# Value of circulating free DNA methylation in the diagnosis of esophageal cancer: A systematic review and meta-analysis

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**Abstract.** Liquid biopsy using circulating biomarkers has shown growing potential in cancer detection. The aim of the present study was to assess the application value of detecting the methylation level of circulating free (cf)DNA in the identification and diagnosis of esophageal cancer (EC), to lay a scientific foundation for its clinical application, and to provide statistically significant references for the detection process. A total of two researchers independently performed a comprehensive search of the PubMed, Web of Science, Cochrane Library, Embase and Scopus databases to identify all relevant studies on cfDNA in EC diagnosis as of July 25, 2024. A meta-analysis was performed using MetaDiSc 1.4, Stata 16.0 and RevMan 5.4 software. Pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic rate (DOR) and area under the curve (AUC) values were determined. According to inclusion and exclusion criteria, a total of 14 studies with a total of 3,936 patients were included in the analysis. The pooled sensitivity and specificity, and PLR, NLR, DOR and AUC of cfDNA methylation in the diagnosis of EC were 0.83 [95% confidence interval (CI), 0.75-0.89], 0.98 (95% CI, 0.95-0.99), 34.57 (95% CI, 18.04-63.44), 0.18 (95% CI, 0.12-0.25), 197.25 (95% CI, 104.53-372.20) and 0.98 (95% CI, 0.96-0.99), respectively. In conclusion, cfDNA methylation demonstrates high diagnostic accuracy for early-stage EC and may serve as a promising non-invasive screening tool.

## Introduction

As the global incidence and mortality rates of cancer continue to rise, it has emerged as a predominant public health concern, the leading cause of death worldwide and a major barrier to increasing life expectancy (1). Esophageal cancer (EC) is a prevalent malignant condition. It ranks among the most frequently diagnosed cancers globally, with the eighth highest incidence rate and the sixth highest mortality rate, with >500,000 new cases diagnosed annually, according to GLOBOCAN (2020) (1). A total of ~80% of new EC cases occur in developing countries, with ~60% of these cases diagnosed in China (2). Despite continuous advancements in diagnostic methods, surgical techniques and adjuvant chemoradiotherapy, most cases of esophageal squamous cell carcinoma (ESCC) are diagnosed at an advanced stage due to a lack of specific symptoms and preventive measures. The overall 5-year survival rate remains low, ranging from 15-50% (3,4). Therefore, effective treatment of EC greatly relies on the identification and prevention of the disease, and implementing screening and early detection methods is crucial for mitigating EC-related fatalities across all demographic groups.

Up to now, the detection of EC has continued to rely on non-molecular methods, which have limited specificity and sensitivity. The use of iodine staining (Lugol's solution) during endoscopic examination remains the gold standard for diagnosing irregularities in the esophageal mucosa (5). However, the widespread use of endoscopic screening in the population faces numerous challenges: Endoscopic targeted biopsy of the upper GI tract is unsuitable for mass screening of the general population due to its invasiveness, limited resources, low patient compliance and high cost, particularly when screening millions of eligible individuals in high-risk areas, such as China (6). Therefore, an urgent need exists to identify a more straightforward, simpler and precise approach for the early detection of EC.

Numerous studies have reported that DNA methylation, as a notable epigenetic alteration, serves a critical role in the progression of EC (7,8). However, traditional DNA methylation detection methods require tissue samples, which limits their clinical application (9). Liquid tumor biopsy, renowned for its procedural simplicity, high sensitivity, specificity and non-invasive or minimally invasive nature, facilitates dynamic monitoring of disease onset, progression and metastasis by detecting relevant tumor markers in the bloodstream, revolutionizing traditional methods of cancer diagnosis and treatment (10). Novel blood biomarkers, such as free DNA, free RNA, metabolites and proteins, have shown potential for the early detection of EC (11-17).

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Circulating free (cf)DNA is a type of extracellular DNA commonly found in the plasma or serum of patients with cancer, which has recently attracted the attention of researchers. It is released into the bloodstream by tumor cells (18) and shares the same genetic signatures and epigenetic modifications as tumor cells (19). This suggests that detecting cfDNA in serum could aid in the early diagnosis and treatment of cancer. Numerous studies have supported the potential of cfDNA as a biomarker for the early detection of cancer, making it a valuable analyte for liquid biopsy assessments (20,21), and offering a promising approach for non-invasive cancer detection. DNA methylation is widely recognized as one of the first molecular alterations occurring during cancer progression (22). Additionally, the biological and diagnostic applications of cell-free DNA have gained increasing popularity due to its minimally invasive, convenient and well-accepted characteristics (23). Abnormal methylated cfDNA detected in blood circulation appears to be a useful biomarker for human cancer (24). However, notable gaps remain in this field, with relatively few studies exploring the potential use of cfDNA methylation for the early detection of EC. Therefore, the present study aimed to thoroughly assess the practical use of cfDNA in diagnosing EC, evaluate the feasibility of cfDNA-based methylation as an early biomarker for EC, and provide a comprehensive reference for its clinical application.

## Materials and methods

*Search strategy and study selection*. A total of two independent investigators performed a search across five databases, PubMed (https://pubmed.ncbi.nlm.nih.gov), Web of Science (https://www.webofscience.com), The Cochrane Library (https://www.cochranelibrary.com), Embase (https://www.embase.com) and Scopus (https://www.scopus.com), for all relevant articles published from the inception of each database until July 25, 2024. The search terms 'esophageal neoplasms', 'esophageal cancers', 'cfDNA', 'circulating cell-free nucleic acid' and 'methylations' were specified (Data S1). In addition, references in all relevant papers were assessed for potential research. The systematic review and meta-analysis were performed in strict accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (25) guidelines and compliance with the established protocol, and is registered with the PROSPERO database (no. CRD42024580147; accessible at https://www.crd.york.ac.uk/prospero/).

*Study selection*. The inclusion criteria were as follows: i) Evaluation of the diagnostic value of cfDNA methylation in EC; ii) inclusion of malignant and benign EC; iii) patients had not undergone adjuvant therapy or received chemotherapy in the recent past, and had no record of other metastatic tumors prior to detection; iv) use of fresh serum samples for testing; v) endoscopy served as a diagnostic criterion for identifying EC; and vi) true positive, false positive, false negative and true negative values were calculated.

Moreover, the exclusion criteria were as follows: i) Non-English literature; ii) non-clinical research literature, including basic experiments, reviews, conference abstracts, laboratory notes and letters to journal editors; and iii) published data was not sufficient to construct a 2x2 table.

*Data extraction*. The literature was independently screened by two researchers, who extracted the data and subsequently cross-validated the results. Any studies with uncertain eligibility were addressed through consultation with an additional researcher. The data gathered encompassed the following: i) Fundamental characteristics of the studies, such as the principal investigator, date of publication, research methodology and the country of origin; ii) details pertaining to the demographic sample of the study, including the participant count and mean age; iii) techniques utilized for assessing methylation; and iv) the reported sensitivity and specificity metrics.

*Quality assessment*. To assess the quality of included studies, two reviewers independently performed quality assessments using the QUADAS-2 tool (version implemented: Original 2011 release with domain-specific signaling questions) (26), which was evidence-based and included patient selection, indicator detection, reference criteria, process and timing.

*Statistical analysis*. MetaDiSc 1.4 and Stata 16.0 software were employed for data analysis, and RevMan 5.4 software was used for quality assessment (27-29). Initially, Meta-Disc was employed to assess the presence of threshold effects and heterogeneity. If the P-value associated with Spearman's correlation coefficient was >0.05, it was concluded that no threshold effect was present. Heterogeneity was evaluated using the Cochran-Q test, focusing on P-values and $I^2$. P>0.05 and $I^2$ ≤50% indicated no significant heterogeneity among the studies. If heterogeneity was detected, a subgroup analysis was performed to determine its source. The factors assessed in the subgroup analysis included the following: i) Ethnicity of the patients; ii) patient count; iii) mean age; and iv) techniques used for methylation detection. When a subgroup demonstrated P>0.05 and $I^2$ ≤50%, covariates were considered as the origin of heterogeneity. The pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR) and diagnostic rate (DOR) were calculated using Stata 16.0, with their corresponding 95% confidence intervals (CI) represented by forest maps. The summary receiver operating characteristic curve was constructed, and the area under the curve (AUC) values were calculated. To assess publication bias, the Deeks funnel plot asymmetry test was employed. In all hypothesis tests, bilateral P<0.05 was considered to indicate a statistically significant difference.

## Results

*Study selection*. In accordance with the research strategy, a total of 310 articles were sourced from online databases, including 2 from The Cochrane Library, 131 from Web of Science, 71 from Scopus, 99 from Embase and 7 from PubMed. These articles were subsequently screened according to the predefined inclusion and exclusion criteria. A total of 34 duplicates were removed, 113 reviews and conference proceedings were excluded, and 105 papers unrelated to the research topic were discarded based on their titles and abstracts. During the screening of the remaining 58 articles for full-text assessment, 44 papers were excluded: 12 were unrelated to cfDNA methylation in EC diagnosis, and 32 studies lacked sufficient data to construct a 2x2 table. Ultimately, a total of 14 studies were included in the analysis (12,30-42). A visual representation

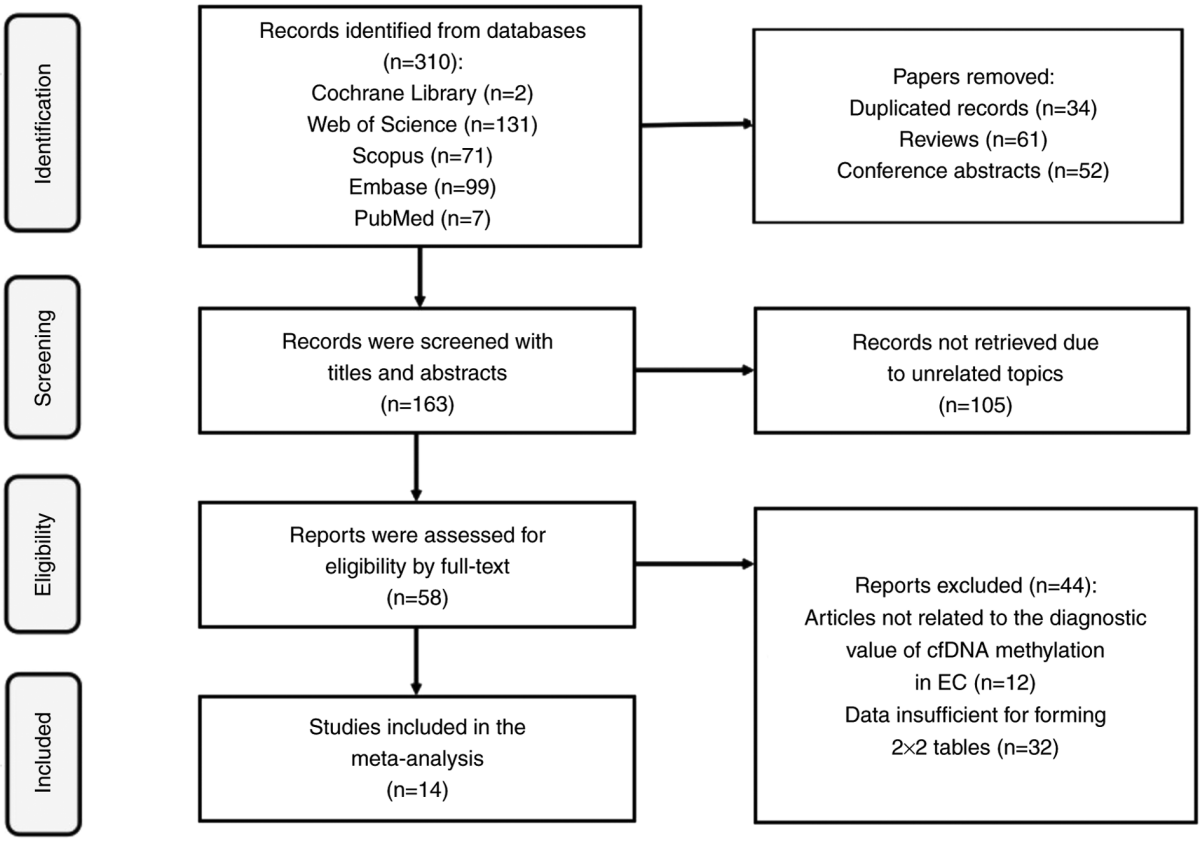


Figure 1. Literature screening process. cfDNA, circulating free DNA; EC, esophageal cancer.

of the literature screening process and its results is presented in Fig. 1.

*Study characteristics*. The meta-analysis incorporated 14 qualifying studies encompassing a collective patient count of 3,936, with an average participant number per study of 218. The articles were published between 2011 and 2024. Most of the articles were retrospective studies, and the majority of the patients were from Asia (8/14) and aged >60 years old on average. All included studies defined controls as allogeneic. Most studies detected cfDNA methylation used methylation-specific polymerase chain reaction (MSP). Table I lists the features of the included studies.

*Quality assessment and risk of bias*. The quality assessment of the included studies, as per the QUADAS-2 scale, revealed that the collective quality of the literature was high (Fig. S1).

*Accuracy of cfDNA methylation for EC*. The pooled sensitivity and specificity were 0.83 (95% CI, 0.75-0.89) and 0.98 (95% CI, 0.95-0.99), respectively (Fig. 2), and the AUC was 0.98 (95% CI, 0.96-0.99; Fig. 3). Combined PLR and NLR were 34.57 (95% CI, 18.04-63.44) and 0.18 (95% CI, 0.12-0.26), respectively (Fig. S2), and combined DOR was 197.25 (95% CI, 104.53-372.20; Fig. S3).

*Subgroup analysis*. In the threshold analysis, a Spearman's correlation coefficient of 0.308 and a corresponding P-value of 0.284 were obtained, indicating the absence of a threshold effect. The $I^2$ sensitivity and specificity results were 94.83 and 80.25%, respectively (Fig. 2), indicating the heterogeneity of sensitivity and specificity due to non-threshold effects. A subgroup analysis was then performed to assess the sources of heterogeneity. Subgroup analysis results indicated notable differences in diagnostic accuracy across subgroups based on sample size, patient age and cfDNA methylation test methods (Table II). Compared with subgroups with a sample size of <200 (DOR, 121.13; 95% CI, 50.83-288.63), those with a sample size of ≥200 exhibited markedly higher diagnostic accuracy (DOR, 172.17; 95% CI, 78.33-378.44). Moreover, compared with individuals aged ≤60 years (DOR, 12.21; 95% CI, 7.79-19.14), those aged >60 years exhibited notably higher diagnostic accuracy (DOR, 360.07; 95% CI, 174.49-743.02). Additionally, among the numerous methylation assessment methods, diagnostic accuracy using MSP (DOR, 66.21; 95% CI, 24.73-177.24) was markedly lower than that of other methods (DOR, 199.83; 95% CI, 83.56-477.85).

*Sensitivity analysis and publication bias*. A funnel plot was generated to assess publication bias, revealing a slope coefficient of P>0.05, and the plot appeared to be symmetrical, suggesting that no significant publication bias existed among the studies (Fig. S4).

## Discussion

As a new diagnostic method for EC, numerous studies have reported that the detection of cfDNA methylation is an

Table I. Main features of the included studies.

| First author, year | Country | TP | FP | FN | TN | No. of patients | Mean age, years | Methods | Sample volume, ml | Control source | Research design | (Refs.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Li *et al*, 2024 | China | 134 | 4 | 34 | 247 | 419 | 62 | EM-seq | 4 | H | Retrospective | (30) |
| Chen *et al*, 2024 | China | 78 | 3 | 4 | 84 | 169 | NR | qMSP | 3 | H | Retrospective | (31) |
| Bian *et al*, 2024 | China | 292 | 48 | 42 | 663 | 1,116 | 55 | NR | 5 | H | Prospective | (32) |
| Vavoulis *et al*, 2023 | UK | 6 | 0 | 2 | 21 | 29 | 67.5 | MSP | NR | H | Retrospective | (33) |
| Nicholson *et al*, 2023 | UK | 37 | 6 | 9 | 969 | 1,021 | 65 | NR | 40 | H | Prospective | (34) |
| Bian *et al*, 2023 | China | 100 | 19 | 18 | 312 | 449 | NR | qMSP | NR | H | Retrospective | (35) |
| Qiao *et al*, 2021 | China | 65 | 3 | 22 | 94 | 185 | 55 | MSP | NR | H | Retrospective | (12) |
| Liu *et al*, 2020 | USA | 47 | 1 | 3 | 20 | 71 | NR | EM-seq | 80 | H | NR | (36) |
| Wang *et al*, 2018 | China | 10 | 0 | 0 | 3 | 13 | 63 | MSP | 200 | H | Retrospective | (37) |
| Boldrin *et al*, 2020 | Europe | 11 | 1 | 7 | 3 | 22 | NR | MSP | 1 | H | Retrospective | (38) |
| Zhai *et al*, 2012 | USA | 10 | 0 | 0 | 28 | 38 | NR | NR | 0.1 | H | Retrospective | (39) |
| Wang *et al*, 2012 | China | 101 | 0 | 108 | 60 | 269 | 60 | MSP | NR | H | NR | (40) |
| Li *et al*, 2011 | China | 37 | 0 | 8 | 15 | 60 | 60 | MSP | NR | H | Retrospective | (41) |
| Andolfo *et al*, 2011 | Europe | 33 | 2 | 8 | 32 | 75 | NR | NR | 500 | H | NR | (42) |

TP, true positive; FP, false positive; FN, false negative; TN, true negative; MSP, methylation-specific polymerase chain reaction; qMSP, quantitative MSP; Control source, origin of baseline for comparison; H, heterogeneous (control samples from other individuals); EM-seq, enzymatic methyl-sequencing; NR, data not reported in the original studies.

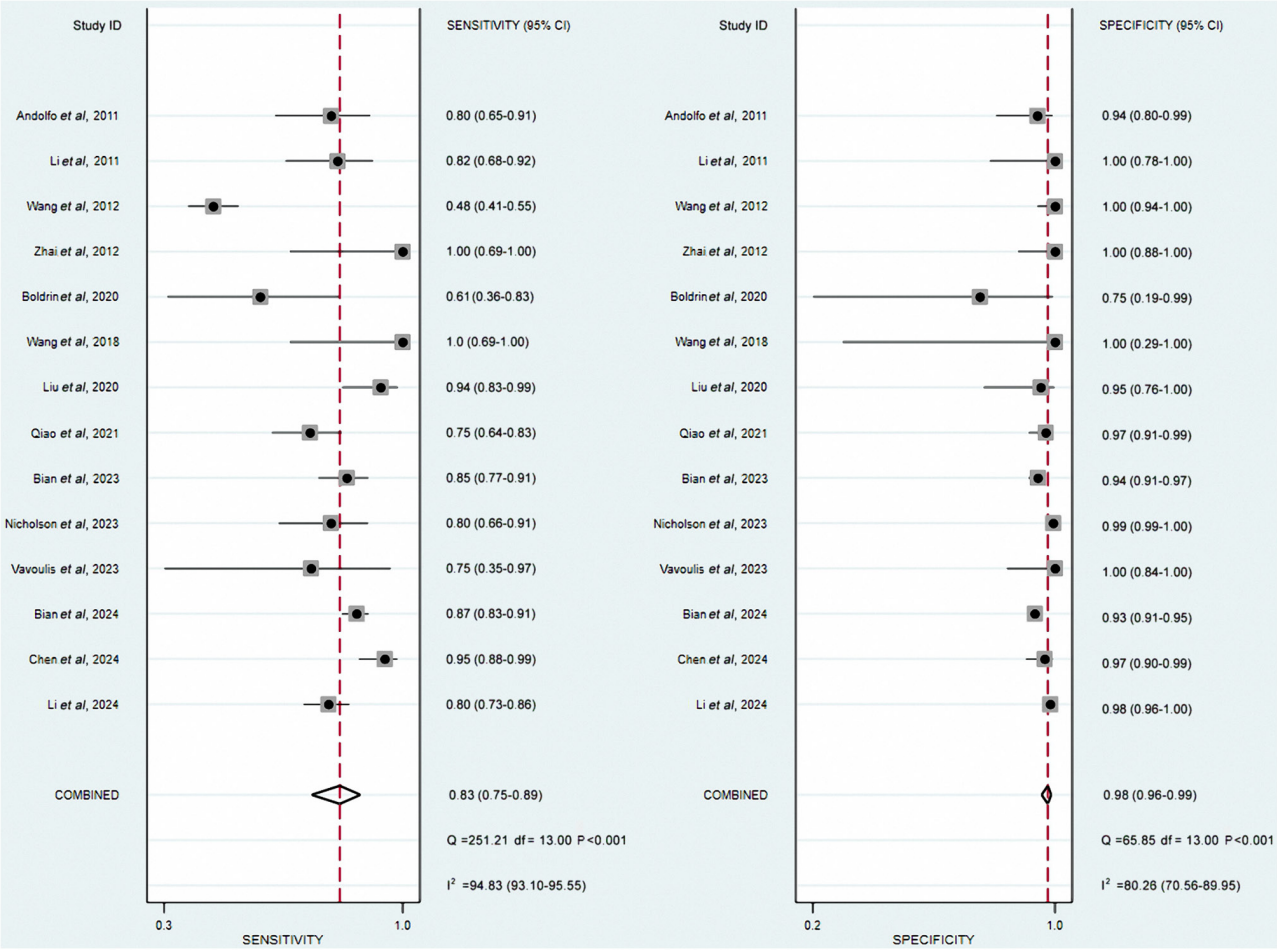


Figure 2. Forest plots of pooled sensitivity and specificity. CI, confidence interval.

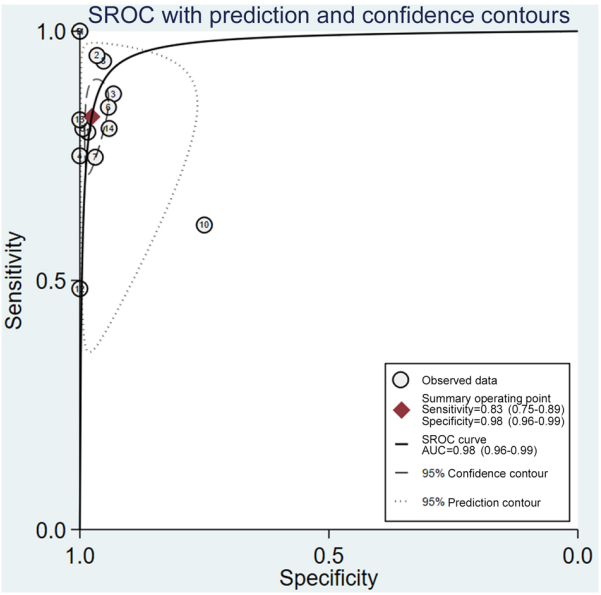


Figure 3. SROC curve of circulating free DNA for the diagnosis of esophageal cancer. The SROC curve is symmetrical with no threshold effect. SROC, summary receiver operating characteristic; AUC, area under the curve.

accurate and effective diagnostic method (19,20,24). Evidence suggests that cfDNA methylation achieves a sensitivity of 75-92% for early-stage detection, comparable with (and in certain cases exceeding) the 70-85% sensitivity of conventional endoscopic screening (43,44). Whilst endoscopy remains the gold standard for morphological assessment, its sensitivity can vary depending on operator experience and lesion characteristics (44). To date, no comprehensive review has yet been performed to assess the accuracy of the diagnosis, to the best of our knowledge. Therefore, the present systematic review and meta-analysis represents the first comprehensive attempt to evaluate the diagnostic efficacy of cfDNA methylation in EC. The findings indicate that the pooled sensitivity and specificity were 0.83 and 0.98, respectively, indicating high sensitivity and specificity for the differential diagnosis of EC, offering substantial support for clinical diagnosis of this disease.

cfDNA consists of small DNA fragments that are released into the bloodstream following cell death, and these fragments circulate throughout the system (45,46). In healthy individuals, cfDNA predominantly originates from blood-forming cells, though its composition can change under specific physiological or pathological conditions. DNA methylation, a widely investigated epigenetic modification, is characterized by the attachment of methyl groups to the carbon-5 position of cytosine, a process catalyzed by DNA methyltransferase enzymes, predominantly occurring at CpG dinucleotides (47).

Table II. Results of the subgroup analysis.

| | | Sensitivity | | | Specificity | | | DOR | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Classification | n | Value (95% CI) | $I^2$, % | P-value | Value (95% CI) | $I^2$, % | P-value | Value (95% CI) | $I^2$, % | P-value |
| Ethnicity | | | | | | | | | | |
| Caucasian | 6 | 0.83 (0.77-0.88) | 66.1 | 0.010 | 0.99 (0.98-1.00) | 60.9 | 0.031 | 136.64 (24.74-754.72) | 74.0 | <0.001 |
| Other | 8 | 0.78 (0.75-0.80) | 94.8 | <0.001 | 0.95 (0.94-0.96) | 68.4 | <0.001 | 114.33 (81.30-160.79) | 3.2 | 0.410 |
| Number of patients | | | | | | | | | | |
| <200 | 10 | 0.71 (0.67-0.75) | 92.2 | <0.001 | 0.97 (0.95-0.99) | 20.3 | 0.250 | 121.13 (50.83-288.63) | 31.8 | 0.150 |
| ≥200 | 4 | 0.85 (0.82-0.87) | 45.7 | 0.140 | 0.97 (0.96-0.97) | 95.1 | <0.001 | 172.17 (78.33-378.44) | 76.8 | <0.001 |
| Mean age, years | | | | | | | | | | |
| ≤60 | 4 | 0.73 (0.70-0.77) | 97.0 | <0.001 | 0.94 (0.92-0.96) | 74.2 | 0.010 | 12.21 (7.79-19.14) | 0.0 | 0.991 |
| >60 | 4 | 0.81 (0.75-0.85) | 33.9 | 0.214 | 0.99 (0.99-1.00) | 0.0 | 0.502 | 360.07 (174.49-743.02) | 0.0 | 0.400 |
| Method | | | | | | | | | | |
| MSP | 6 | 0.61 (0.56-0.66) | 87.8 | <0.001 | 0.98 (0.95-0.99) | 36.7 | 0.160 | 66.21 (24.73-177.24) | 7.2 | 0.374 |
| Other | 4 | 0.86 (0.82-0.89) | 80.8 | <0.001 | 0.96 (0.94-0.97) | 58.7 | 0.062 | 199.83 (83.56-477.85) | 50.7 | 0.112 |

CI, confidence interval; DOR, diagnostic rate; MSP, methylation-specific polymerase chain reaction.

An increasing body of evidence indicates that aberrant DNA methylation serves a notable role in the development and progression of cancer, primarily characterized by global hypomethylation, localized hypermethylation across several genomic regions (predominantly at CpG sites), and direct mutations in methylated cytosines (48-50), which positions cfDNA as a potential biomarker for early cancer detection. Concurrently, research has demonstrated that the genetic data encapsulated within cfDNA can accurately identify tumor mutations and gene expression patterns (51). Moreover, the short half-life of cfDNA (ranging from 16 min to 2 h) enables dynamic monitoring of cancer progression (52,53). The methylation pattern of cfDNA is also reflective of the tissue or cell of origin, indicating that detecting aberrant DNA methylation patterns specific to tumors in the plasma of a patient could serve as a method for early cancer diagnosis and prognosis (54). Azad *et al* (55) reported an association between cfDNA levels in the blood of patients undergoing chemoradiotherapy for EC and tumor progression, metastasis and disease-specific survival rates. Similarly, Egyud *et al* (56) highlighted the ability of cfDNA to track treatment response and detect relapses in patients with esophageal adenocarcinoma (EAC). These findings align with the conclusions of the present review and provide the foundation for the present research.

Subgroup analyses based on sample size revealed that groups with larger sample sizes demonstrated improved diagnostic performance compared with those with smaller sample sizes. This could be attributed to the fact that elevated cfDNA levels are not exclusive to cancer but can also occur in conditions such as injury, heart attacks, strokes and chronic diseases such as diabetes. Several factors can influence cfDNA levels, including tumor size, location, vascularization and treatments such as surgery, chemotherapy, radiotherapy, and liver and kidney clearance mechanisms (57). Detection sensitivity is also affected by cfDNA molecule quantity, sequencing efficiency and the number of methylation markers. As sample size increases, the influence of these factors is diminished, enhancing diagnostic accuracy. In analyses of DNA methylation assay methods (using techniques such as enzymatic methyl-sequencing), diagnostic accuracy and sensitivity (other, 0.86; 95% CI, 0.82-0.89) and specificity (other, 0.96; 95% CI, 0.94-0.97) were markedly higher than those achieved with MSP methylation assay subgroups. It is recognized that cfDNA methylation identification methods generally fall into two categories: Bisulfite-based and bisulfite-free techniques. Bisulfite treatment involves converting unmethylated cytosines to uracil whilst leaving methylated cytosines unaffected, and the methylation status is determined using PCR amplification and sequencing (58). Bisulfite-free techniques refer to methodologies for detecting DNA methylation that do not rely on bisulfite conversion. Instead, they utilize alternative biochemical strategies to directly distinguish methylated cytosines (5mC/5hmC) from unmodified cytosines. Whole Genome Bisulfite Sequencing (WGBS) is considered the most comprehensive method for assessing DNA methylation, which constructs libraries and performs sequencing following the bisulfite conversion, yielding methylation data for every cytosine, encompassing areas with sparse CpG concentrations and non-CpG locations (CpA, CpT and CpC). Compared with WGBS, although WSP has a lower cost, there are few sites for study, and the design of primers or probes is complex, which may contribute to its lower sensitivity and specificity in certain subgroups (59).

Subgroup analysis by age revealed higher diagnostic accuracy in individuals aged >60 years compared with those aged <60 years. Despite a trend towards cancer incidence in younger individuals, EC remains more prevalent in middle-aged and older populations. The highest incidence peak occurs between 80-84 years of age (60). As age increases, the risk of precancerous lesions, multiple cancers and tumor heterogeneity rises, which may affect cfDNA concentration and methylation levels, influencing diagnostic accuracy.

In addition, EAC and ESCC are two distinct types of EC, with different origins and genetic characteristics (61). Although the present study was unable to perform subgroup analyses on using this variable due to insufficient data, preliminary judgments based on existing literature suggest that there are differences between EAC and ESCC. EAC demonstrates higher sensitivity (85-90%) using markers such as SRY-box transcription factor 17 and hyperplastic polyposis protein 1, whilst ESCC shows moderate sensitivity (75-82%) with markers such as cysteine dioxygenase type 1 or retinoic acid receptor β2 (62). These differences in sensitivity and the specific methylation markers highlight the distinct molecular pathways involved in the development of these two subtypes. However, future studies need to further explore these differences in larger sample sizes to provide more clinically meaningful guidance for subtype-specific applications.

Previous studies have reported that cfDNA methylation has strong diagnostic performance, with an early screening model achieving 74.7% sensitivity and 95.9% specificity (12,30). This outperforms traditional cytology and compares favorably with microRNA (miRNA)-based methods such as the combination of RUNX family transcription factor 3 methylation and carbohydrate antigen-19-9, which offer higher sensitivity in pancreatic cancer but lower specificity compared with cfDNA methylation (63). The superior stability of methylated DNA, compared with the susceptibility of miRNAs to degradation, may explain this advantage. Additionally, emerging technologies, such as machine learning-enhanced cfDNA methylation sequencing, further improve early detection accuracy (AUC, 0.91) and could markedly reduce cancer mortality (64). However, large-scale prospective trials are essential to validate these models across diverse populations and optimize detection thresholds.

Furthermore, despite its high diagnostic accuracy, the cost of cfDNA methylation testing remains a critical barrier to large-scale implementation. Technological advancements and economies of scale may help alleviate this challenge in the future. Furthermore, pre-analytical factors, such as blood collection methods, processing time and storage conditions, can affect test reproducibility. Standardized protocols are, therefore, crucial for clinical adoption. Whilst cfDNA methylation shows strong specificity, integrating it with other biomarkers, such as miRNAs or proteins, may offer a balanced approach for clinical implementation, particularly in resource-limited settings where cost-effectiveness is critical.

Moreover, there are certain limitations of the present study: i) EAC and ESCC were not classified in the study; ii) the impact of different stages of EC on diagnostic results was not taken into

account; iii) due to the novelty of several detection techniques, there was no uniform cut-off value, which affected the accuracy of results; vi) the inclusion of English-only literature may have introduced selection bias; v) the findings of the present study were primarily derived from retrospective studies, which may have introduced selection bias and limits generalizability. Only two included studies employed prospective designs, underscoring the need for validation in large, multicenter prospective cohorts; and vi) most of the included studies involved patients from China, USA and UK, which may limit the generalizability of the findings to other populations.

In conclusion, the present study highlights the advancements in utilizing cfDNA for early EC diagnosis, substantiating the feasibility and efficacy of cfDNA methylation sequencing as a diagnostic modality. To the best of our knowledge, the present study is the first meta-analysis evaluating cfDNA methylation for EC diagnosis, demonstrating that the cfDNA methylation detection method has high sensitivity, specificity and AUC values. Moreover, analyzing serum cfDNA is a minimally invasive, rapid and efficient detection method and has potential diagnostic value for EC. However, diagnostic accuracy varies depending on several factors, such as the methylation sequencing methodology, cfDNA yield and cancer stage at detection. Therefore, additional prospective studies are required to validate the precision of cfDNA methylation detection across diverse parameters, facilitating optimization for clinical implementation. Future clinical trials should validate the clinical utility of cfDNA methylation for early EC detection, with potential integration into preventive healthcare paradigms. Such integration may notably improve early detection rates whilst reducing costs and enhancing patient safety.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

CM contributed to the conception and design of the study, data collection, data analysis, figure creation and drafting of the manuscript. YW and ZD confirm the authenticity of all raw data, contributed to the study design and participated in figure creation and critical revision of the manuscript. XingL, MY, XuanL, QL, HL and NQ contributed to the conception and design of the study and reviewed the manuscript. XinL contributed to the conception, design, review and revision of the manuscript. All authors read and approved the final manuscript.

**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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