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REVIEW ARTICLE

Gastric cancer detection based on cell-free DNA in blood: A systematic review and meta-analysis

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Abstract

Objective: Screening and early diagnosis of gastric cancer (GC) are crucial for improved prognosis. However, gastroscopic screening is not feasible in large populations due to its high cost and invasive nature. The detection of circulating cell-free DNA (cfDNA) provides an attractive minimally-invasive alternative for screening of GC. In this systematic review and meta-analysis, we evaluate the diagnostic value of cfDNA-based markers for GC, including the detection of total concentration, mutations, and methylation alterations.

Methods: We performed a systematic search of four literature databases (PubMed, Embase, Web of Science, and Cochrane Library) for articles published before November 2022. The revised tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) was used to evaluate the quality of included studies. PROSPERO registration number: CRD42021210830.

Results: A total of 15 original articles involving 2849 individuals were included in this meta-analysis, comprising five studies on concentration, nine studies on methylation alterations, and one study on mutation biomarkers of cfDNA. Among these studies, seven selected early-stage GC subjects. For the diagnoses of overall stages and early-stage GC, the pooled sensitivities with 95% confidence interval were 0.74 (0.66–0.82) and 0.64 (0.51–0.76), and the pooled specificities

Mona Wang and Xiaohan Fan contributed equally to this work.

were 0.92 (0.84–0.96) and 0.94 (0.87–0.98) with summary areas under the curve (SAUCs) of 0.89 (0.86–0.91) and 0.86 (0.83–0.89), respectively.

Conclusions: This meta-analysis suggests that cfDNA-based biomarkers show diagnostic value for GC early detection.

KEYWORDS
circulating cell-free DNA, early diagnosis, gastric cancer, liquid biopsy

1 | INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer and the ~~fourth~~ primary cause of cancer-related deaths globally according to the GLOBOCAN database 2020.[1] The 5-year survival rate depends greatly on the stage at diagnosis. For advanced-stage patients, the 5-year survival rate is 6%–10.2% but for early-stage subjects 72%–77%.[2,3] An early detection followed by endoscopic resection of the tumour can even elevate the 5-year survival rate to over 90%.[4,5] The insidious onset and asymptomatic early stage of GC result in most clinical patients being diagnosed at advanced stages with poor prognosis. Currently, the gold standard for GC diagnosis relies on gastroscopic examination and pathological analysis of biopsy tissue. However, it is difficult to implement large-scale gastroscopic screenings with these methods because of their invasive nature, the necessity for experienced endoscopists and pathologists, and the high costs of the diagnostic procedure. Therefore, the identification of novel biomarkers using minimally invasive, and more effective and feasible methods is urgently needed for large-scale screening of GC. In this regard, the detection of biomarkers in blood has emerged as an interesting alternative. However, biomarkers investigated for GC screening, such as pepsinogen (PG) levels, gastrin-17 (G-17), and *Helicobacter pylori* antibodies, have not shown sufficient sensitivity and specificity.[6,7]

Recent advances in liquid biopsy technology have enabled the identification of novel biomarkers based on circulating cell-free DNA (cfDNA), circulating tumour cells (CTC), long non-coding RNA (lncRNA), or exosomes. CfDNA may contain DNA fragments released from precancerous or tumor cells into the bloodstream and therefore aid in monitoring local mucosal abnormalities in a non-invasive manner. Previous studies have used chemiluminescence or fluorescence-based quantitative polymerase chain reaction (PCR)[8–11] or AluPCR[12] to evaluate the associations between cfDNA concentration and tumours. Furthermore, the discovery of novel genetic variations or epigenetic alterations, such as mutations or methylation changes in cfDNA provides new possibilities for non-invasive cancer biomarker investigation. Although several studies have focused on the identification of cfDNA markers of GC, the diagnostic value of cfDNA biomarkers for GC screening has not been systematically evaluated.

In this review, we summarized the currently available studies on the three kinds of cfDNA biomarkers for GC screening (concentration, methylation, and mutation alterations) and comprehensively evaluated their performance in diagnosing GC, especially early-stage GC.

2 | METHODS

2.1 | Literature search strategy

Using four major databases including PubMed (https://pubmed.ncbi.nlm.nih.gov/), EMBASE (https://www.embase.com/), Web of Science (https://www-webofscience.com/), and Cochrane Library (https://www.cochranelibrary.com/), we conducted a comprehensive and systematic literature search for studies on cfDNA biomarker evaluation for GC screening published before November 2022. Keywords and search strategies are shown in Table S1.

2.2 | Inclusion and exclusion criteria

The inclusion criteria for eligible studies were as follows: (i) studies focusing on human patients (over 18 years old, any gender) with confirmed GC; (ii) studies using serum or plasma samples for cfDNA biomarker investigation; (iii) studies focusing on biomarkers including cfDNA concentration, methylation and mutation alterations; (iv) studies concentrating on screening or early detection of GC; (v) studies publishing sufficient data for direct or indirect calculation of true positive (TP), true negative (TN), false positive (FP) and false negative (FN) statistics; and (vi) studies with full text available in English or Chinese.

The exclusion criteria were: (i) studies not related to GC; (ii) studies using tissue samples rather than plasma or serum; (iii) studies that did not include cfDNA concentration, methylation, or mutation biomarkers; (iv) studies

focusing on GC prognosis, efficacy assessment and recurrence monitoring; (v) studies that did not provide enough parameters for the calculation of TP, TN, FP, and FN; (vi) studies conducting only animal or in vitro experiments without human cases; (vii) non-original studies, such as reviews, conference abstracts or patents; (viii) duplicates.

cfDNA concentration is defined as the percentage of cfDNA measured in the blood. A healthy individual has a cfDNA concentration of 0–100 ng/mL, with a mean of 30 ng/mL, whereas the cfDNA concentration in cancer patients varies between 0–1000 ng/mL with an average of 180 ng/mL.[13–15] cfDNA methylations are characterized when a methyl group is added to the cytosine residues, specifically at cytosine-guanine (CpG) dinucleotide sites by DNA methyltransferases (DNMT).[16]

2.3 | Data extraction and quality assessment

The retrieved literature was imported into the reference manager software EndNote (Clarivate). Two researchers (Mona Wang and Xiaohan Fan) read the literature independently, checked that inclusion and exclusion criteria were met and extracted data from the studies finally included. The view of a third investigator (Boyang Huang) was sought if the opinions of the first two researchers were inconsistent. Data extracted from the selected studies included the name of the first author, year of publication, country, sample size, number of GC patients, number of controls, type of samples, type of cfDNA biomarkers, detection method for biomarker investigation, and statistical parameters such as sensitivity, specificity, TP, FP, FN, and TN. For a study with an independent validation set, the corresponding values of TP, FP, FN, and TN from the validation set were also extracted. For studies including early GC cases, statistical parameters were calculated for both early GC and all-stage GC subjects. If a study evaluated multiple biomarker combinations, the biomarker combination with the best diagnostic performance was chosen.

2.4 | Study quality assessment

The quality of the included studies was evaluated using the revised tool for the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2).[17] QUADAS-2 evaluates four key aspects, including patient selection, index test, reference standard, and flow and timing. All components were evaluated for "risk of bias" and "concerns regarding applicability". The risk of bias was determined as "low," "high," or "uncertain" for each aspect of the study. All studies were assessed independently by two investigators (Mona Wang and Xiaohan Fan). Any discrepancies between the opinions of the two investigators were discussed and reassessed by the third investigator (Boyang Huang).

2.5 | Statistical analysis

Statistical analysis was performed using RevMan 5.3 and the Midas package of Stata 17.0 software to calculate sensitivity, specificity, pooled positive likelihood ratio (PLR), pooled negative likelihood ratio (NLR), pooled diagnostic odds ratio (DOR), and corresponding 95% confidence interval (CI) based on TP, FP, FN, and TN indicators. The LRT_I2 (I-square) statistic and LRT_Q2 (Chi-square) statistic were used to test the heterogeneity of the studies. A fixed-effect model was used when heterogeneity was considered low ($I^2 \leq 50\%$ and $p > 0.05$). Otherwise, a random-effects model was selected when the heterogeneity was high with $I^2 \geq 50\%$ and $p < 0.05$. The sources of the heterogeneity were further explored by subgroup analysis, sensitivity analysis, or meta-regression. The summary receiver operating characteristic (SROC) curve was plotted and the area under the curve (AUC) was calculated. Publication bias was analyzed using Deek's funnel plot with $p < 0.10$ indicating the presence of publication bias.

2.6 | Subgroup analysis and meta-regression analysis

Subgroup analysis by stage of GC (early or all stage), as well as type of cfDNA biomarker (concentration and methylation), were conducted. All GC cases in stages I and II were defined as early GC. No subgroup analysis of mutation biomarkers was performed as only 1 associated publication was eligible for inclusion.

Meta-regression analysis was performed to assess potential factors that may lead to heterogeneity and bias, including the country where the study was conducted, the type of samples, the type of biomarkers, the total sample size of the study, and the throughput of the detection method (Table 1).

3 | RESULTS

3.1 | Baseline study characterization

Following the literature search strategy, 1217 publications were preliminarily selected from the four databases. The retrieved literature was imported into EndNote with 666 articles remaining after duplicates were removed. Accord-

TABLE 1 Study characteristics and extracted statistical data.

First author	Year	Country	Sample type	Biomarker type	Total Population (P/C)	Age (Control)	Age (GC)	Stage	TP	FP	FN	TN	Sen	Spe	AUC	cfDNA marker detection method	Molecular target (gene)
Park[10]	2009	Korea	tissue plasma	concentration	96(57/39)	N/A	61.30	all	43	9	14	30	0.75	0.77	0.82	quantitative real-time PCR	MYC/GAPDH
Park[12]	2012	Korea	tissue plasma	concentration	113(54/59)	61.60	61.80	all	41	22	13	37	0.75	0.63	0.78	Alu81-qPCR test	N/A
Yang[26]	2013	China	plasma	methylation	62(40/22)	N/A	60.66	all	17	0	23	22	0.44	1.00	N/A	PCR sequencing	BCL6B
Zhang[27]	2014	China	plasma	methylation	99(57/42)	57.21	61.49	all	49	22	8	20	0.86	0.48	N/A	MSP	RNF180, SFRP2 promoter
Kim[37]	2014	South Korea	plasma	concentration	64(30/34)	63.79	66.72	all	29	2	1	32	0.97	0.94	0.99	qPCR	N/A
Wu[11]	2016	China	serum	concentration	199(99/100)	N/A	N/A	all	75	3	24	97	0.76	0.97	0.94	ECLIA-IIS chemiluminescence assay	N/A
Li[9]	2017	China	plasma, serum	concentration	161(81/80)	N/A	N/A	all	63	1	18	42	0.78	0.98	0.99	Fluorescence quantitative PCR	ALU247
Anderson[18]	2018	America, South Korea	plasma, tissue	methylation	74(36/38)	69.00	70.00	all	30	0	6	38	0.83	1.00	0.91	QuARTS	ELMO1, ZNF569, C13orf18
Cohen[28]	2018	America	plasma, tissue FFPE, WBC	mutation	880(68/812)	49.00	63.00	all	49	7	19	805	0.72	0.99	N/A	PCR NGS	16-gene panel
Wei[25]	2019	China	serum, tissue, cell line	methylation	73(53/20)	57.00	69.00	all	47	5	6	15	0.89	0.75	N/A	MSP	SPG20
Saliminejad[24]	2020	Iran	plasma	methylation	184(96/88)	56.10	59.50	all	64	6	32	82	0.67	0.93	0.80	MSP	RPRM
Chen[20]	2020	China	plasma	methylation	518(104/414)	61.62	63.95	all	89	19	15	395	0.86	0.95	N/A	PanSeer assay	595 genomic region panel
Hideura[21]	2020	Japan	serum	methylation	111(50/61)	58.00	72.2	early	25	12	25	49	0.50	0.80	0.70	CORD	RUNX3
Cao[19]	2020	China	plasma	methylation	230(74/156)	N/A	N/A	early	30	8	44	49	0.41	0.85	0.65	MSP	SEPT9, RNF180
Ren[23]	2022	China	plasma, adjacent tissue	methylation	171(42/42)	61.1	59.2	all	28	4	14	38	0.67	0.91	0.86	MCTA-Seq	153-gene panel

Abbreviations: AUC, area under the curve; CORD, combined restriction digital PCR; MSP, methylation-specific PCR; P/C, patient/control; Sen, sensitivity; Spe, specificity; WBC, white blood cells; WGS, whole genome sequencing.

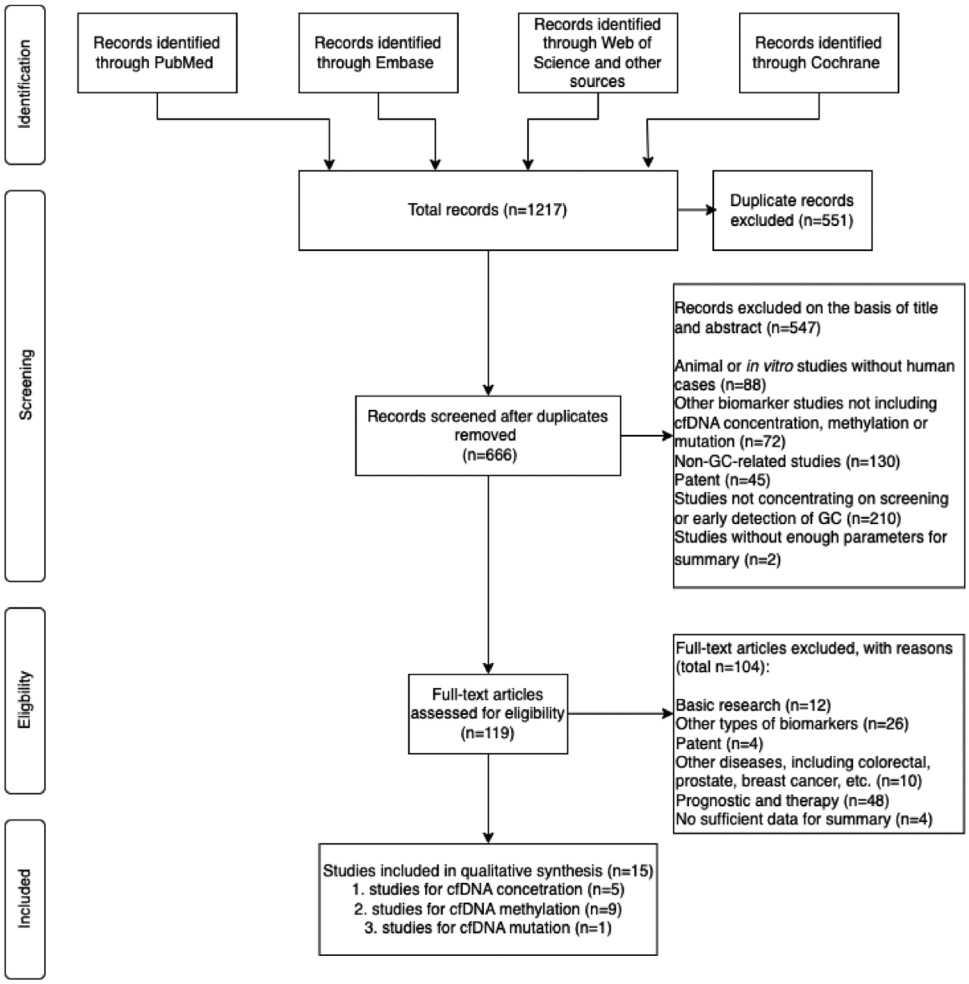

FIGURE 1 PRISMA flow chart of the literature selection process. 1217 studies were filtered from four different databases: PubMed, Embase, Web of Science, and Cochrane. In the screening process, 551 duplicated studies and 547 studies not matching the inclusion criteria were removed. The remaining 119 studies were screened for eligibility and 104 studies were removed after full-text screening. 15 studies were included for qualitative analysis.

ing to the inclusion and exclusion criteria, 508 articles were initially excluded based on the title and abstract content, and 104 articles were further excluded after subsequent full-text intensive reading. Finally, 15 eligible studies were selected for meta-analysis (Figure 1). All of the 15 studies investigated cfDNA biomarkers in GC subjects in all stages, with eight publications further specifically describing the diagnostic value of cfDNA biomarkers for early GC. Among the 15 studies, 5 focused on cfDNA concentration alterations,[8–12] nine evaluated cfDNA methylation biomarkers,[18–27] and 1 reported mutation variations.[28]

The publication years of the 15 studies ranged from 2009 to 2022. The sample sizes of the study population were from 49 to 880, covering patients from nine different countries (Iran, Japan, China, America, Denmark, Netherlands, South Korea, Bulgaria, and Lithuania). Six studies included gastric tissue and plasma samples simultaneously with the other nine studies applying only plasma or serum samples. A total of 2849 study subjects, including 941 GC patients and 1908 non-GC controls, were included in the 15 studies. The detection methods for the cfDNA biomarkers included PCR, methylation-specific PCR (MSP), and high-throughput second-generation sequencing technologies. After intensive reading of the articles, TP, FP, TN, and FN were extracted from early and other-stage GC subjects (Table 2).

3.2 | Quality of the included studies

Each selected study was assessed according to QUADAS-2 criteria for its Risk of Bias (RoB) and quality in four aspects, namely *Patient Selection, Index Test, Reference Standard*, and *Flow and Timing* (Figure 2). Concerns regarding the reference standard in both RoB and applicability were

TABLE 2 Summary results of subgroup analysis in different diagnosis stages and liquid biopsy methods.

Subgroup of liquid biopsy	Sensitivity (95%CI)	Specificity (95%CI)	PLR (95%CI)	NLR (95%CI)	DOR (95%CI)	AUC for SROC	Reference
Overall liquid biopsy	0.74 (0.66–0.82)	0.92 (0.84–0.96)	10.77 (5.24–22.15)	0.27 (0.20–0.37)	34.82 (14.64–2.82)	0.89 (0.86–0.91)	2, 8, 10–12, 18–21, 23–28
Stages of GC							
Early stage	0.64 (0.51–0.76)	0.94 (0.87–0.98)	11.47 (4.27–30.49)	0.38 (0.26–0.55)	30.27 (8.45–108.49)	0.86 (0.83–0.89)	18–20, 23, 24, 28
Other stages	0.78 (0.71–0.83)	0.94 (0.85–0.97)	12.21 (5.13–29.08)	0.24 (0.18–0.31)	51.22 (21.13 –124.16)	0.89 (0.86–0.91)	8–12, 18, 20, 23–28
Liquid biopsy methods							
Methylation	0.71 (0.57–0.81)	0.90 (0.79–0.96)	7.34 (3.23–16.66)	0.33 (0.21–0.50)	22.48 (8.22–61.51)	0.87 (0.84–0.90)	18–21, 23–28
Concentration	0.78 (0.73–0.82)	0.91 (0.75–0.97)	8.59 (2.82–26.23)	0.24 (0.19–0.31)	35.62 (9.83–129.09)	0.80 (0.76–0.83)	8–12

Abbreviations: AUC, area under the curve; DOR, diagnostic odds ratio; NLR, negative likelihood ratio; PLR, positive likelihood ratio; SROC, summary receiver operating characteristic.

FIGURE 2 Quality assessment of the selected studies by Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2). All studies were assessed by QUADAS-2 using the Revman tool. (A) Risk of bias in all studies. (B) Risk of summary categorised as "high", "unclear" and "low" based on the rating of two independent reviewers.

low (presented in green) since almost all studies used the gold standard of endoscopic results for diagnosis. There were a few applicability concerns regarding the Patient Selection and Index Test as per the inclusion criteria of the review. However, the selection of patients was characterized as high RoB in eight studies because of the high heterogeneity of the studies without randomization in subject selection and case-control design. The RoB

of the Index Test was described as high or unclear in 5 studies, which may be due to conducting and interpreting the index test after knowledge of the reference standard. Nevertheless, the overall quality of the literature included in this review is high and qualified for further analysis.

3.3 | Overall performance of cfDNA biomarkers in predicting GC

The diagnostic value of cfDNA biomarkers for GC subjects in all stages was evaluated using sensitivity and specificity. After the heterogeneity test, we applied a random-effects model for meta-analysis and revealed a pooled sensitivity (95% CI) of 0.74 (0.66–0.82) and specificity (95% CI) of 0.92 (0.84–0.96). The combined parameters were further calculated, giving a pooled PLR (95% CI) of 9.67 (4.66–20.05), pooled NLR (95% CI) of 0.28 (0.20–0.38), pooled DOR (95% CI) of 34.82 (14.64–82.82), and AUC in SROC (95% CI) of 0.89 (0.86–0.91). Forest plots for the respective statistical values are shown in Figure 3.

3.4 | Diagnostic performance of cfDNA biomarkers for early GC subjects

Seven studies included the subgroup of early GC subjects. Accordingly, we analyzed the alterations of cfDNA biomarkers in early GC subjects. Based on the results of the heterogeneity test, we used a random-effects model for the meta-analysis and calculated a pooled sensitivity (95% CI) of 0.64 (0.51–0.76) and a pooled specificity (95% CI) of 0.94 (0.87–0.98) (Figure S1). Furthermore, the pooled PLR, NLR, DOR, and AUC of SROC curve analysis were 11.41 (4.27–30.49), 0.38 (0.26–0.55), 30.27 (8.45–108.49), and 0.86 (0.83–0.89), respectively.

3.5 | Diagnostic performance of cfDNA methylation biomarkers for GC subjects

Among the included studies, nine evaluated cfDNA methylation biomarkers for GC diagnosis. The random-effects model was selected for meta-analysis according to the results of the heterogeneity test. Corresponding forest plots in Figure S2 show a pooled sensitivity of 0.71 (0.57–0.81), specificity of 0.90 (0.79–0.96), PLR of 7.34 (3.23–16.66), NLR of 0.33 (0.21–0.50), DOR of 22.48 (8.22–61.51) and AUC of SROC curve analysis of 0.87 (0.84–0.90), respectively. The performance of cfDNA methylation biomarkers exhibited their applicability for GC diagnosis.

3.6 | Diagnostic performance of cfDNA mutation biomarkers for GC subjects

Only one article by Cohen et al. reported cfDNA mutation biomarkers for early GC detection.[28] The mutation biomarkers in that study presented a sensitivity and specificity of 0.686 and 0.991, respectively, for early-stage GC diagnosis.[28] Taking all stages of GC diagnosis into consideration, the sensitivity and specificity were increased to 0.72 and 0.99.[28]

In the study of Cohen, et al.[28] a panel containing 16 genes was sequenced in both plasma and tissue samples. Amongst all mutated genes, TP53 mutation was most frequently detected in plasma samples and the mutation site of *TP53 K372fs* presented the highest detection rate in cfDNA. In tissue samples, the genes of TP53, PIK3CA, KRAS, and CTNNB1 were most commonly mutated (cut-off value = mutation frequency/ mutation count > 3.5%) (Table S3). The mutation sites of *PIK3CA E545K, KRAS G12D, TP53 V272M, and PIK3CA H1047R* were most frequently observed in tissue samples amongst the cohort of Cohen et al.[28]

3.7 | Diagnostic performance of cfDNA concentration for GC subjects

Of all included studies, five focused on the relationship between cfDNA concentration and GC. Based on the results of the heterogeneity test a random-effects model was used for meta-analysis which showed a pooled sensitivity and specificity of 0.78 (0.73–0.82) and 0.91 (0.75–0.97), respectively (Figure S3). Furthermore, pooled PLR, NLR, DOR, and AUC were 8.59 (2.82–26.23), 0.24 (0.19–0.31), 35.62 (9.83–129.09), and 0.80 (0.76–0.83), respectively.

3.8 | Publication bias

The Deek's funnel-plot asymmetry test was performed on all 15 studies and indicated a symmetrical plot with a regression curve of $p = 0.18$, suggesting no significant publication bias in the included literature (Figure 4).

3.9 | Meta-regression

According to the calculation of the I^2 value in the Diagnostic Odds Ratio, considerably high heterogeneity was detected across the total studies (100%). To reveal the possible sources of heterogeneity, we performed a meta-regression analysis assessing potential co-variates among

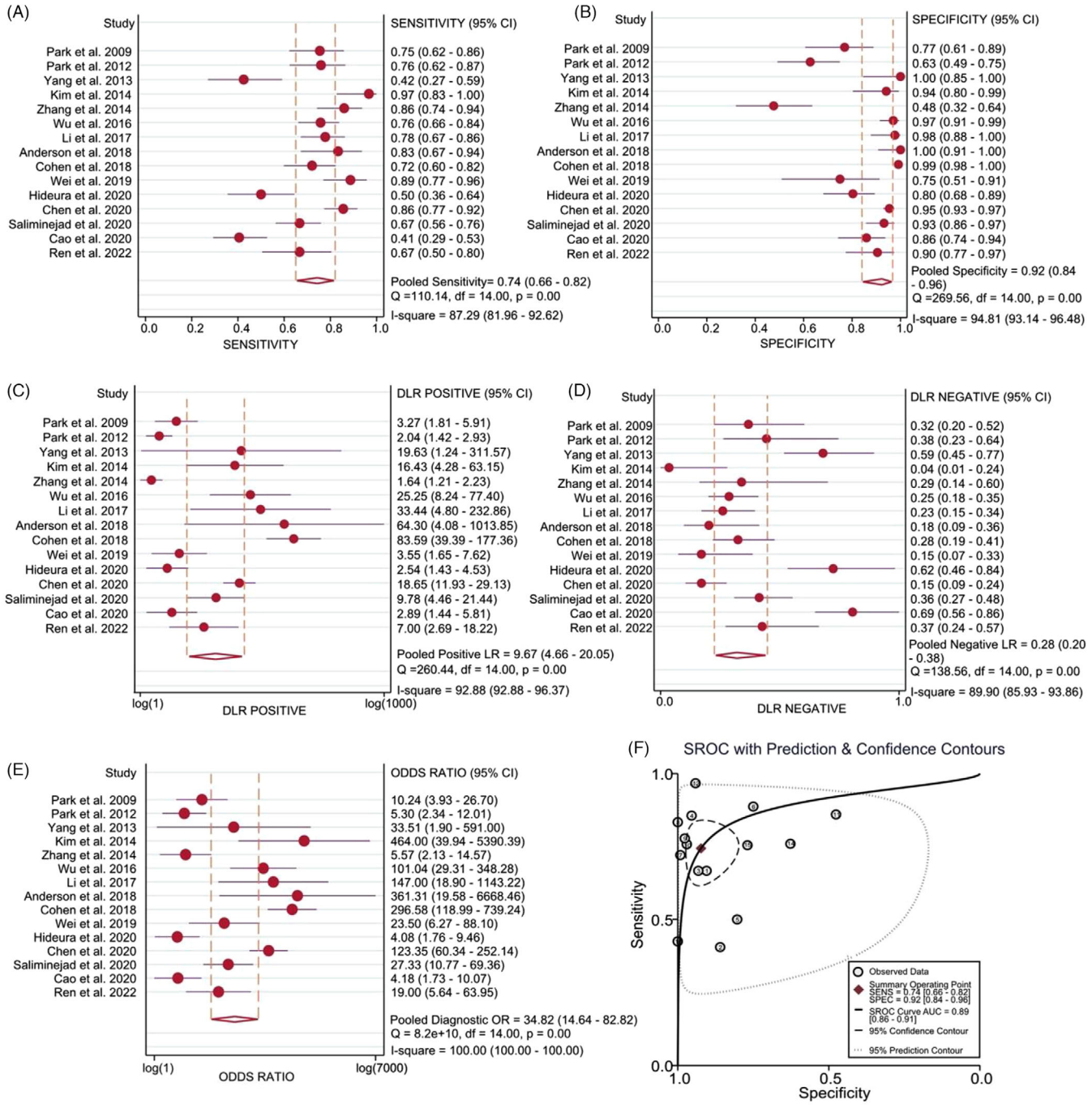

FIGURE 3 Meta-analysis of cell-free DNA (cfDNA) markers in the diagnosis of gastric cancer (GC). Sensitivities (A), specificities (B), positive likelihood ratios (C), negative likelihood ratios (D), diagnostic ratios (E), and areas under the curve (AUCs) (F) of cfDNA markers in diagnosing GC. Horizontal lines indicate 95% confidence intervals.

these 15 studies. The potential co-variates included countries where the studies were conducted (China or other countries), publication year (before or after 2015), types of samples (plasma or serum), types of biomarkers (cfDNA methylation or others), detection throughput (high or low), GC patient size (number of GC subjects ≥50 or < 50) and total study population size (total number of study population ≥100 or < 100).

The meta-regression analysis found statistically significant relationships ($p < 0.05$) between the heterogeneity of sensitivity and type of biomarker or total study population size. However, no statistically significant relationships were found between the heterogeneity of specificity and all co-variates (Figure 5). Generally, three different types of studies were included: cfDNA concentration, methylation, and mutation. Amongst them, the majority were methyla-

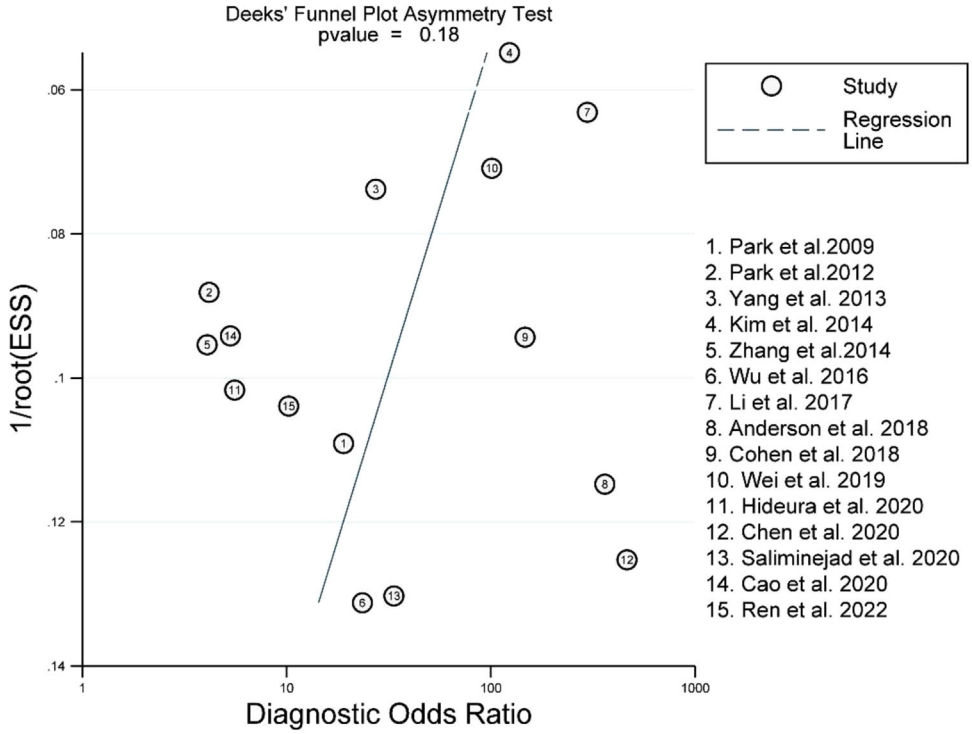

FIGURE 4 Deek's funnel-plot analysis test. Deek's funnel plot presented the symmetry of the effect size measure (diagnostic odds ratio) over the range of sample sizes presented an unlikeliness of publication bias in this review.

tion studies (9/15). Other studies describing cfDNA concentration (5/15) and mutation (1/15) presented a higher sensitivity compared to methylation studies. Excepting the study on mutation with 880 participants, the average population size for cfDNA concentration studies (127) was lower than that for methylation studies (169). This observation is in accordance with the second finding of the meta-regression analysis that studies with a smaller total population size presented a higher sensitivity compared to studies with a larger population size. Detailed analysis found that most of the studies (3/5) on cfDNA concentration were published before 2015 and more studies focusing on cfDNA methylation and mutation biomarkers (8/15) were published after 2015, perhaps due to the development of novel technologies. Other factors, such as the country where the study was conducted and sample type, did not show significant effects on heterogeneity ($p > 0.05$).

4 | DISCUSSION

Early diagnosis is crucial for the prevention and treatment of cancer, especially for cancers located in the gastrointestinal tract. Early detection and treatment of GC may increase the 5-year survival rate up to 90%.[4,5] A non-invasive method for early diagnosis may be more cost-effective and easier to conduct than conventional upper GI endoscopy. The present systematic review and meta-analysis summarized current advances in the early detection of GC using cfDNA biomarkers. To our knowledge, this is the first review analyzing the performance of cfDNA biomarkers for early detection of GC in a systematic and meta-analytical manner.

In recent decades, the frequently studied, non-invasive cfDNA biomarkers for GC diagnosis can be divided into three categories, including cfDNA concentration, methylation, and mutation changes. AUC of ROC curve analysis is a commonly used assessment index for comparison of the accuracy of diagnostic tests. In this systematic review, we calculated overall AUCs for GC subjects in all stages as well as in the early stage, which reached 0.89 and 0.86, respectively. This suggests that cfDNA biomarkers are potentially efficient diagnostic tools for GC and early GC detection.

cfDNA biomarkers showed a high pooled sensitivity (0.74, 0.66–0.82) and high pooled specificity (0.92, 0.84–0.96) in diagnosing subjects at all GC stages. However, in diagnosing GC in early-stage subjects, a high pooled specificity (0.94, 0.87–0.98) but lower pooled sensitivity (0.64, 0.51–0.76) was observed. The lower sensitivity of the cfDNA biomarkers for early-stage GC detection may be due to the limited amount of cfDNA released from tumour tissue in early-stage development,[29–31] a key hurdle for the clinical application of non-invasive biomarkers. Thus, future use of cfDNA biomarkers for screening pop-

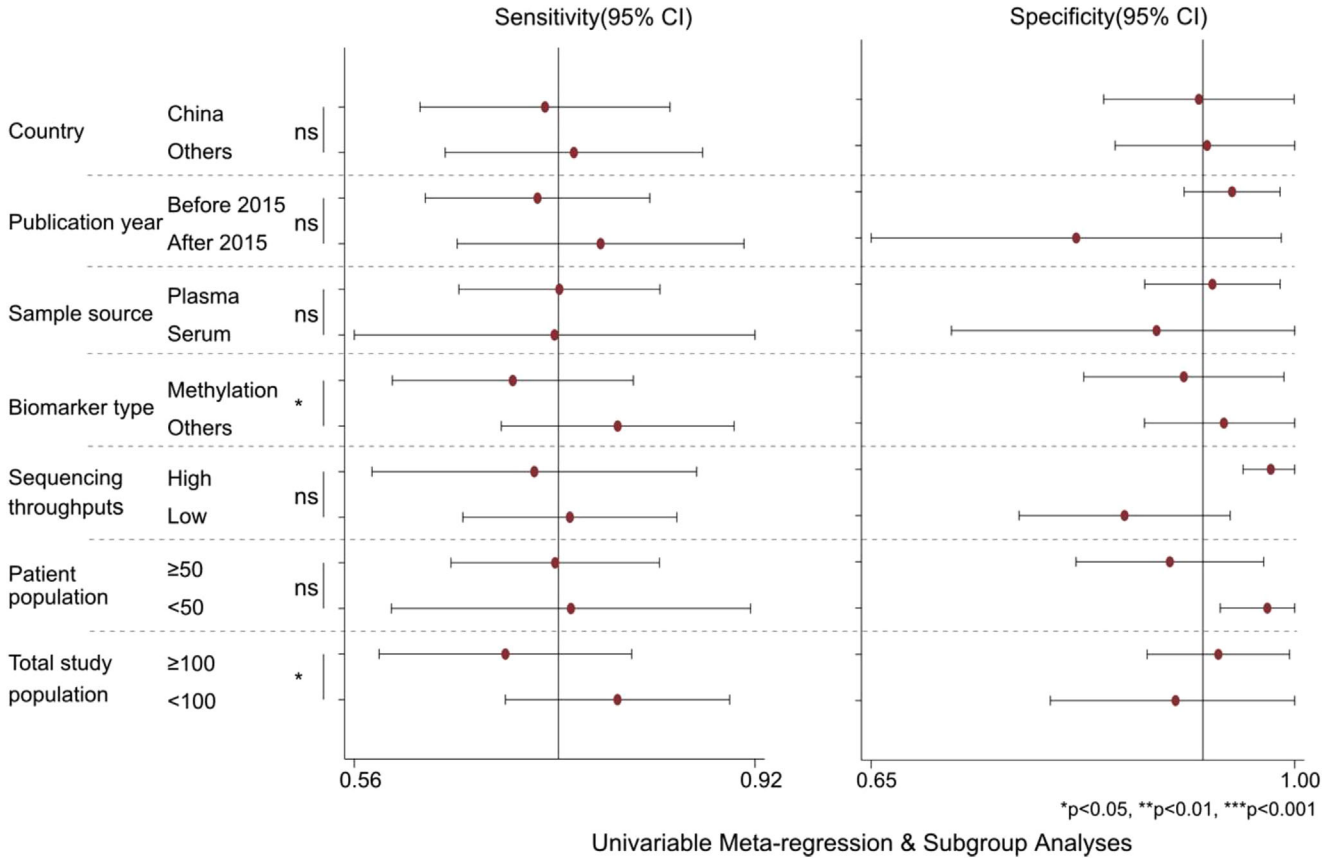

FIGURE 5 Meta-regression forest plot showing sources of heterogeneity between studies. Selected publications were examined for the source of high heterogeneity. The following covariates were analyzed for their significance in sensitivity (95% confidence interval [CI]) and specificity (95% CI): Country of origin, Publication year, sample source, biomarker type, sequencing throughput, patient population, and total study population. Statistical significance was determined with a two-way analysis of variance (ANOVA). *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$, and ns: no statistical significance.

ulations may require not only an improved selection of biomarkers but also more sensitive detection technologies to capture trace cfDNA alterations. Benefiting from novel developed technologies like NGS, multiple potential cfDNA biomarkers have been investigated to date. Nevertheless, the specific functions and interplays of the great number of biomarkers remain not fully understood.

With not enough credible mutation biomarkers identified, applying mutation biomarkers remains challenging, particularly for early diagnosis of GC. In this systematic review, only one study on cfDNA mutation was found eligible and provided statistical data. The other studies showed no detailed sensitivity and specificity information, making an accurate assessment of the candidate mutation biomarkers difficult. While comparing this study with four other mutation publications, which were not eligible for systematic analysis,[32–35] we noticed that only one study[34] had tried to combine different types of plasma biomarkers (methylation and mutation) to improve the accuracy of GC diagnosis. Tomeva et al.[34] applied cfDNA methylation and mutation biomarkers, and additional miRNA biomarkers to establish an integrative non-invasive panel for GC screening. The comprehensive performance of the integrative panel was optimized to an accuracy of 95.4%, sensitivity of 97.9%, and specificity of 80%.[34] Recently, a population-based cohort study explored noninvasive multi-analytical biomarkers and constructed integrative models for preliminary risk assessment and GC detection.[36] This study found that the integrated model including methylation and mutation biomarkers showed improved sensitivities (0.72 and 0.63) and AUCs (0.83 and 0.82) in training and test sets to discriminate GC from IM/LGIN. The integrated GC model had better performance in training and test sets compared to the traditional model including PG ratio and *H. pylori* infection status (AUCs, 0.60 and 0.68, p-values < 0.001 and 0.005, respectively, DeLong test). The outstanding performance of the multi-biomarker panel suggested a promising direction for non-invasive GC screening studies.

Most cfDNA mutation studies, including the one analyzed in this systematic review, used targeted gene panels containing 16–73 genes.[28,33–35] Cristiano et al.[32] performed

whole genome sequencing analysis. The most frequently reported mutated genes in plasma samples from GC subjects included *TP53*, *KMT2C*, *MUC16*, *RP1B*, *PIK3CA*, *CTNNB3*, *KRAS*, *ERBB2*, SMAD4, AR, EGFR, VHL, and SYNE1. The most commonly detected mutated genes in GC tissue samples were TP53, MUC16, KMT2C, PIK3CA, SYNE1, KRAS, ARID1A, BRCA2, RNF43, PKHD1, *RIMS2*, *CTNNB1*, *FAT4*, and *ZIC4*. Amongst all the mutations found in cfDNA of GC subjects, the mutations of *KMT2C c.2652+62A > G*, and *c.5009-19C > T* presented the highest detection rates,[35] followed by EGFR L858R, FGFR3 Y375C, APC T1556N, TP53 K372fs, PIK3CA E545K, KRAS G12D and ERBB2 R678Q, TP53 R248W, APC A381fs. In the tissue of GC patients, the mutations RNF43 G659Vfs, ACVR2A K437Rfs, KMT2C c.1012+76G > T, ARID1A P146fs, MUC16 P13555C, PIK3CA E545K, TP53 G245S, R248W, R282W, and KRAS G12D were most frequently detected amongst all the 4 cohorts. High variations in the most frequently detected mutations were observed across different cohorts. These variations might partly result from discrepancies in detection technologies, as well as differences in study populations. To summarize, although many prominent mutation biomarkers for GC have been identified, there is still potential for improvement in terms of addressing their heterogeneity, sensitivity, and specificity.

In attempting to identify possible sources of heterogeneity in this systematic review, we found that the number of GC patients or total study population may greatly affect heterogeneity between the different studies. The most significant heterogeneity may derive from different types of biomarkers, such as methylation or non-methylation markers, which is reasonable considering the great discrepancies between genetic and epigenetic mechanisms for GC development. On the other hand, the significant heterogeneity between cfDNA methylation and non-methylation biomarker studies may suggest the necessity to establish a multiple biomarker panel for efficient early detection of GC. In addition to the factors examined, heterogeneity may also be related to factors such as gender, age, tumour size, histological tumour type, and tumour, node, metastasis (TNM) stage of patients, which could not be analyzed due to lack of data. Another reason for the substantial variability in this study could be the small number of included studies. Nevertheless, the possibility of publication bias is not to be neglected, as it remains a prevalent issue in meta-analyses.

Finally, although no significant differences were found between the studies applying plasma or serum samples, concordance analysis of biomarkers (methylation, mutation, and concentration) between cfDNA and tumour samples is important to discern alterations reflecting the genomic landscape from the tumour itself.

5 | CONCLUSION

In this study, we conducted a systematic review and meta-analysis on the value of cfDNA concentration, methylation, and mutations for GC diagnosis. Our findings suggest that cfDNA biomarkers may show high sensitivity and specificity for GC diagnosis. However, for early detection of GC, the sensitivity of cfDNA biomarkers still needs further improvement. Most of the current studies only focus on one single cfDNA biomarker type, such as methylation, mutation, or concentration. High-quality, well-designed, multi-omics integration studies are still needed for accurate GC screening and early detection.

AUTHOR CONTRIBUTIONS

Mona Wang and Xiaohan Fan designed the study; Mona Wang and Xiaohan Fan coordinated the study; Mona Wang, Xiaohan Fan, and Boyang Huang performed the acquisition of data and the statistical analysis; Mona Wang, Xiaohan Fan, and Boyang Huang interpreted the data; Mona Wang and Xiaohan Fan drafted the manuscript. All authors revised the final manuscript and approved this version to be published.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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