Systematic Review and Meta-Analysis

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Diagnostic value of cell-free DNA in thyroid cancer

A systematic review and meta-analysis

Fei Hou, MD[a], Xiao-Dan Sun, MD[b], Zhi-Yong Deng, MD[a,*]

Abstract

Objective: An increasing number of studies have shown the potential diagnostic value of cell-free DNA (cfDNA) as a new biomarker in the management of thyroid cancer (TC); however, the accuracy of research results is inconsistent. This meta-analysis is the first to synthesize published results and evaluate the application value of circulating cfDNA in the diagnosis of TC.

Methods: A search strategy was developed according to PICO (P: Patient; I: Intervention; C: Comparison; O: Outcome) principles. We searched 5 databases until October 2022. Original studies that examined cfDNA for the diagnosis of TC and used pathology as the gold standard were included in this meta-analysis. A random-effects model was used to pool the data extracted from individual studies, including the number of patients and the numbers of true positives, false positives, true negatives, and false negatives.

Results: A total of 622 patients with TC, 547 patients with benign thyroid nodules, and 98 healthy individuals were included in 20 studies reported in 14 articles. The types of cfDNA included in the research include specific mutations of cfDNA, methylation of cfDNA, the content of cfDNA, and cfDNA index. After rigorous statistical analysis, the pooled sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, diagnostic odds ratio, and area under the summary receiver operating characteristic curve were 0.76 (95% confidence interval [CI] 0.62–0.85), 0.87 (95% CI 0.78–0.93), 5.08 (95% CI 3.3–10.3), 0.28 (95% CI 0.17–0.46), 21 (95% CI 9–49), and 0.89 (95% CI 0.86–0.91), respectively. The meta-regression results showed that the number of cfDNAs, cfDNA methylation status, and sample size were the sources of heterogeneity in the specificity of the study. A subgroup analysis showed that the quantitative analysis group (cfDNA level) had a higher diagnostic accuracy than that of the qualitative analysis group (cfDNA methylation, mutation, or integrity index), with a sensitivity of 0.84, specificity of 0.89, and area under the curve of 0.91.

Conclusions: The results of this meta-analysis suggest that cfDNA has value as an adjunct for the diagnosis of TC. Quantitative detection of cfDNA can achieve relatively high diagnostic accuracy. However, due to heterogeneity, the test results based on cfDNA for TC should be interpreted with caution.

Abbreviations: AUC = area under the curve, BTN = benign thyroid nodule, cfDNA = cell-free DNA, CI = confidence interval, CNKI = China National Knowledge Infrastructure, ctDNA = circulating tumor DNA, DOR = diagnostic odds ratio, HCC = hepatocellular carcinoma, NLR = negative likelihood ratio, PLR = positive likelihood ratio, qPCR = quantitative real-time polymerase chain reaction, SROC = summary receiver operating characteristic, TC = thyroid cancer, Tg = thyroglobulin.

Keywords: cell-free DNA, diagnosis, liquid biopsy, meta-analysis, thyroid cancer

1. Introduction

Thyroid cancer (TC) is the most common head and neck tumor and mainly includes papillary and follicular carcinomas. The global incidence of TC has increased yearly over the past 20 years. In 2020, the number of new TC cases was approximately 586,202 worldwide, accounting for 3.0% of all new cancer cases, and the incidence of TC ranked 9th among all cancers.[1] The clinical importance of thyroid nodule diagnosis rests with the need to exclude TC, which occurs in 7 to 15% of the population, depending on age, sex, radiation exposure history, family history, and other factors.[2] Thus, it

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

Ethical approval was not needed because this is a meta-analysis.

Trial registration: INPLASY (202170002).

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is very important to correctly diagnose TC and prevent unnecessary surgery.

The current assessment methods of thyroid nodules mainly include a thyroid function test, radionuclide scan of the thyroid, computed tomography, thyroid ultrasonography, and ultrasound-guided fine-needle aspiration cytology.[3] Ultrasonography and fine-needle aspiration biopsy are accurate and cost-effective methods to assess the nature of thyroid nodules.[4,5] In terms of serological examination, with the development of biotechnology, many tumor markers related to the occurrence, development, and outcome of TC have been found, such as thyroglobulin (Tg), TgAb, calcitonin (Calcitonin), and galactose Lectin-3, cytokeratin-19, vascular endothelial growth factor, and human bone marrow endothelial cell markers, etc. However, the main tumor markers used clinically for TC are Tg, TgAb, and calcitonin. Serum Tg is elevated in most thyroid diseases. It is an insensitive and nonspecific indicator of TC.[6–8] It is mainly used for the diagnosis of disease recurrence and metastasis after surgery in differentiated TC patients. However, serum Tg cannot be used to identify TC. Moreover, calcitonin can only be used to diagnose medullary TC.[9] Currently, there is no effective and comprehensive tumor marker for the diagnosis of TC, and it is thus of great significance to find sensitive and objective biomarkers for the diagnosis of TC.

Degraded DNA fragments in the plasma are termed free DNA, circulating free DNA, or cell-free DNA (cfDNA). cfDNA was first discovered by Mandel and Metais in 1947.[10] cfDNA exists in various human body fluids, and its concentration changes with tissue damage, cancer, and inflammation. Both mothers and fetal-placental units produce cfDNA. cfDNA in the normal human body is mainly represented by small and uniform, 185 to 200 bp fragments produced during apoptosis.[11] Tumor tissue necrotic cells are due to abnormal apoptosis processes, resulting in DNA fragments of different sizes and >200 bp. In the circulation, cfDNA molecules are quickly eliminated, with a half-life of 1 hour or less. cfDNA derived from tumors is termed circulating tumor DNA (ctDNA), and it has cancer-specific genetic and epigenetic traits.[12] Thus, peripheral blood allows easy and noninvasive analysis of the complex and dynamic molecular characteristics of cancer. cfDNA can be used for tumor diagnosis, prognosis, and even monitoring of the tumor treatment response and can be applied in the clinic as a novel tumor marker. cfDNA has been evaluated as a biomarker for cancers such as pancreatic, colon, and breast cancer.[13] The analysis includes the detection of specific gene mutations in cfDNA, integrity assessment, DNA methylation detection, or the estimation of total cfDNA.[14] Many studies have reported that cfDNA can be used as a molecular marker to diagnose TC, and the level of cfDNA is closely related to the occurrence and progression of TC.[15]

However, the research results on the diagnostic performance of cfDNA for TC are controversial, and there are no relevant meta-analysis reports. Therefore, to clarify the diagnostic performance of cfDNA for TC, this meta-analysis was conducted to comprehensively assess the available data, and its results may provide a reference for further cfDNA research and clinical applications.

2. Methods

2.1. Search strategy and inclusion criteria

The protocol for this systematic review was registered on INPLASY (202170002) and is available in full at https://inplasy.com/inplasy-2021-7-0002/. This study was conducted in strict accordance with the criteria stated in PRISMA. The search strategy was based on the principle of PICO (P: Patient; I: Intervention; C: Comparison; O: Outcome): (a) P is a patient suspected of having TC or thyroid nodules (search terms: "Thyroid Neoplasms" OR " Thyroid Carcinoma" OR "Thyroid Cancers" OR "Thyroid Nodule"); (b) I is cfDNA (search terms: "Cell-Free Nucleic Acids" OR "Circulating Cell-Free Nucleic Acid" OR "Circulating Tumor DNA"); (c) C is the pathological diagnosis method for TC (the search term was not used, as it greatly limits the number of articles obtained, and colleagues used this as the selection criterion when screening the literature); (d) O is for diagnostic accuracy evaluation, such as sensitivity, specificity, etc. (the search term was not used because it greatly limits the number of articles obtained, and our colleagues used this as the selection criterion when screening the literature). We searched 8 databases from their establishment to October 29, 2022, including PubMed, EMBASE, Web of Science, Cochrane Library, China National Knowledge Infrastructure (CNKI), SinoMed (Chinese biomedical literature service system), Chinese VIP, and Wan Fang databases. The search did not limit the year of publication or language. We used the following keywords in the search: "Cell-Free Nucleic Acids" OR "Circulating Cell-Free Nucleic Acid" OR "Circulating Tumor DNA" and "Thyroid Neoplasms" OR "Thyroid Carcinoma" OR "Thyroid Cancers" OR "Thyroid Nodule." Articles that met all of the following inclusion criteria were included the following: cfDNA/ctDNA was used to diagnose TC; pathological examination was the reference standard for diagnosing TC; and true-positive, false-positive, false-negative, and true-negative data were available for a 2×2 table. Articles that met any of the following criteria were excluded: case reports, reviews, meeting abstracts, and letters; research using cells or animal models; documents from which 2×2 table data could not be directly or indirectly extracted; and repeated publications.

2.2. Data extraction and quality assessment

Three researchers independently and carefully performed literature screening and data extraction. When there were any inconsistencies, a fourth researcher was available to help. The content of the extracted data included the first author's name, year of publication, country, sample size, age and sex of subjects, research type, specimen type, cfDNA detection method, cfDNA type, sensitivity, specificity, cutoff value, and required information for quality assessment. If an article reported >1 type of cfDNA studied, we considered each cfDNA test as an independent study. The research results for multiple cfDNA combinations were regarded as independent research results, similar to the research results for a single cfDNA. The Quality Assessment of Diagnostic Accuracy Studies 2 evaluation standard was used to assess the quality of the literature. We used the RevMan 5.3 software to assess the quality of the literature.

2.3. Data analysis

First, we used RevMan 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014), Meta-Disc (developed by the Unit of Clinical Biostatistics team of the Ramón y Cajal Hospital in Madrid, Spain), and STATA 15 software (Copyright 1985–2017 StataCorp LLC) to perform the analysis. Spearman rank correlation analysis or summary receiver operating characteristic (SROC) curve analysis was used to determine whether the SROC graph was shoulder arm-like and to explore whether there was a threshold effect. When there was no threshold effect, we calculated the pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), and diagnostic odds ratio (DOR). Heterogeneity tests among the included studies were analyzed using the Cochran Q test and I^2 test, and the appropriate model was selected according to the degree of heterogeneity as follows: if P was >0.100 and I^2 was <50%, the fixed-effects model was selected; otherwise, the random-effects model was selected to conduct the meta-analysis. To identify the source of heterogeneity, the type of research (prospective or retrospective), cfDNA analysis (single or multiple cfDNAs),

quantitative analysis (cfDNA level) or qualitative analysis (cfDNA methylation, mutation or integrity index), cfDNA methylation or nonmethylation, sample size (large [≥100] or small [<100]) and type of the control group (benign thyroid nodule [BTN] group or healthy control group) were analyzed by meta-regression. Potential sources of heterogeneity for this study were identified by sensitivity analysis. In addition, the included studies were examined for publication bias by Deeks funnel plot asymmetry test, and significant publication bias was considered when the *P* value was <0.1.

3. Results

3.1. Basic characteristics of the included literature

We retrieved 901 articles (64 from PubMed, 254 from EMBASE, 25 from Cochrane Library, 468 from Web of Science, 30 from CNKI, 9 from SinoMed, 6 from the VIP database, and 45 from the Wan Fang database). First, 282 duplicate articles were excluded, and then, we chose to exclude the latter 605 articles for the following reasons: full texts could not be downloaded, and only abstracts were available for 6 articles; 118 articles were in the form of a meta-analysis, systematic review, review, conference report, or case report; 403 articles had irrelevant research topics, 18 articles did not have relevant results; 33 articles were cell- or animal-based studies; 19 articles were on RNA research; and 8 articles were on studies that were not rigorously designed. Finally, 14 papers were included (3 in Chinese and 11 in English), and Figure 1 illustrates the selection flowchart.

3.2. Characteristics and quality analysis of the included studies

The 14 articles were published between 2013 and 2021 (20 studies in total) and included 622 patients with TC, 547 patients with BTNs, and 98 healthy controls. The diagnosis of TC in all studies was accomplished by pathological examination. Of the 14 included articles, 4 articles were from China; 2 articles were from Iran; 2 articles were from Italy; 1 article was from India; 1 article was from South Korea; 1 article was from Poland; 1 article was from the US; 1 article was from the UK; and 1 article was from Egypt. Eleven articles used patients with BTNs as controls, and 3 articles used healthy people as controls. Eleven articles used a prospective approach, and 3 articles used a retrospective approach. Thirteen articles focused on plasma samples, but this information was unknown for 1 article. Seventeen of the 20 studies focused on a single cfDNA, and the other 3 studies focused on multiple cfDNAs. In addition, all patients in the 20 studies had their cfDNA samples collected prior to surgery. The characteristics of the eligible

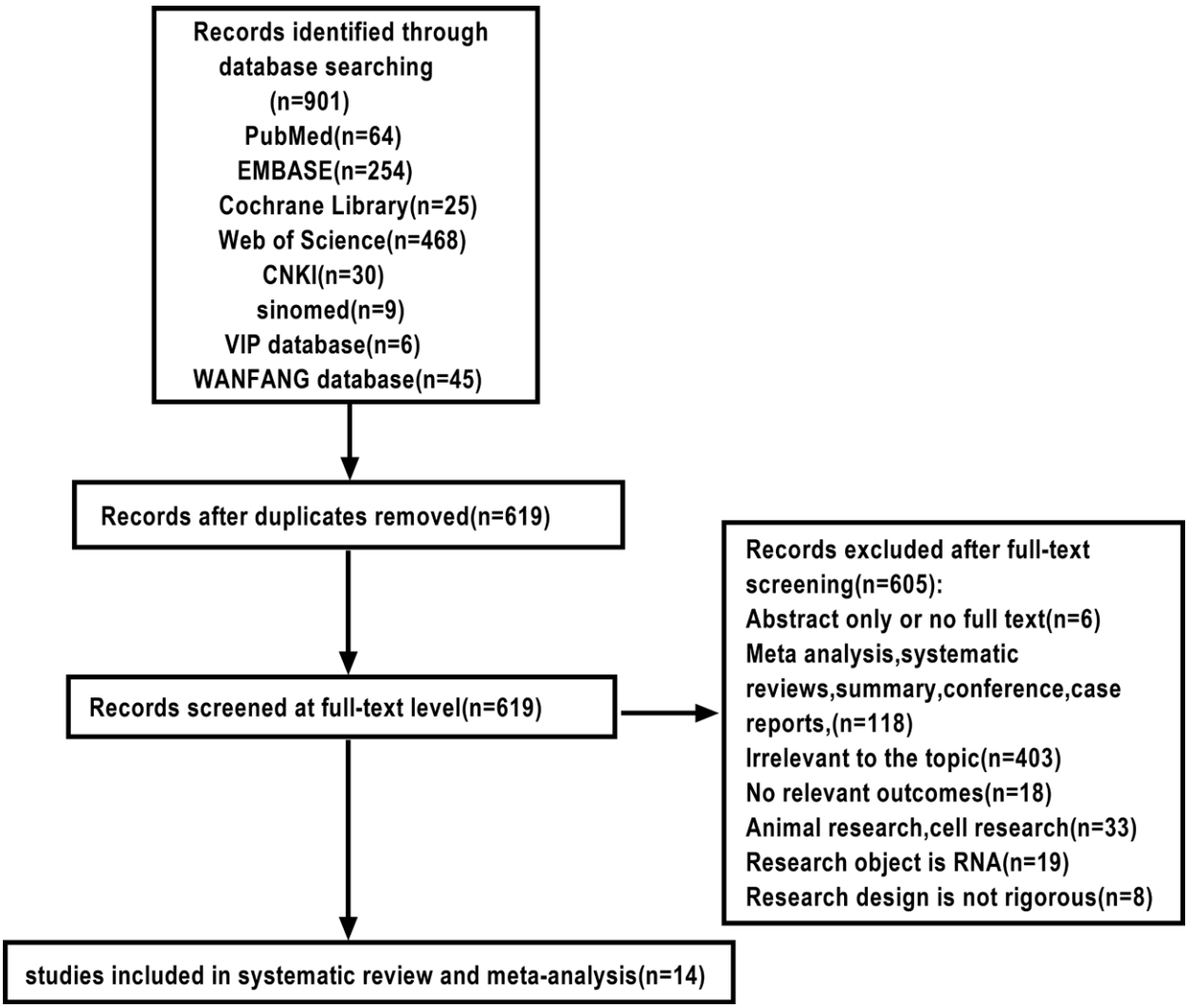

Figure 1. A flowchart of the article selection process.

Table 1

Basic characteristics of the research in this meta-analysis.

ID	Author	Year	Country	Case/control group	Size	Pathological type				Tumor stage				Mean age	Gender (male:female)	Type	Specimen	Method
						PTC	FTC	MTC	ATC	I	II	III	IV					
1	Ewelina Perdas[16]	2019	Poland	Case HS	32 30	32	0	0	0	26	5	1	0	48.57 ± 15.84 47.00 (40.75–55.25)	5:27 11:19	Retro	Plasma	qPCR
2	C Pupilli[17]	2013	Italy	Case HS	29 49	29	0	0	0	NA	NA	NA	NA	NA 53 (28–89)	NA 23:26	Pro	Plasma	qPCR
3	Mariangela Zane[18]	2013	Italy	Case HS	158 19	86	5	58	9	67	2	45	28	56 (5–92) NA	61:97 NA	Retro	Plasma	qPCR
4	Fatemeh Khatami[19]	2019	Iran	Case BTN	57 45	57	0	0	0	11	27	15	1	42.28 ± 16.36 43.16 ± 11.29	18:39 5:40	Pro	Plasma	MS-HRM
5	M, H. A.[37]	2021	Egypt	Case BTN	60 75	18	21	21	0	34		26		45 ± 18.5 43.6 ± 10.9	13:47 18:57	Pro	NA	qPCR
6	Fatemeh Khatami[21]	2020	Iran	Case BTN	57 45	57	0	0	0	11	27	15	1	(18–86)	NA NA	Pro	Plasma	MS-HRM
7	Mark Lupo[22]	2018	USA	Case BTN	13 43	9	1	0	0	7	NA	2	1	57 (26–83)	12:44	Pro	Plasma	NA
8	Susmita Dutta[14]	2021	India	Case (DTC) BTN	37 82	NA	NA	NA	NA	5	20	9	NA	34.32 ± 11.25	26:93	Pro	Plasma	NA
9	Jia Meng[23]	2020	China	Case BTN	44 31	NA	NA	NA	NA	NA	NA	NA	NA	51 50.5	10:34 2:29	Pro	Plasma	qPCR
10	SiShuang huang[24]	2021	China	Case BTN	22 60	NA	NA	NA	NA	NA	NA	NA	NA	42.00 ± 8.95 41.75 ± 7.85	10:12 24:36	Pro	Plasma	qf-PCR
11	Huang Youxin*.[25]	2017	China	Case BTN	16 59	16	0	0	0	NA	NA	NA	NA	46 (26–65)	7:68	Pro	Plasma	MS-PCR
12	Hyeon-Gun Jee[26]	2019	Korea	Case BTN	10 10	7	3	0	0	NA	NA	NA	NA	46.5 (28–63) 48.4 (18–70)	2:8 2:8	Retro	Plasma	RT-PCR
13	Huang Youxin†.[27]	2017	China	Case BTN	16 59	16	0	0	0	NA	NA	NA	NA	46 (26–65)	7:68	Pro	Plasma	RT-PCR
14	Krupal B. Patel[39]	2021	U.K.	Case BTN	71 38	68	3	0	0	NA	NA	NA	NA	NA	33:67	Pro	Plasma	qPCR

ATC = anaplastic thyroid carcinoma, BTN = benign thyroid nodules, DTC = differentiated thyroid cancer, FTC = follicular thyroid carcinoma, HS = healthy subjects, MTC = medullary thyroid carcinoma, PTC = papillary thyroid carcinoma, qf-PCR = quantitative real-time fluorescence polymerase chain reaction, qRT-PCR = quantitative real-time polymerase chain reaction.

*Research object: Combined detection method of ctDNA (TSHR + RARβ2 + RASSF1A).

† Research object: ctDNA.

studies and the extracted data are shown in Tables 1 and 2. Based on the Quality Assessment of Diagnostic Accuracy Studies 2 evaluation tool, the RevMan 5.3 software was used to evaluate the quality of the included studies. The results are shown in Figure 2.

3.3. Accuracy of cfDNA in diagnosing TC patients

Spearman rank correlation analysis with Meta-Disc showed that the sensitivity and 1 – specificity were negatively correlated ($rs = 0.105$, $P = .659 > 0.05$), and there was no threshold effect. Furthermore, the I^2 values of the sensitivity, specificity, PLR, NLR, and DOR were all >50% ($P < .01$, $I^2 = 90.4\%$; $P < .01$, $I^2 = 90.6\%$; $P < .001$, $I^2 = 91.6\%$; $P < .001$, $I^2 = 94.4\%$; and $P < .001$, $I^2 = 82.0\%$, respectively) in this study. Thus, the random-effects model was used for meta-analysis. The results showed that the pooled sensitivity of cfDNA in diagnosing TC was 0.76 (95% confidence interval [CI] 0.62–0.85); the pooled specificity was 0.87 (95% CI 0.78–0.93); the pooled PLR was 5.80 (95% CI 3.3–10.3); the pooled NLR was 0.28 (95% CI 0.17–0.46); and the pooled DOR was 21 (95% CI 9–49). The SROC curve showed that the corresponding area under the curve (AUC) was 0.89 (95% CI 0.86–0.91) (Figs. 3 and 4A).

3.4. Meta-regression, subgroup, and sensitivity analyses

The search for the source of heterogeneity of the research data was performed using meta-regression analysis. The meta-regression analysis results showed that the number of cfDNA studies, the methylation status of cfDNA, and the sample size may have been the sources of heterogeneity in the meta-analysis specificity (Fig. 5A). We performed a subgroup analysis based on the amount of cfDNA, quantitative/qualitative analysis, methylation status of cfDNA, and sample size. The subgroup analysis showed that the sensitivity, specificity, PLR, DOR, and AUC in the quantitative analysis group (cfDNA level) were higher than those in the qualitative analysis group (cfDNA methylation, mutation, or integrity index); the specificity, PLR, and DOR of multiple cfDNAs that were combined to diagnose TC were higher than those of individual cfDNAs; the specificity, PLR, DOR, and AUC of the studies on cfDNA nonmethylation were higher than those of the studies on cfDNA methylation; and the sensitivity of

Table 2

Data extracted from the article.

ID	Author	Year	Research object	TP	FP	FN	TN
1	Ewelina Perdas[16]	2019	Nuclear cf-DNA (cf-nDNA)	16	1	16	29
			Mitochondrial cf-DNA (cf-mtDNA)	20	6	12	24
2	C Pupilli[17]	2013	BRAFV600E mutation	19	10	10	39
3	Mariangela Zane[18]	2013	cf-DNAALU83	116	1	42	18
			cf-DNAALU244	106	0	52	19
4	Fatemeh Khatami[19]	2019	cf-DNA methylation of MGMT (C)	40	24	17	21
			cf-DNA methylation of MGMT (D)	44	13	13	32
5	M, H. A.[37]	2021	cf-DNAALU83	43	7	17	68
			cf-DNAALU244	60	7	0	68
6	Fatemeh Khatami[21]	2020	cf-DNA methylation of SLC5A8 (c)	38	33	19	12
			cf-DNA methylation of RASSF1 (b)	48	26	9	19
7	Mark Lupo[22]	2018	cf-DNA (BRAF, CTNNB1, EGFR, FOXL2, GNAS, KRAS, NRAS, PIK3CA and TP53) More than 5 mutations are considered positive	1	2	12	41
8	Susmita Dutta[14]	2021	Mutation from cf-DNA (BRAF, NRAS, KRAS, HRAS, RET-PTC3, TERT, RET-PTC1, PAX8-PPARγ)	37	6	0	76
9	Jia Meng[23]	2020	cf-DNAALU115	25	7	19	24
			cf-DNA integrity index = (qPCR-Alu247 value/qPCR-Alu115 value)	33	5	11	26
10	SiShuang huang[24]	2021	ctDNA	19	11	3	49
11	Huang Youxin[a][25]	2017	Combined detection methylation of ctDNA (TSHR + RARβ2 + RASSF1A)	14	2	2	57
12	Hyeon-Gun Jee[26]	2019	ctDNA SLC5A5	9	1	1	9
13	Huang Youxin[b][27]	2017	ctDNA	15	7	1	52
14	Krupal B. Patel[39]	2021	BRAFV600E mutation	15	0	56	38

ctDNA = circulating tumor DNA, FN = false negative, FP = false postive, qPCR = quantitative real-time polymerase chain reaction, TN = ture negative, TP = ture positive.

large-sample research was higher than that of small-sample research. Table 3 shows the detailed subgroup analysis results. The results of the sensitivity analysis showed that there were 5 original studies with strong sensitivity, while the other original studies did not affect the sensitivity of the calculated results (Fig. 4B). Overall, the results of this study were relatively stable.

3.5. Publication bias

Publication bias was tested by linear regression, and Deeks funnel plot was drawn. The results showed no obvious publication bias (P = .73) (Fig. 4C).

3.6. Clinical application value

The probability of identifying or excluding patients with TC by cfDNA testing is presented in the Fagan nomogram. For anyone who had a 20% probability of having TC before the test, if cfDNA was positive in the tumor test, the probability of having TC after the test would reach 59%; however, a negative cfDNA test result meant that the probability would drop to 7% after the test. Therefore, cfDNA testing plays a significant role in the initial screening of patients with TC (Fig. 5B).

4. Discussion

Liquid biopsy techniques mainly include free circulating tumor cell detection, cfDNA detection, exosome detection, and circulating RNA detection.[28] Undoubtedly, tissue biopsy has some weaknesses. For example, it is an invasive test and has a limited role in understanding the risk of cancer metastasis, disease progression, and the treatment effect. Compared with traditional tumor detection methods (such as imaging examinations and tissue examinations), liquid biopsy has developed more rapidly owing to its flexible and safe sampling methods, noninvasiveness and convenience, regular and repeated sampling, and high compliance. Liquid biopsy is expected to be used in the early stage of tumors. Early warning and auxiliary diagnosis, real-time monitoring of curative effects, drug guidance and exploration of the drug resistance mechanism, prognosis judgment and risk classification, and targeted drug companion diagnosis play important roles.[29,30] Among all liquid biopsy analyses, circulating cfDNA may be the most promising tool for identifying small tumor residues, evaluating treatment responses and prognosis, and identifying disease resistance mechanisms[31]; thus, cfDNA has become a focus of current research.

Liquid biopsy technology is highly sensitive, and its corresponding markers can often be used for early tumor screening before clinical manifestations occur and imaging shows lesions.[28] For example, Lam WKJ screened 20,174 middle-aged men in Southeast Asia for nasopharyngeal cancer by testing Epstein–Barr virus plasma cfDNA. The sensitivity for the diagnosis of nasopharyngeal cancer was 97.1%, and the specificity was 98.6%.[32] In addition, when differences in the Epstein–Barr virus cfDNA methylation were included in the test, the positive predictive value of cfDNA could be significantly improved.[33] The detection of epidermal growth factor receptor mutations in plasma ctDNA can be used as a reference standard for choosing gefitinib as a first-line treatment option for patients with lung adenocarcinoma (BENEFIT study).[34] cfDNA is considered to be a prognostic biomarker for advanced hepatocellular carcinoma (HCC). The study of Park et al found that after radiotherapy, the serum cfDNA levels significantly decreased in patients with HCC, indicating that the cfDNA level after HCC radiotherapy is a good predictor of the treatment response and local control.[35]

For TC, the study of Salvianti et al showed that the cfDNA integrity index 180/67 could be used as a biomarker for the diagnosis of thyroid nodules. They adopted a quantitative real-time polymerase chain reaction (qPCR) method to evaluate the integrity index 180/67, which is based on quantifying 2 amplicons of different lengths (180 and 67 bp, respectively). The serum cfDNA content in patients with thyroid nodules was higher than that in healthy controls. It is important that the cfDNA integrity index of patients with a cytological diagnosis of TC (Thy4/Thy5) is higher than that of patients with benign nodules (Thy2).[36,37] Although there have been studies on cfDNA in TC, there is currently no meta-analysis focusing on the diagnostic efficacy of cfDNA in TC. To address this problem,

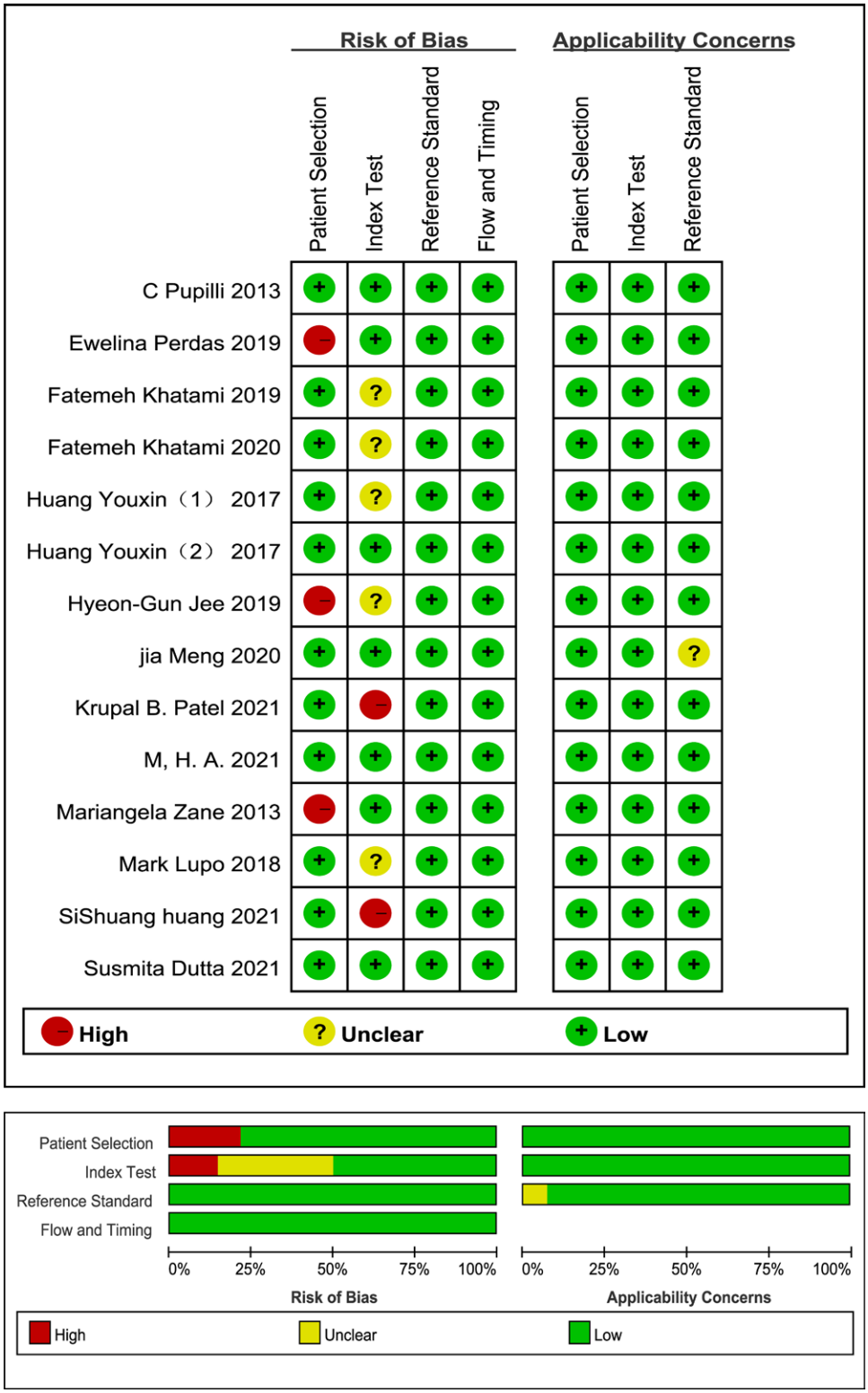

Figure 2. QUADAS-2 entries for evaluation of literature quality. QUADAS-2 = Quality Assessment of Diagnostic Accuracy Studies 2.

in this meta-analysis, strict inclusion and exclusion criteria were used to select published articles as comprehensively as possible, and meta-regression was performed to analyze the sources of heterogeneity. Importantly, this is the first meta-analysis to evaluate the diagnostic value of cfDNA in TC, showing that cfDNA is a novel circulating biomarker for the diagnosis of TC.

This meta-analysis included 20 studies from 14 articles, including 622 patients with TC, 547 patients with BTNs, and 98 healthy controls. The study results showed that the pooled sensitivity of cfDNA in the diagnosis of TC was 0.76 (95% CI 0.62–0.85) and the pooled specificity was 0.87 (95% CI 0.78–0.93). The SROC curve showed that the corresponding AUC was 0.89 (95% CI 0.86–0.91), which was much higher than the common diagnostic criteria (>0.8). The results showed that cfDNA was a novel potentially promising biomarker with high sensitivity and specificity for the diagnosis of TC. The PLR and NLR have also been

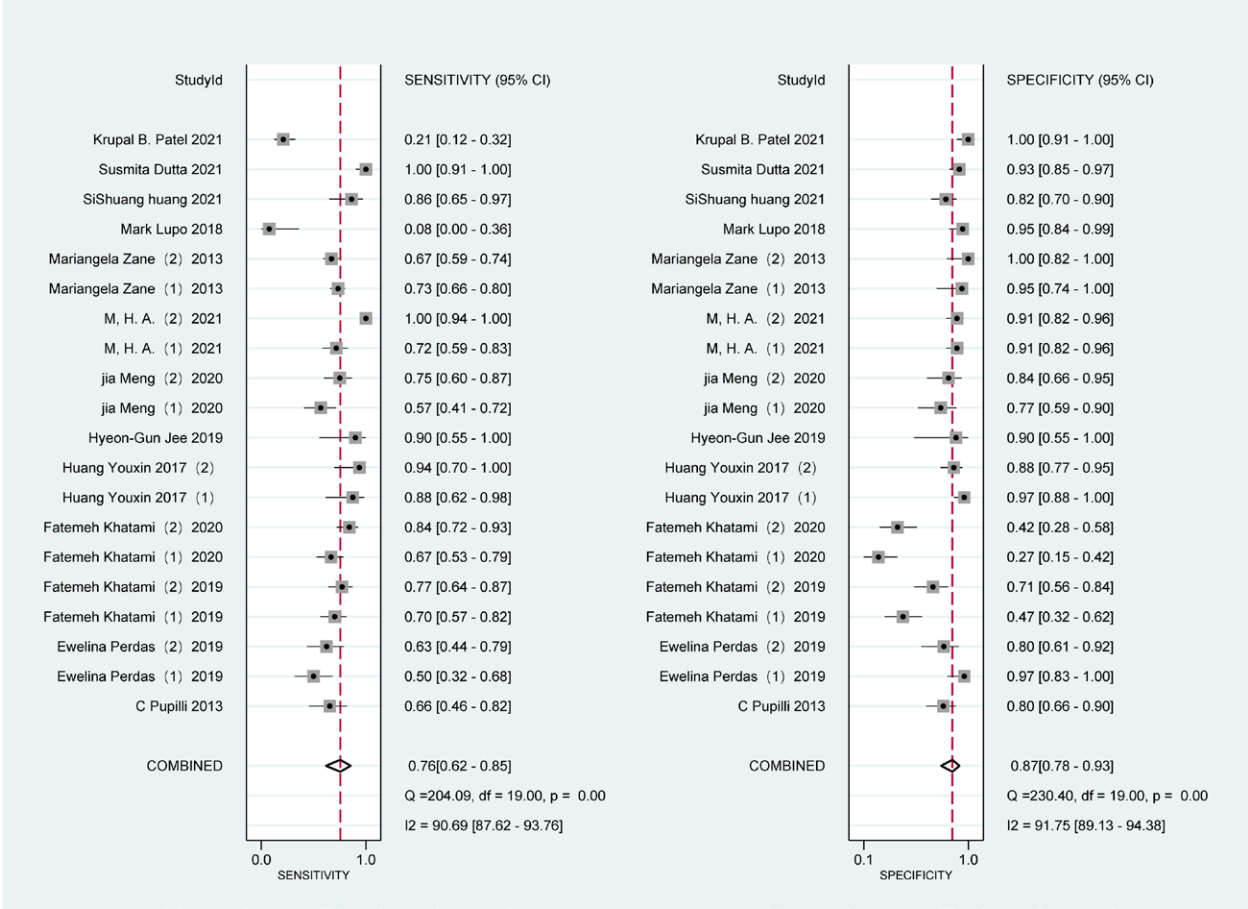

Figure 3. Forest plot of the sensitivity and specificity of cfDNA in the diagnosis of TC. cfDNA = cell-free DNA, TC = thyroid cancer.

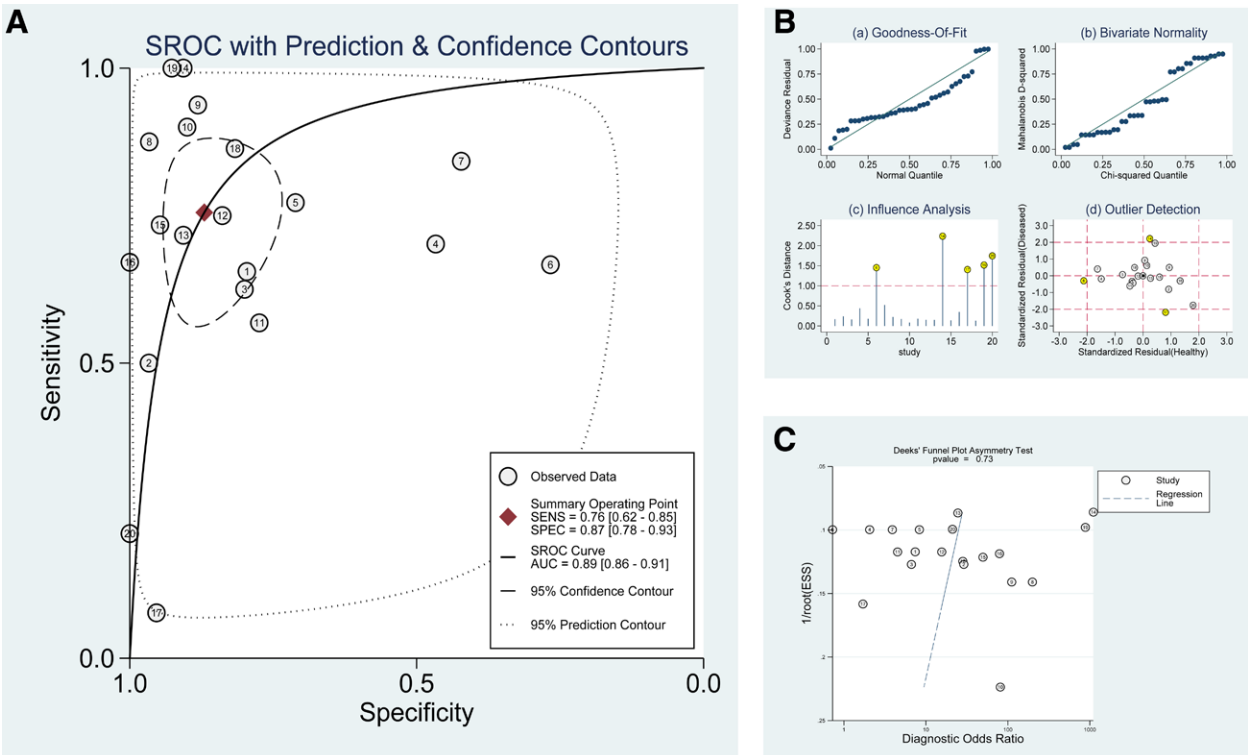

Figure 4. (A) Summary receiver operating curve of the diagnostic performance of cfDNA for TC in include studies. (B) Sensitivity analysis of cfDNA in the diagnosis of TC. (C) Deek funnel plot assessing the publication bias of included studies. cfDNA = cell-free DNA, TC = thyroid cancer.

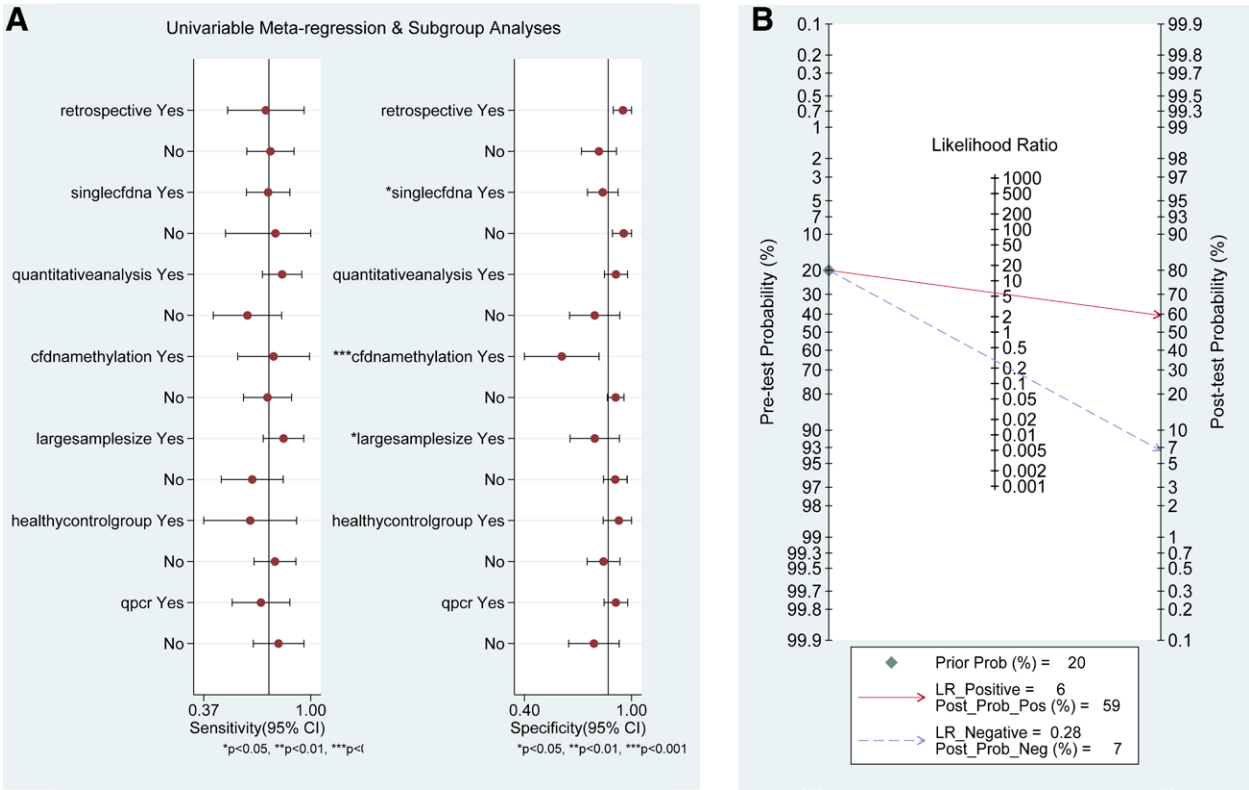

Figure 5. (A) Meta-regression analysis of the sensitivity and specificity of cfDNA in the identification of TC. (B) Fagan nomogram shows the ability of cfDNA testing to confirm or exclude TC. cfDNA = cell-free DNA, TC = thyroid cancer.

Table 3

Subgroup analysis of diagnostic value of cfDNA for thyroid cancer.

Subgroup	N	Sensitivity (95% CI)	Specificity (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	DOR (95% CI)	AUC (95% CI)
Total number of studies	20	0.76 (0.62–0.85)	0.87 (0.78–0.93)	5.8 (3.3–10.3)	0.28 (0.17–0.46)	21 (9–49)	0.89 (0.86–0.91)
Quantitative/qualitative							
Quantitative analysis	11	0.84 (0.67–0.94)	0.89 (0.85–0.92)	7.6 (5.2–11.0)	0.18 (0.08–0.41)	43 (14–129)	0.91 (0.88–0.93)
Qualitative analysis	9	0.63 (0.44–0.78)	0.82 (0.56–0.94)	3.5 (1.4–8.4)	0.45 (0.31–0.67)	8 (3–21)	0.76 (0.73–0.80)
CfDNA profiling							
Single cfDNA	17	0.69 (0.66–0.72)	0.77 (0.74–0.81)	4.02 (2.45–6.61)	0.38 (0.27–0.54)	12.88 (6.24–26.60)	0.84
Multiple cfDNAs	3	0.79 (0.67–0.88)	0.95 (0.90–0.97)	11.33 (3.80–33.83)	0.12 (0.00–305.53)	65.15 (1.87–2268.89)	0.98
Methylation							
CfDNA methylation	5	0.78 (0.68–0.85)	0.62 (0.31–0.86)	2.1 (0.9–5.0)	0.36 (0.17–0.78)	6 (1–30)	0.79 (0.75–0.82)
CfDNA non–methylation	15	0.76 (0.55–0.89)	0.90 (0.86–0.93)	7.7 (5.1–11.6)	0.27 (0.14–0.54)	29 (11–73)	0.92 (0.89–0.94)
Sample size							
Large (n ≥ 100)	9	0.84 (0.68–0.93)	0.80 (0.58–0.92)	4.2 (1.7–10.2)	0.20 (0.09–0.46)	21 (4–100)	0.89 (0.86–0.92)
Small (n < 100)	11	0.65 (0.45–0.80)	0.90 (0.83–0.94)	6.5 (4.0–10.4)	0.39 (0.24–0.64)	17 (8–36)	0.90 (0.87–0.92)

AUC = area under the curve, cfDNA = cell-free DNA, CI = confidence interval, DOR = diagnostic odds ratio, Negative LR = negative likelihood ratio, Positive LR = positive likelihood ratio.

shown to measure overall diagnostic accuracy.[20] Generally, PLR > 10 and NLR < 0.1 indicate a high accuracy. The PLR value in this meta-analysis was 5.8, which indicates that compared with the control group, the probability of positive cfDNA determination in patients with TC is approximately 5.8 times higher. The NLR was found to be 0.28, which means that cases with negative test results have about a one-third chance of developing TC. Unsatisfactory likelihood ratio results obtained in a meta-analysis may indicate its poor robustness and accuracy. To further evaluate the effectiveness of a diagnosis, we also analyzed the DOR, which is a single indicator of test accuracy. A DOR value >10 indicates a good discriminant test performance.[38] The results of our meta-analysis showed that the DOR of the cfDNA test used to distinguish patients with TC from the control group was 21, indicating good overall accuracy.

Of the 20 studies included in this meta-analysis, 4 studies focused on specific mutations in cfDNA, such as circulating BRAF V600E, CTNNB1, EGFR, FOXL2, GNAS, KRAS, NRAS, HRAS, RET-PTC3, TERT, RET-PTC1, PAX8-PPARγ, PIK3CA, and TP53 mutations[14,17,22,39]; 4 studies used methylation-sensitive high-resolution melting to identify the methylation status of the promoter regions of genes such as MGMT(C), MGMT(D), SLC5A8(c) and RASSF1(b)[19,21]; 1 study used methylation-specific PCR to identify the methylation of the TSHR, RARβ2, and RASSF1A genes[25]; 9 studies used real-time fluorescence qPCR to detect the content of cfDNA, such as the concentrations of cfDNA ALU83 and cfDNA ALU244, in patients with TC[16,18,24,26,27,37]; and 1 study used qPCR to calculate the numbers of longer and shorter gene fragments in the plasma and then calculate the ratio of the absolute concentrations of longer and

shorter fragments, such as qPCR-Alu247 value/qPCR-Alu115 value, to calculate the cfDNA index.[23] Finally, all patients in these 20 studies had cfDNA samples collected prior to surgery.

In our meta-analysis, cfDNA showed a high diagnostic performance; however, there was heterogeneity in this study. Meta-regression was used to explore the possible sources of heterogeneity. The regression results showed that the number of cfDNAs, cfDNA methylation status, and sample size were the sources of heterogeneity in the specificity of the study. In addition, using a subgroup analysis, we found that the quantitative analysis group (cfDNA level) had a higher diagnostic accuracy than that of the qualitative analysis group (cfDNA methylation, mutation, or integrity index), with a sensitivity of 0.84 (0.67–0.94), specificity of 0.89 (0.85–0.92), PLR of 7.6 (5.2–11.0), NLR of 0.18 (0.08–0.41), DOR of 43 (14–129), and AUC of 0.91 (0.88–0.93). This is consistent with the findings of Yin, who concluded that the diagnostic accuracy of quantitative cfDNA analysis was higher than that of qualitative analysis.[40] In addition, the specificity, PLR, DOR, and AUC of multiple cfDNAs in the diagnosis of TC were higher than those of a single cfDNA. These results indicated that the combination of multiple cfDNAs might be more accurate in diagnosing TC than a single cfDNA. Consistently, meta-analyses of miRNAs in TC led to the same conclusion, indicating that multiple miRNA detection provides better diagnostic performance than that of single miRNA detection,[41] although these meta-analyses studied miRNAs, not cfDNA. The reason for this may be that cancer occurs as a result of multiple genetic mutations and epigenetic abnormalities. The combined detection of multiple cfDNA sequences can easier diagnose TC. The subgroup analysis also showed that the pooled specificity, PLR, DOR, and AUC of studies on cfDNA methylation were lower than those of studies on cfDNA nonmethylation. We believe that the difference may be due to fewer studies included in the cfDNA methylation group. In general, the diagnostic accuracy of cfDNA is higher if the study involves a larger number of participants. Our subgroup analysis showed a higher sensitivity in large-sample studies (0.84) than in small-sample studies (0.65). Wenli Xie's meta-analysis led to similar conclusions, as their subgroup analysis demonstrated a higher concordance rate for studies with large samples (>100), which also had a higher SROC AUC (0.9757) than that of studies with small samples (0.9325).[42]

The results of the sensitivity analysis showed that there were 5 original studies with strong sensitivity. When these 5 studies were excluded, the effect value of the meta-analysis was affected, and the other original studies did not affect the sensitivity of the calculated results. Overall, the results of this meta-analysis are relatively stable. A Fagan nomogram was used to evaluate the clinical value of cfDNA diagnostic tests, and it showed the probability for patients with positive or negative results to have TC. Compared with the prior probability (20%), the positive posttest probability (59%) is much higher, and the negative posttest probability is quite low (7%), indicating that cfDNA has a certain diagnostic potential in distinguishing patients with TC from controls. Thus, it can be used as a suitable screening method for TC.

In a systematic review of 9 studies, J. M. Fussey concluded that cfDNA was related to more advanced TC.[43] For the evaluation of cfDNA as a diagnostic tool, J. M. Fussey conducted a qualitative analysis of only 3 original studies. However, compared with J. M. Fussey's systematic review, our research not only included a more comprehensive and timely selection of original studies, but we also conducted a meta-analysis and obtained accurate quantitative results. Therefore, as this was the first meta-analysis to evaluate the diagnostic value of cfDNA for TC, our findings are promising and may guide future research.

However, there are several limitations to this study. First, this meta-analysis involved a variety of cfDNA types, and different cfDNA types have various different detection methods and exhibit different characteristics that affect their sequencing depth not only in terms of their detection range, different resolution limits, and exposure to different types of genetic and epigenetic variants but also depending on the amount of sample source material.[44,45] Therefore, direct aggregation of results may lead to hidden biases, which can lead to non-negligible heterogeneity. Unfortunately, there are few studies on cfDNA for the diagnosis of TC, and we look forward to more high-quality prospective studies on cfDNA in the future when we can focus on a particular type of cfDNA study for the pooling of results. Second, the original studies did not subgroup pathological TC type, nor did our meta-analysis. Different subtypes have vastly different growth rates, metastatic potentials, and patterns, with differences in potential cfDNA release, which can lead to heterogeneity in research. Third, in the subgroup analyses, the numbers of studies included in the multiple cfDNA groups were relatively small, which could easily lead to deviations in the results. Therefore, caution should be taken when interpreting the results of the subgroup analyses. Fourth, we observed significant heterogeneity. The meta-regression results indicated that no analyzed factors other than the number of cfDNA studies, cfDNA methylation status, and sample size explained most of the heterogeneity. Except for these 3 analysis characteristics, many other factors that may be a potential source of heterogeneity, such as race, country, cfDNA cutoff value, control group, and sample type, were not included because of insufficient information for analysis. Fifth, because of the lack of complete TNM staging information for TC in the original studies, we, unfortunately, were not able to assess the accuracy of cfDNA in diagnosing TC at different pathological stages. Moreover, although we searched authoritative English databases (PubMed, EMBASE, Web of Science, and Cochrane Library) and Chinese databases (CNKI, SinoMed, VIP, and Wan Fang), it is possible that a few relevant studies were missed. In addition, we used Deeks funnel plot asymmetry test to detect publication bias in the included studies, and the results showed that there was no significant publication bias ($P = .96$).

In conclusion, the results of this meta-analysis suggest that cfDNA has value as an adjunct to the diagnosis of TC. The quantitative detection of cfDNA can obtain relatively high diagnostic accuracy. However, due to heterogeneity, especially because the types of cfDNA included in this study were not all the same, the test results based on cfDNA for TC should be interpreted with caution. In general, more large-scale original studies are needed to verify the diagnostic potential of different types of cfDNA in TC. This study contributes to research on cfDNA in TC.

Author contributions

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