studies on GC compared to studies on HCC. A subsequent meta-regression was carried out. The results suggested that the type of cancer, sample size and publication year were not the origin of the heterogeneity. Owing to insufficient data, we could not further evaluate the other important variates that might have contributed to the between-study heterogeneity, including age, gender, genetic background, severity of disease, socioeconomic status and study design.

In spite of our efforts to accomplish a comprehensive and accurate analysis, this meta-analysis still has certain limitations. First, all of the enrolled subjects were from Asia, which decreased the applicability of the results across different ethnicities. Further studies investigating the diagnostic accuracy of circRNAs among different populations of patients in terms of genetics, ethnicity and geography are duly warranted. Second, several other variables including gender, age, subtype of certain cancer and socioeconomic status are closely associated with the tumorigenesis and progression of digestive system neoplasms. Due to limited data provided in the included studies, further subgroup analyses on these variables could not be carried out in this meta-analysis. Third, since the significance of circRNAs as diagnostic biomarkers has been explored only in recent years, sample sizes have been relatively small. As a result, small-study effects might be present [50]. Fourth, most of the investigated circRNAs were extracted from tissue samples; only one study combined tissue and plasma to assess the diagnostic value of circRNAs [32]. As previously reported, circRNAs are enriched and stable in exosomes [51, 52], which indicates that it might be possible to diagnose cancer by detecting plasma or serum circRNAs. It is believed that circRNAs could be useful as a noninvasive tumor screening tool for clinical practice in the future [53]. More large-scale studies are needed to evaluate the diagnostic accuracy of plasma or serum circRNAs for digestive system malignancies.

Conclusion

In summary, this study identified circRNAs associated with digestive system malignancy and indicated that circRNAs could distinguish patients from controls with high sensitivity and specificity. Deaths from digestive system tumors are among the leading causes of cancer deaths around the world, and there is an emphasis to identify earlier and less invasive stages of disease to achieve better clinical outcomes. For this purpose, circRNAs may be potential diagnostic biomarkers for the detection of human digestive system neoplasm. Studies with a larger sample size are needed to further verify the diagnostic importance of circRNAs.

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Disclosure Statement

The authors declare no Disclosure Statement.

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