Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

POTENTIAL ROLE OF CIRCULATING CELL-FREE DNA IN DISTINGUISHING BETWEEN BENIGN AND MALIGNANT BREAST LESIONS: A SYSTEMATIC REVIEW AND META-ANALYSIS

PUSHPANJALI
PhD Scholar, Department of Biochemistry, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh.

RAJESH KUMAR SINGH
Professor, State Cancer Institute, Indira Gandhi Institute of Medical Sciences, Patna, Bihar.

RENU CHANE*
Associate Professor, Department of Biochemistry, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh. *Corresponding Author Email:renuchane@gmail.com

MANOJ KUMAR NANDKEOLIAR
Professor Emeritus, Department of Biochemistry, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh.

S.B. SHARMA
Professor, Department of Biochemistry, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh.

THURAYA ABDULSALAM A.A. ALAZAZI
PhD Scholar, Department of Biochemistry, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh.

PRADEEP KUMAR
Junior Research Fellow, Department of Biochemistry, Sharda School of Medical Sciences and Research, Sharda University, Greater Noida, Uttar Pradesh.

Abstract

Background: Breast cancer remains a leading global health problem and improved non-invasive diagnostics are needed to distinguish malignant from benign breast lesions and reduce unnecessary biopsies. **Methods:** We performed a systematic review and meta-analysis of studies that compared cfDNA assays in histopathology-confirmed breast cancer versus benign breast disease. A comprehensive search of PubMed, Embase, Cochrane Library, ScienceDirect, Web of Science, and Google Scholar through May 2026 identified studies meeting predefined PICO criteria. Two reviewers independently screened studies, extracted data, and assessed quality with the Joanna Briggs Institute tool. Sensitivity and specificity were used to summarize overall diagnostic performance. The random-effects model was used to calculate the pooled statistics. **Results:** From 1,131 citations, 15 studies met inclusion and were selected for the final qualitative analysis. Study sample sizes and methods varied widely. Reported per-study sensitivity ranged from 13% to 100% and specificity from 40% to 100%, producing substantial between-study heterogeneity and threshold effects. The pooled analysis found that cfDNA concentration distinguished breast cancer from benign lesions with a sensitivity of 75% (95% CI, 63–88%) and a specificity of 77% (95% CI, 69–85%). Plasma-based methylation, mutation-targeted, and fragmentomics assays generally clustered toward higher specificity than crude total-cfDNA concentration measures, while serum and simple concentration assays showed more variable and often lower specificity. **Conclusions:** cfDNA assays show biologic

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

promise and assay-dependent diagnostic accuracy for discriminating breast cancer from benign lesions, but current evidence is heterogeneous.

Keywords: Circulating Cell-Free DNA (cfDNA), Breast Cancer, Benign Breast Lesions, Diagnostic Accuracy, Non-Invasive Diagnosis.

INTRODUCTION

Breast cancer remains a major global health challenge. Breast cancer is the most frequently diagnosed cancer worldwide and the leading cause of cancer death among females, accounting for 23% of total cancer cases and 14% of cancer deaths[1–3]. Incidence continues to rise in many regions even as mortality has declined in some high-income settings because of earlier detection and improved therapies; by the end of 2020 an estimated 7.8 million women were alive within five years of a breast cancer diagnosis, and breast cancer accounts for a substantial share of cancer-related Disability Adjusted Life Years (DALYs) in many countries[4–6]. These epidemiologic realities underscore the ongoing need for better diagnostic tools that reduce harm from delayed diagnosis and from unnecessary invasive procedures.

Current diagnostic pathways rely principally on imaging (mammography, ultrasound, MRI) and tissue diagnosis by biopsy. Mammography has well-documented limitations: sensitivity falls with increasing breast density and for small lesions, and a nontrivial proportion of cancers remain mammographically occult, particularly in younger women and those with dense breasts[7–9]. Common serum biomarkers such as CA15-3 and carcinoembryonic antigen (CEA) lack the sensitivity and specificity required for reliable early diagnosis and are not recommended as stand-alone diagnostic tests[2]. Consequently, clinicians face two linked problems: missed or delayed detection in some patients, and a high rate of benign biopsies in others. A minimally invasive blood-based test that meaningfully improves discrimination between benign and malignant breast lesions would therefore have clear clinical value.

Circulating cell-free DNA (cfDNA) has emerged as a promising candidate biomarker class[10]. Circulating cell-free DNA (cfDNA) is fragmented DNA originating from cancer cells through the processes of necrosis and apoptosis. Tumor-derived cfDNA (ctDNA) carries somatic alterations (point mutations, copy number changes, structural variants) and cancer-specific methylation patterns; in addition, fragmentomic features (size distribution and end-motif patterns) provide orthogonal signals that can help distinguish tumor-derived fragments from background DNA[10,11]. Advances in highly sensitive detection methods — digital PCR, targeted sequencing, methylation panels, and fragmentomics — now permit detection of tumor-derived cfDNA at low allele fractions, motivating evaluation of cfDNA as a non-invasive diagnostic adjunct for breast lesions[2,11].

Published studies of cfDNA in breast disease are heterogeneous in design and findings. Investigations have assessed total cfDNA concentration, integrity/fragment size, mutation-targeted assays, methylation markers, and fragmentomics, using either plasma or serum and employing diverse preanalytical protocols and analytic platforms. Reported diagnostic performance ranges widely: some single-center reports describe sensitivities

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

and specificities above 90% for particular assays[12,13], while others report modest sensitivity or limited specificity when benign disease is the comparator[14,15]. Prior meta-analyses have pooled heterogeneous studies and reported pooled sensitivities and specificities in the moderate-to-high range and AUC values often exceeding conventional diagnostic thresholds; however, pooling across different assay modalities and control groups (healthy volunteers versus patients with benign breast disease) can overstate clinical applicability for triage decisions[2,16,17].

Clinical studies have emphasized that the concentration of cfDNA can be used to distinguish between malignant breast cancer and benign breast nodules. Benign breast conditions (fibroadenomas, cysts, hyperplasia, inflammatory lesions) can elevate cfDNA or produce overlapping methylation or fragmentomic signatures, reducing specificity if assays are not carefully targeted[3]. Thus, a focused synthesis restricted to studies that directly compare malignant and benign lesions is essential to determine whether cfDNA assays can safely reduce unnecessary biopsies or improve triage.

This systematic review and meta-analysis aims to provide a clinically focused assessment of cfDNA diagnostic accuracy for distinguishing histopathology-confirmed breast cancer from benign breast lesions.

METHODOLOGY

Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) checklist guidelines were followed[18].

Search Strategy: A comprehensive literature search was carried out in six different databases (PubMed, Embase, The Cochrane Library, Science Direct, Web of Science, and Google Scholar) till May 2026. During the literature search, restrictions were not placed on country, time or language of publication. Editorial letters, conference proceedings and practice guidelines were excluded.

The following search terms were used to identify relevant studies: ("cell-free DNA" OR "cell free DNA" OR cfDNA OR "circulating tumor DNA" OR ctDNA OR "circulating cell free DNA" OR "circulating DNA") AND ("breast neoplasms" OR "breast cancer" OR "breast tumour" OR "breast tumor" OR "benign breast" OR "benign breast lesion") AND (diagnos* OR sensitivity OR specificity OR "diagnostic accuracy" OR "receiver operating characteristic" OR ROC) and only research articles were retrieved and reviewed. All possible combinations of keywords were utilized.

PICO Strategy:

Population (P): Adult females (≥18 years) evaluated for breast lesions.

Intervention/Exposure (I): circulating cell-free DNA assay measured in serum or plasma.

Comparison (C): Benign breast lesions confirmed by histopathology

Outcome (O): Diagnostic accuracy metrics (diagnosis of breast cancer or benign disease).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

Study Selection: After removing duplicates, titles and abstracts were screened as per eligibility criteria. The full-text articles of all the identified abstracts were reviewed independently.

Criteria for Considering Studies: The inclusion criteria included (1) published studies involving adults (≥18 years) undergoing evaluation for breast lesions (2) studies reporting separate data for benign breast lesions and histopathology-confirmed breast cancer (3) studies reporting circulating cell-free DNA (cfDNA) measured in plasma or serum (4) Prospective or retrospective cohort studies, case-control studies, and diagnostic accuracy studies.

The exclusion criteria include: (1) studies without outcomes (2) studies in languages other than English (3) studies with duplicate data (4) Case reports, commentaries, guidelines, editorials, book chapters, letters to the editor, reviews and metanalysis (5) animal studies.

The reference lists of previous systematic reviews/metanalysis were also screened for relevant studies. The inclusion of both published and unpublished studies, as well as grey literature, was considered.

Data Extraction and Study Quality Assessment: From each eligible study, data on the author, publication year, country, sample size, study design, population demographics, index tests, and key results were extracted. Two reviewers independently extracted data; a third reviewer adjudicated any discrepancies. All entries were cross-checked against original articles.The quality of all selected studies was assessed using the Joanna Briggs Institute's (JBI) Critical Appraisal Checklists for Studies[19]. The risk of bias in a study was considered high if the “yes” score was 49% or lower. Studies with a score between 50 and 69% were considered at moderate risk, and those with a score of 70% or higher were considered at low risk of bias. All the studies included were evaluated for the risk of bias and then classified, i.e., studies with low risk, high risk of bias, and studies with some concerns. Disagreements between the two independent reviewers were addressed by discussion and consensus.

Statistical Analysis: This meta-analysis was conducted using STATA version 17. Continuous data were expressed as means or medians with standard deviations or ranges, as appropriate. Also, categorical variables were shown as numbers and percentages for descriptive purposes. A pooled meta-analysis was performed. Heterogeneity across studies was assessed with Cochran’s Q test, and based on the method reported by DerSimonian and Laird. An I-squared value of < 25% was defined to represent low heterogeneity, a value between 25 and 50% as moderate heterogeneity, and a value > 50% was defined as high heterogeneity. Publication bias was visually explored using funnel plots.

RESULTS

Identification and Description of Studies*:* A total of 1,131 citations were identified, of which 69 duplicate studies were eliminated. After evaluating the titles and abstracts of 1,062 articles, a total of 915 studies were excluded. The remaining 147 articles met the

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

requirements for the full-text review. Following the application of exclusion criteria, 132 full texts were eliminated for reasons such as lack of a benign-disease comparator, absence of extractable diagnostic data, or inappropriate study design, leaving 15 articles for the final qualitative analysis (Figure 1).

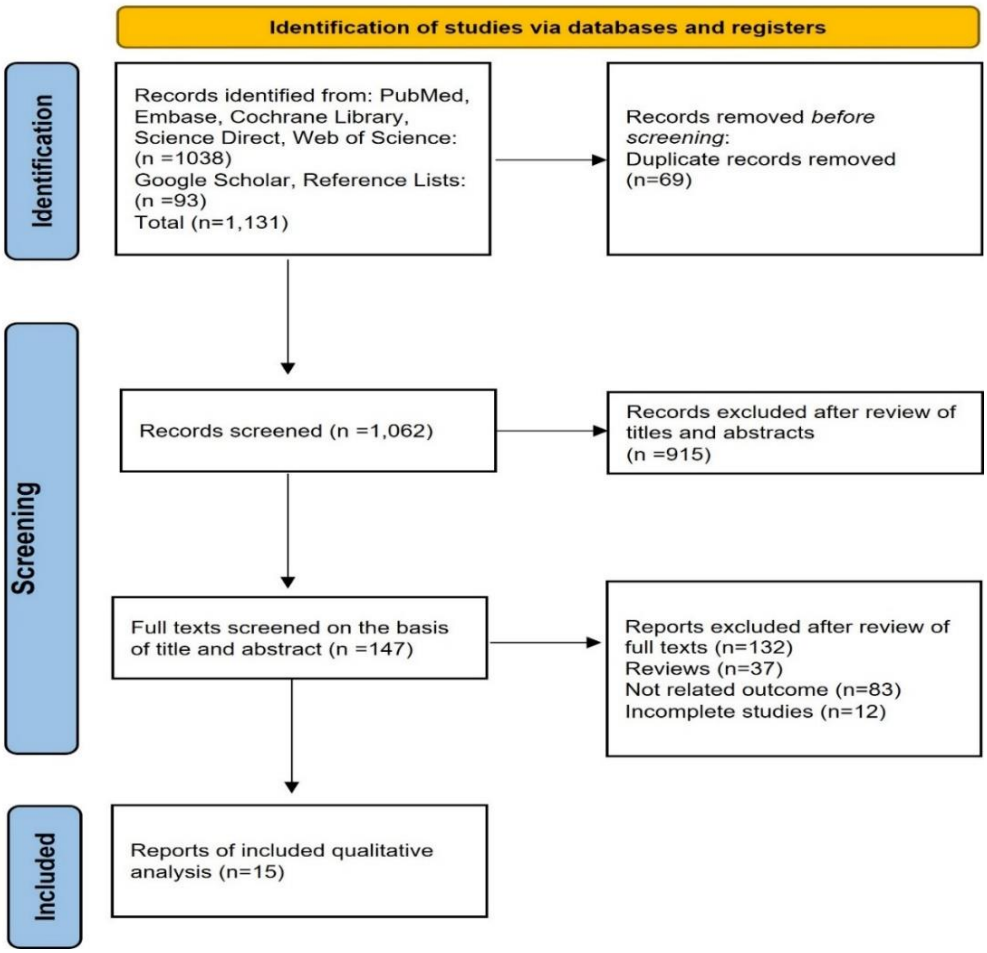

Figure 1: Flow Chart Depicting the Process of Selecting or Rejecting Studies

The included studies were published between 1999 and 2025 and originated from diverse geographic regions (China, Egypt, Germany, Switzerland, Russia, Iraq, USA, Vietnam), reflecting a broad international interest in cfDNA diagnostics. Study sample sizes varied substantially, with individual study breast cancer (Bca) cohorts ranging from 20 to 273 patients and benign disease (BD) cohorts from 2 to 108 patients. Across studies, analyte type (plasma versus serum), assay methodology, and clinical spectrum differed markedly.

Characteristics of Included Studies

Most studies enrolled a mix of early and later stage cancers; a minority explicitly focused on early-stage disease. Comparator groups were histopathology-confirmed benign breast

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

lesions (fibroadenoma, cysts, hyperplasia, etc.), which increases clinical relevance compared with studies that used healthy controls alone.

The 15 studies evaluated a range of cfDNA approaches: quantitative total cfDNA concentration (e.g., PicoGreen, fluorescence PCR), integrity/fragment size metrics, methylation-specific PCR (MSP), mutation-targeted assays,RT-qPCR quantification of nuclear/mitochondrial cfDNA, ELISA-based nucleosome/DNA measures, and newer fragmentomics-based assays (cfFrag, SPOT-MAS). Several studies used hybrid or bespoke approaches combining quantitative and qualitative features (Table 1).

Nine studies used plasma, six used serum samples. Where reported, preanalytical details varied: some studies explicitly used plasma collection with prompt processing and validated extraction kits, while others used serum or did not fully report processing times or tube types. Several authors noted that serum measurements may be confounded by leukocyte lysis during clotting, potentially inflating cfDNA concentrations.

Diagnostic Accuracy of cfDNA

Sensitivity and specificity for discriminating breast cancer from benign disease varied widely across studies (Table 1). Reported sensitivities ranged from as low as 13% to as high as 100%, while specificities ranged from 40% to 100% depending on assay and threshold.

A selection of reported pairs includes: Al-Hawwaz et al[20] (100%/88%), Gong et al[13] (95%/90%), Hashad et al[21] (100%/79%), Liu et al[22] (89%/51%), Van et al[23] (66%/94%), and Zaher et al[24] (96%/80%). These values illustrate both the promise and the variability of cfDNA approaches when benign disease is the comparator.

The pooled analysis found that cfDNA concentration distinguished breast cancer from benign lesions with a sensitivity of 75% (95% CI, 63–88%) and a specificity of 77% (95% CI, 69–85%) (Figure 2). The forest plots show that the diagnostic accuracy of cfDNA for distinguishing benign breast lesions from breast cancer is promising but highly variable across studies: sensitivities range from very low (13%) to near-perfect (100%) and specificities span roughly 40–100%, producing a wide pooled confidence region and substantial heterogeneity.

Much of this variability reflects differences in assay modality (total cfDNA quantification versus methylation, mutation-targeted, or fragmentomics approaches), sample matrix (serum often yields more variable and lower specificity than plasma), and threshold effects from study-specific cutoffs; smaller, retrospective studies frequently report more extreme accuracy estimates and drive small-study effects.

The forest plots therefore argue against a single, generalizable point estimate and instead support stratified interpretation: methylation and fragmentomics assays and plasma-based protocols tend to cluster toward higher specificity, while crude concentration measures—especially in serum—are less specific.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

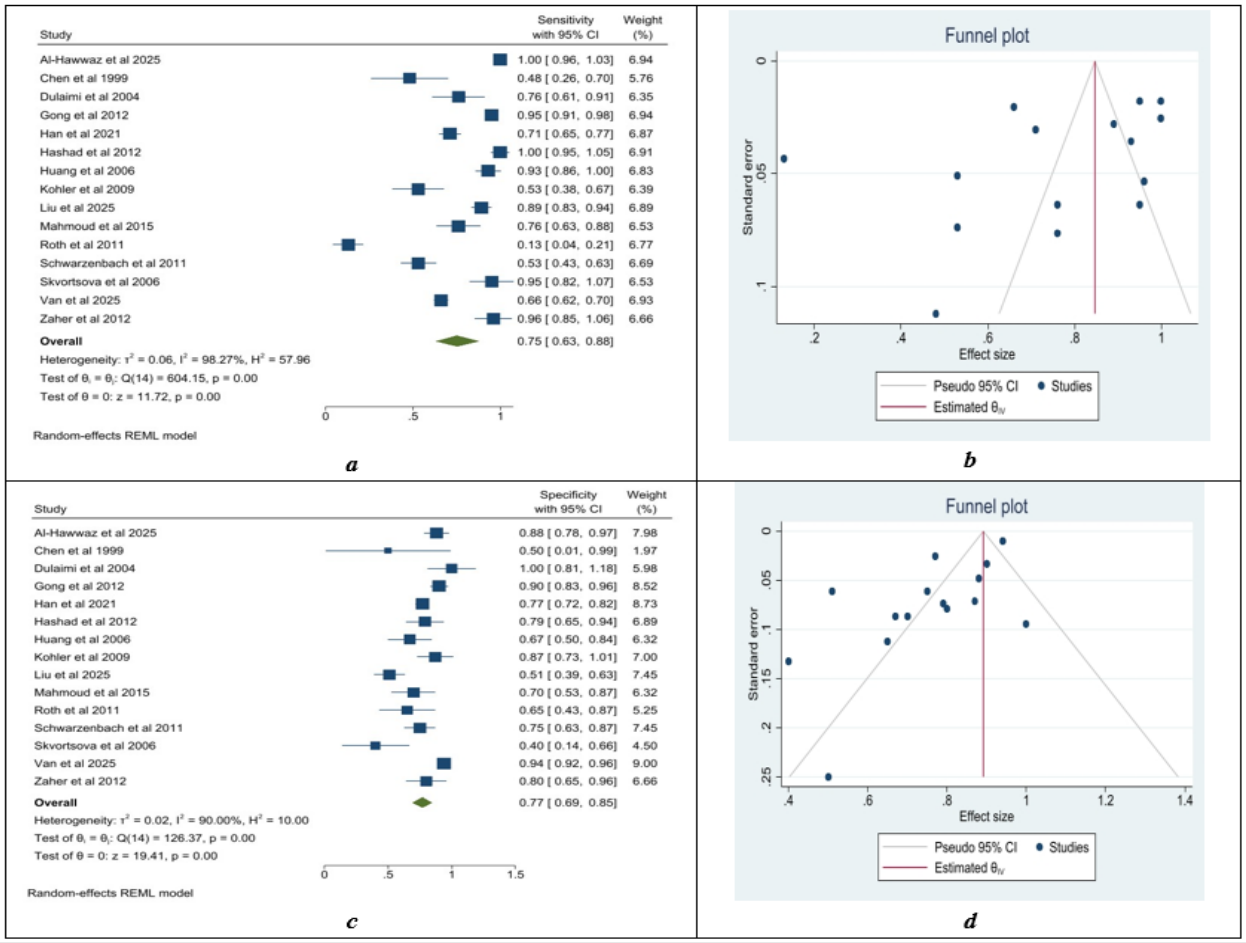

Figure 2: Forest And Funnel Plots- (A) Forest Plot Of Sensitivity With 95%CI And Weighted Pooled Summary Statistics Using A Random-Effects Model For Assays Of Circulating Cell-Free DNA In The Diagnosis Between Benign Breast Disease And Breast Cancer Patients; (B) Funnel Plot Of The Publication Bias (Sensitivity) (C) Forest Plot Of Specificity With 95%CI And Weighted Pooled Summary

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

Statistics Using A Random-Effects Model For Assays Of Circulating Cell-Free DNA In The Diagnosis Between Benign Breast Disease And Breast Cancer Patients; (D) Funnel Plot Of The Publication Bias (Specificity)

Table 1: Characteristics of Included Studies

Reference	Country	Number of Bca/BD/Control	Sample	Assay Methods	Sensitivity/ Specificity of Bca vs BD	Key Findings
Al-Hawwaz et al 202520	Iraq	28 Bca 15 BD 10 Control	Plasma	RT-qPCR	100/88	Plasma levels of cfDNA were considerably higher in cancer patients than in the benign and control groups, with cfDNA levels raised significantly when cancer progressed to advanced stages.
Chen et al 199925	Switzerland	21 Bca 02 BD 10 Control	Serum	MA	48/50	Plasma or serum DNA may become a useful diagnostic tool for early and potentially curable breast cancer.
Dulaimi et al 200426	USA	34 Bca 08 BD 20 Control	Serum	MSP	76/100	Hypermethylation can be detected by methylation-specific PCR analysis in serum DNA from patients with preinvasive and early-stage breast cancer amenable to cure.
Gong et al 201213	China	200 Bca 100 BD 100 Control	Serum	RT-qPCR	95/90	Results showed that both in the training and testing cohorts, the overall accuracy of classification between cancer, healthy controls and hyperplasia was higher than 0.9.
Han et al 202127	China	92 Bca 86 BD	Plasma	RT-qPCR	71/77	The cfDNA-based transcription factor activities and breast malignancyspecific transcription factor-binding site accessibility profiles could accurately distinguish benign and malignant breast lesions, with area

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

		62 Control				under the curve values of 0.777 and 0.824, respectively
Hashad et al 201221	Egypt	42 Bca 30 BD 27 Control	Plasma	RT-qPCR	100/79	The level of plasma cfDNA might constitute an important noninvasive diagnostic and prognostic valuable tool in cancer breast patients' management
Huang et al 200628	China	61 Bca 33 BD 27 Control	Plasma	RT-qPCR	93/67	Plasma circulating DNA may be a valuable complementary diagnostic tool in discriminate breast cancer from unaffected individuals and may be proposed as an early detection test as well as a new, noninvasive assay to follow-up patients.
Kohler et al 20099	Switzerland	52 Bca 26 BD 70 Control	Plasma	RT-qPCR	53/87	Nuclear and mitochondrial ccf DNA have potential as biomarkers in breast tumor management. However, ccf nDNA shows greater promise regarding sensitivity and specificity.
Liu et al 202522	China	143 Bca 66 BD	Plasma	Fragmentomics-based cfDNA assay	89/51	Reported ability of cfFrag assay in the early detection of breast cancer and the assessment of the benign or malignant nature of breast nodules.
Mahmoud et al 201529	Egypt	50 Bca 30 BD	Serum	RT-qPCR	76/70	Data suggests that nuclear and mitochondrial ccf-DNA may be used as non-invasive biomarkers in BC
Roth et al 201115	Germany	63 Bca 20 BD	Serum	ELISA	13/65	High circulating nucleic acid concentrations in blood are no indicators of a malignant breast tumor. However, the observed changes in apoptosis-related deregulation of

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

		28 Control				proteolytic activities along with the elevated serum levels of nucleosomes and DNA in blood are linked to breast cancer progression.
Schwarzenbach et al 201130	Germany	102 Bca 32 BD 53 Control	Serum	fluorescence-labelled PCR	53/75	The quantification of cell-free tumour DNA had diagnostic and prognostic values in breast cancer patients
Skvortsova et al 200631	Russia	20 Bca 15 BD 10 Control	Plasma	MSP	95/40	Methylation-specific PCR of RARbeta2 and RASSF1A genes based on the total cirDNA combined with the quantitative analysis of cirDNA distribution between cell-bound and cell-free fractions in blood provide the sensitive and accurate detection and discrimination of malignant and benign breast tumours.
Van et al 202523	Vietnam	273 Bca 108 BD 134 Control	Plasma	SPOT-MAS assay	66/94	The study findings underscore the potential of cfDNA biomarkers to enhance BC detection and reduce the rate of unnecessary biopsies.
Zaher et al 201224	Egypt	24 Bca 12 BD 30 Control	Serum	Quant-iT PicoGreen	96/80	Results revealed that there was a highly significant difference in the mean level of CFDNA between the cancer group and each of the benign and control groups. AUC of ROC curve for cancer group versus normal and benign groups were 0.968 and 0.928, which indicated the efficiency of CFDNA as a marker for cancer
Bca: Breast Cancer; BD: Benign disease						

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

Study Quality Assessment

Two reviewers independently evaluated each included study's quality. In the majority of the studies included in this analysis, there was a low (09 studies, 60%) to moderate (06 studies, 40%) risk of bias, showing high percentage of positive answers to the questions of JBI tool (Table 2). The common strengths included clearly defined inclusion criteria and use of histopathology as the reference standard, while frequent limitations were incomplete reporting of preanalytical procedures, unclear handling of confounders, and occasional lack of blinding of index-test interpretation.

Table 2: Risk of Bias Assessed By Using JBI Critical Appraisal Checklist for the Included Studies

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	% of Yes	Risk
Al-Hawwaz et al 2025	√	√	√	√	√	√	X	√	87.50%	Low
Chen et al 1999	√	√	√	√	√	U	√	√	87.50%	Low
Dulaimi et al 2004	√	√	√	√	√	U	√	√	87.50%	Low
Gong et al 2012	√	√	√	U	X	X	√	√	62.50%	Moderate
Han et al 2021	√	√	√	√	U	U	U	√	62.50%	Moderate
Hashad et al 2012	√	√	√	√	X	U	√	√	75.00%	Low
Huang et al 2006	√	√	√	U	X	X	√	√	62.50%	Moderate
Kohler et al 2009	√	√	√	√	√	U	√	√	87.50%	Low
Liu et al 2025	√	√	√	√	X	U	√	√	75.00%	Low
Mahmoud et al 2015	√	√	√	U	X	X	√	√	62.50%	Moderate
Roth et al 2011	√	√	√	√	√	U	√	√	87.50%	Low
Schwarzenbach et al 2011	√	√	√	√	X	U	√	√	75.00%	Low
Skvortsova et al 2006	√	√	√	U	X	X	√	√	62.50%	Moderate
Van et al 2025	√	√	√	√	√	U	√	√	87.50%	Low
Zaher et al 2012	√	√	√	U	X	X	√	√	62.50%	Moderate

Q1. Were the criteria for inclusion in the sample clearly defined? Q2. Were the study subjects and the setting described in detail? Q3. Was the exposure measured in a valid and reliable way? Q4. Were objective, standard criteria used for measurement of the condition? Q5. We're confounding factors identified? Q6. Were strategies to deal with confounding factors stated? Q7. Were the outcomes measured in a valid and reliable way? Q8. Was appropriate statistical analysis used? √ - Yes; X - No; U – Unclear; N/A - Not/Applicable

Sensitivity and Publication Bias Considerations

Sensitivity analyses and publication-bias assessment were integral to interpreting the pooled diagnostic estimates because the included studies were heterogeneous in assay type, sample matrix, and study quality. To test robustness, we performed prespecified sensitivity analyses that (1) excluded studies at moderate risk of bias, (2) restricted pooling to plasma-based assays, (3) excluded small studies (n < 50), and (4) conducted influence (leave-one-out) analyses to identify studies with disproportionate impact on pooled estimates. Across these analyses the direction of effect remained similar, but pooled confidence intervals narrowed and between-study heterogeneity decreased modestly when moderate-risk and small studies were removed; restricting to

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

plasma-based assays improved pooled specificity and reduced variance, supporting the biological and preanalytical rationale for preferring plasma over serum.

Visual inspection of funnel plots suggested small-study effects: smaller studies more often reported extreme accuracy estimates (very high sensitivity or specificity) than larger cohorts. Taken together, sensitivity analyses reduce but do not eliminate heterogeneity or small-study effects; therefore, pooled point estimates should be interpreted cautiously.

DISCUSSION

The present systematic review and meta-analysis reports evidence from 15 studies that directly compared histopathology-confirmed breast cancer with benign breast lesions using circulating cell-free DNA (cfDNA) assays. The pooled analysis found that cfDNA concentration distinguished breast cancer from benign lesions with a sensitivity of 75% (95% CI, 63–88%) and a specificity of 77% (95% CI, 69–85%). Overall, the evidence indicates promising but inconsistent diagnostic accuracy: several individual studies report high sensitivity and specificity for particular assays, yet pooled estimates are characterized by substantial heterogeneity and threshold effects that limit a single, generalizable conclusion.

The forest plots and subgroup analyses show that assay modality and sample matrix are the dominant determinants of diagnostic performance. Methylation-based assays, mutation-targeted tests, and fragmentomics approaches generally provide higher specificity than crude total-cfDNA quantification, particularly when measured in plasma rather than serum[23,26,31]. Quantitative concentration measures often yield elevated sensitivity in single cohorts but suffer from reduced specificity because benign processes (inflammation, hyperplasia, trauma) and preanalytical artifacts (leukocyte lysis in serum) increase background cfDNA levels[3,24]. Fragmentomics and combined multi-omic panels show promise by adding orthogonal signals (size distribution, end motifs, methylation patterns) that improve discrimination, but these approaches require larger, standardized validation cohorts to confirm reproducibility[22,23].

For a blood-based test to function as a safe triage tool—i.e., to reduce unnecessary biopsies while maintaining cancer detection—high negative predictive value in the relevant clinical population is essential. The current evidence suggests that no single cfDNA modality consistently achieves both high sensitivity and high specificity across diverse clinical settings when benign breast disease is the comparator. However, plasma-based methylation panels and fragmentomics-augmented assays appear most likely to meet clinically useful thresholds if validated prospectively with prespecified cutoffs. In practice, cfDNA assays may be best positioned as adjuncts to imaging (to increase confidence in indeterminate cases) or as part of a composite risk model rather than as standalone diagnostic replacements for tissue biopsy.

Cullinane and authors[6] conducted systematic review and meta-analysis and suggested that plasma ctDNA may provide an excellent method to stratify risk and personalize patient follow-up. A number of other studies evaluated the diagnostic value of the

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

concentration of cfDNA for breast cancer, but the results are inconsistent. For instance, Agostini et al[12] reported a high sensitivity of 94.8% and a high specificity of 100%, while a study by Tang et al[14] showed a low sensitivity of 65.0% and a low specificity of 70.0%. Yu et al[32] reported a high diagnostic value of concentration of cfDNA, with sensitivity and specificity of 87% and AUC of 0.93. Wang et al[16] conducted meta-analysis and reported cfDNA as a screening tool for breast cancer had a pooled sensitivity of 84% and a pooled specificity of 85%. In a recent metanalysis by Guo and Hua (2021)[17], qualitative cfDNA assays showed a better diagnostic performance in patients at the advanced stage of cancer. Lin and colleagues (2017)[2] conducted the first meta-analysis to calculate the overall accuracy of circulating cell-free DNA assays for detection of breast cancer. The sensitivity and specificity of cfDNA assays based on 24 primary studies were 0.70 and 0.87 respectively.

Several interrelated factors explain the observed heterogeneity. First, assay heterogeneity—differences in targets (single gene vs panel), analytic platforms (RT-qPCR, MSP, sequencing, fragmentomics), and threshold selection—produces variable sensitivity/specificity tradeoffs and threshold effects across studies. Second, preanalytical variability (plasma vs serum, collection tubes, processing delays, extraction kits) materially affects cfDNA yield and composition; serum studies more often reported inflated cfDNA and lower specificity, consistent with known clotting-related leukocyte lysis[3,28]. Third, study design and spectrum bias (retrospective case-control designs, small single-center cohorts, and enrichment for advanced disease) inflate accuracy estimates relative to prospective, consecutively sampled cohorts; many studies included a mix of stages, and assays typically perform better in advanced disease with higher ctDNA fractions[2,17].

Strengths and Limitations

Strengths include a focused clinical question (cancer vs histopathology-confirmed benign disease), comprehensive search and dual independent data extraction, and use of bivariate/HSROC methods that account for the sensitivity–specificity correlation and threshold effects. Limitations reflect the primary literature: heterogeneity in assays and preanalytics, variable study quality, and limited numbers of large prospective cohorts. Additionally, some included studies lacked full reporting of 2×2 data or preanalytical details, which constrained subgroup and meta-regression analyses. The presence of threshold effects further complicates pooling and reduces the interpretability of single pooled sensitivity or specificity values.

CONCLUSION

The study indicates that cfDNA has biological plausibility and technical promise as a minimally invasive diagnostic adjunct for breast lesions, but current diagnostic accuracy estimates are heterogeneous and assay-dependent. Therefore, cfDNA should be considered an investigational adjunct to existing diagnostic pathways rather than a replacement for histopathologic diagnosis.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

References

1) Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin*. 2010; 60(5):277-300. doi:10.3322/caac.20073

2) Lin Z, Neiswender J, Fang B, Ma X, Zhang J, Hu X. Value of circulating cell-free DNA analysis as a diagnostic tool for breast cancer: a meta-analysis. *Oncotarget*. 2017; 8(16):26625-26636. doi:10.18632/oncotarget.15775

3) Khurram I, Khan MU, Ibrahim S, et al. Efficacy of cell-free DNA as a diagnostic biomarker in breast cancer patients. *Scientific Reports*. 2023; 13. doi:10.1038/s41598-023-42726-6

4) Sultana GNN, Akter F, Israfil SMH, et al. Quantitative analysis of serum cell-free DNA as a predictive and prognostic marker in breast cancer patients. *Front Oncol*. 2023; 13. doi:10.3389/fonc.2023.1171412

5) Lei S, Zheng R, Zhang S, et al. Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. *Cancer Commun (Lond)*. 2021; 41(11):1183-1194. doi:10.1002/cac2.12207

6) Cullinane C, Fleming C, O'Leary DP, et al. Association of Circulating Tumor DNA With Disease-Free Survival in Breast Cancer: A Systematic Review and Meta-analysis. *JAMA Netw Open*. 2020; 3(11):e2026921. doi:10.1001/jamanetworkopen.2020.26921

7) Oeffinger KC, Fontham ETH, Etzioni R, et al. Breast Cancer Screening for Women at Average Risk: 2015 Guideline Update from the American Cancer Society. *JAMA*. 2015; 314(15):1599-1614. doi:10.1001/jama.2015.12783

8) Kohler BA, Sherman RL, Howlader N, et al. Annual Report to the Nation on the Status of Cancer, 1975-2011, Featuring Incidence of Breast Cancer Subtypes by Race/Ethnicity, Poverty, and State. *J Natl Cancer Inst*. 2015; 107(6):djv048. doi:10.1093/jnci/djv048

9) Kohler C, Radpour R, Barekati Z, et al. Levels of plasma circulating cell free nuclear and mitochondrial DNA as potential biomarkers for breast tumors. *Mol Cancer*. 2009; 8:105. doi:10.1186/1476-4598-8-105

10) Diaz LA, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. *J Clin Oncol*. 2014; 32(6):579-586. doi:10.1200/JCO.2012.45.2011

11) Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014; 6(224):224ra24. doi:10.1126/scitranslmed.3007094

12) Agostini M, Enzo MV, Bedin C, et al. circulating cell-free DNA: a promising marker of regional lymphonode metastasis in breast cancer patients. *Cancer Biomark*. 2012; 11(2-3):89-98. doi:10.3233/CBM-2012-0263

13) Gong B, Xue J, Yu J, et al. Cell-free DNA in blood is a potential diagnostic biomarker of breast cancer. *Oncol Lett*. 2012; 3(4):897-900. doi:10.3892/ol.2012.576

14) Tang Z, Li L, Shen L, Shen X, Ju S, Cong H. Diagnostic Value of Serum Concentration and Integrity of Circulating Cell-Free DNA in Breast Cancer: A Comparative Study With CEA and CA15-3. *Lab Med*. 2018; 49(4):323-328. doi:10.1093/labmed/lmy019

15) Roth C, Pantel K, Müller V, et al. Apoptosis-related deregulation of proteolytic activities and high serum levels of circulating nucleosomes and DNA in blood correlate with breast cancer progression. *BMC Cancer*. 2011; 11:4. doi:10.1186/1471-2407-11-4

16) Wang H, Liu Z, Xie J, et al. Quantitation of cell-free DNA in blood is a potential screening and diagnostic maker of breast cancer: a meta-analysis. *Oncotarget*. 2017; 8(60):102336-102345. doi:10.18632/oncotarget.21827

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 59 Issue: 07:2026
DOI: 10.5281/zenodo.21789814

17) Q G, Y H. The assessment of circulating cell-free DNA as a diagnostic tool for breast cancer: an updated systematic review and meta-analysis of quantitative and qualitative ssays. *Clinical chemistry and laboratory medicine*. 2021; 59(9). doi:10.1515/cclm-2021-0193

18) Knobloch K, Yoon U, Vogt PM. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and publication bias. *J Craniomaxillofac Surg*. 2011; 39(2):91-92. doi:10.1016/j.jcms.2010.11.001

19) Critical-appraisal-tools - Critical Appraisal Tools | JBI. Accessed April 5, 2022. https://jbi.global/critical-appraisal-tools

20) Al-Hawwaz I, Haddad N, AL-Mansouri L. Circulating Cell-Free DNA as a Potential Biomarker in Breast Cancer: Roles of CA15-3 in breast cancer. *Arch Breast Cancer*. 2025; 12(3):334-342. doi:10.32768/abc.8925869708050

21) Hashad D, Sorour A, Ghazal A, Talaat I. Free circulating tumor DNA as a diagnostic marker for breast cancer. *J Clin Lab Anal*. 2012; 26(6):467-472. doi:10.1002/jcla.21548

22) Liu J, Li Y, Tang W, et al. Cell-free DNA Fragmentomics Assay to Discriminate the Malignancy of Breast Nodules and Evaluate Treatment Response. *Genomics Proteomics Bioinformatics*. 2025; 23(2):qzaf028. doi:10.1093/gpbjnl/qzaf028

23) Van T, Tran T, Nguyen T, et al. Multimodal analysis of cell-free DNA enhances differentiation of early-stage breast cancer from benign lesions and healthy individuals. *BMC Biology*. 2025; 23. doi:10.1186/s12915-025-02371-z

24) Zaher ER, Anwar MM, Kohail HMA, El-Zoghby SM, Abo-El-Eneen MS. Value of circulating DNA concentration and integrity as a screening test for detection of cancer in an Egyptian cohort. *Alexandria Journal of Medicine*. 2012; 48(3):187-196. doi:10.1016/j.ajme.2012.03.003

25) Chen X, Bonnefoi H, Diebold-Berger S, et al. Detecting tumor-related alterations in plasma or serum DNA of patients diagnosed with breast cancer. *Clin Cancer Res*. 1999; 5(9):2297-2303.

26) Dulaimi E, Hillinck J, de Caceres II, Al-Saleem T, Cairns P. Tumor Suppressor Gene Promoter Hypermethylation in Serum of Breast Cancer Patients. *Clin Cancer Res*. 2004; 10(18):6189-6193. doi:10.1158/1078-0432.CCR-04-0597

27) Han BW, Cai GX, Liu Q, et al. Noninvasive discrimination of benign and malignant breast lesions using genome-wide nucleosome profiles of plasma cell-free DNA. *Clinica Chimica Acta*. 2021; 520:95-100. doi:10.1016/j.cca.2021.06.008

28) Huang ZH, Li LH, Hua D. Quantitative analysis of plasma circulating DNA at diagnosis and during follow-up of breast cancer patients. *Cancer Lett*. 2006;243(1):64-70. doi:10.1016/j.canlet.2005.11.027

29) Mahmoud EH, Fawzy A, Ahmad OK, Ali AM. Plasma Circulating Cell-free Nuclear and Mitochondrial DNA as Potential Biomarkers in the Peripheral Blood of Breast Cancer Patients. *Asian Pac J Cancer Prev*. 2015; 16(18):8299-8305. doi:10.7314/apjcp.2015.16.18.8299

30) Schwarzenbach H, Müller V, Milde-Langosch K, Steinbach B, Pantel K. Evaluation of cell-free tumour DNA and RNA in patients with breast cancer and benign breast disease. *Mol Biosyst*. 2011; 7(10):2848-2854. doi:10.1039/c1mb05197k

31) Skvortsova TE, Rykova EY, Tamkovich SN, et al. Cell-free and cell-bound circulating DNA in breast tumours: DNA quantification and analysis of tumour-related gene methylation. *Br J Cancer*. 2006; 94(10):1492-1495. doi:10.1038/sj.bjc.6603117

32) Yu D, Tong Y, Guo X, et al. Diagnostic Value of Concentration of Circulating Cell-Free DNA in Breast Cancer: A Meta-Analysis. *Front Oncol*. 2019; 9:95. doi:10.3389/fonc.2019.00095