GMR
The Original

THE ROLE OF CFDNA, CTDNA, METHYLATION AND FRAGMENTOMICS IN EARLY CANCER DETECTION: A SYSTEMATIC REVIEW

Shalu Singh[1]*, Sanjeev Kumar Bansal[2], Kishor Khodke[3]

[1]PhD Scholar, Department of Biochemistry, SGT University, Gurugram, Haryana, India.
[2]Professor and HOD, Department of Biochemistry, SGT University, Gurugram, Haryana, India
[3]Consultant Pathologist, Department of Pathology, Apollo Hospitals, Nashik, Maharashtra, India
***Corresponding Author:** Shalu Singh, Email ID - singhshalu4296@gmail.com

ABSTRACT

Background: Early cancer detection is a major strategy for reducing cancer-related morbidity and mortality. Circulating cell-free DNA (cfDNA) provides a minimally invasive substrate for detecting tumor-associated quantitative, genetic, epigenetic, and structural abnormalities. This systematic review evaluates the roles of total cfDNA, circulating tumor DNA (ctDNA), DNA methylation/hydroxymethylation, and cfDNA fragmentomics in early and multi-cancer detection.

Methods: A PRISMA 2020-aligned systematic review was conducted using structured electronic searches and citation chaining for human studies evaluating blood-based cfDNA approaches in localized, preclinical, screening-detected, or otherwise potentially curable cancer. Studies restricted to treatment monitoring, minimal residual disease, or metastatic disease without an early-detection component were excluded. Fourteen primary studies were included. Study quality was assessed using QUADAS-2. Owing to heterogeneity in tumor types, assay platforms, populations, and thresholds, results were synthesized qualitatively.

Results: Mutation-based ctDNA assays established the feasibility of detecting early-stage malignancy but were limited by low tumor fraction, genomic heterogeneity, sequencing error, and clonal hematopoiesis. Methylation-based assays provided broader tumor-derived signal, very high specificity, and tissue-of-origin information, although stage I sensitivity remained substantially lower than later-stage sensitivity. Fragmentomic approaches based on genome-wide fragment-size distributions, coverage, end motifs, and nucleosome-related patterns showed strong diagnostic performance using low-coverage whole-genome sequencing and were complementary to mutation analysis. Prospective studies demonstrated that cfDNA-based multi-cancer early detection can be integrated into clinical pathways, but positive tests may trigger substantial diagnostic evaluation. QUADAS-2 assessment identified patient-selection bias as the most frequent methodological concern.

Conclusion: cfDNA-based early cancer detection is progressing from single-analyte mutation testing toward integrated genomic, epigenomic, and fragmentomic profiling. Methylation and fragmentomics are particularly promising for low-burden disease, while multi-omic approaches may provide the best balance of sensitivity and specificity. Large prospective population studies demonstrating stage shift, acceptable diagnostic harms, cost-effectiveness, and ultimately reduced cancer mortality are still required.

KEYWORDS: cell-free DNA; circulating tumor DNA; ctDNA; liquid biopsy; DNA methylation; hydroxymethylation; fragmentomics; cancer screening; multi-cancer early detection; MCED

1. INTRODUCTION

Cancer mortality is strongly influenced by stage at diagnosis because localized malignancies are more likely to be amenable to curative surgery, radiotherapy, or other definitive treatment. However, established population screening exists for only a limited number of cancer types, leaving many lethal malignancies without an effective average-risk screening strategy.

Liquid biopsy has emerged as a potential solution to this diagnostic gap. Cell-free DNA (cfDNA) consists of extracellular DNA fragments released into the circulation, predominantly from normal hematopoietic cell turnover. In patients with cancer, a variable fraction of cfDNA originates from tumor cells and is termed circulating tumor DNA (ctDNA). Early work showed that tumor-derived mutations can be detected in plasma across multiple cancer types, but detection declines substantially in localized disease because tumor-derived molecules may constitute only a minute fraction of total plasma DNA [1].

More recent work has demonstrated that cfDNA contains multiple layers of tumor-associated information beyond sequence mutations. These include aberrant methylation and hydroxymethylation, copy-number abnormalities, altered fragment lengths, genome-wide fragmentation distributions, fragment-end motifs, and nucleosome-related signatures. These properties have shifted the field from mutation-only liquid biopsy toward multidimensional analysis of the circulating DNA molecule [4,6,8,9].

This systematic review synthesizes the evidence for four major cfDNA strategies—total cfDNA-related features, mutation-defined ctDNA, DNA methylation/hydroxymethylation, and fragmentomics—with particular attention to early-stage detection, multi-cancer applications, prospective screening feasibility, and methodological quality.

2. MATERIALS AND METHODS

2.1 Review design and reporting framework

The review was structured according to PRISMA 2020 principles [15]. The review question was framed around the diagnostic performance and clinical utility of blood-based cfDNA assays for detection of localized, preclinical, screening-detected, or otherwise potentially curable cancers.

2.2 Search strategy

Structured electronic searches were conducted using four concept groups: (1) cfDNA concentration/characteristics and cancer screening, (2) mutation-based ctDNA and early cancer detection, (3) cfDNA methylation or hydroxymethylation and early/multi-cancer detection, and (4) cfDNA fragmentomics or fragmentation patterns and early cancer detection. Citation chaining from seminal and validation studies was used to capture major studies not returned among the initial candidate records. Searches were restricted to human clinical studies and the evidence set was updated through January 2026.

The structured searches yielded 40 candidate records. Two duplicate records were removed. Citation chaining added 12 unique records, resulting in 50 records for title/abstract screening. Twenty-two records were excluded at screening, 28 full-text reports were assessed, and 14 studies met the final inclusion criteria. The PRISMA-style flow is shown in Figure 1.

2.3 Eligibility criteria

Studies were included when they: (1) enrolled human participants; (2) evaluated blood- or plasma-derived cfDNA; (3) assessed cancer detection or discrimination; (4) included localized, stage I–III, preclinical, asymptomatic, screening, or potentially resectable malignancy; (5) evaluated sequence mutations, methylation/hydroxymethylation, fragmentomic features, or multi-analyte models containing a cfDNA component; and (6) reported diagnostic performance or clinically interpretable detection outcomes.

Studies were excluded when they focused exclusively on treatment response, minimal residual disease, relapse monitoring, or advanced metastatic disease without an early-detection component; were non-human or model-only investigations; were reviews/commentaries; or were conference abstracts/preprints without adequate independent clinical detail.

2.4 Outcomes and data extraction

Extracted variables included study design, population, cancer types and stages, sample size, cfDNA analytical strategy, validation approach, sensitivity, specificity, area under the receiver operating characteristic curve (AUC), cancer-signal or tissue-of-origin performance, and major study limitations. Stage-specific performance was prioritized whenever reported.

2.5 Risk-of-bias assessment

Methodological quality was assessed with QUADAS-2, covering patient selection, index test, reference standard, and flow/timing [16]. Judgments were categorized as low, high, or unclear risk of bias. Applicability was considered separately when a study population was substantially enriched compared with the intended screening population.

2.6 Data synthesis

Because the included studies differed substantially in cancer spectrum, disease stage, participant selection, assay chemistry, sequencing depth, classifier architecture, and diagnostic threshold, statistical pooling was considered inappropriate. A qualitative synthesis was therefore performed by biomarker strategy.

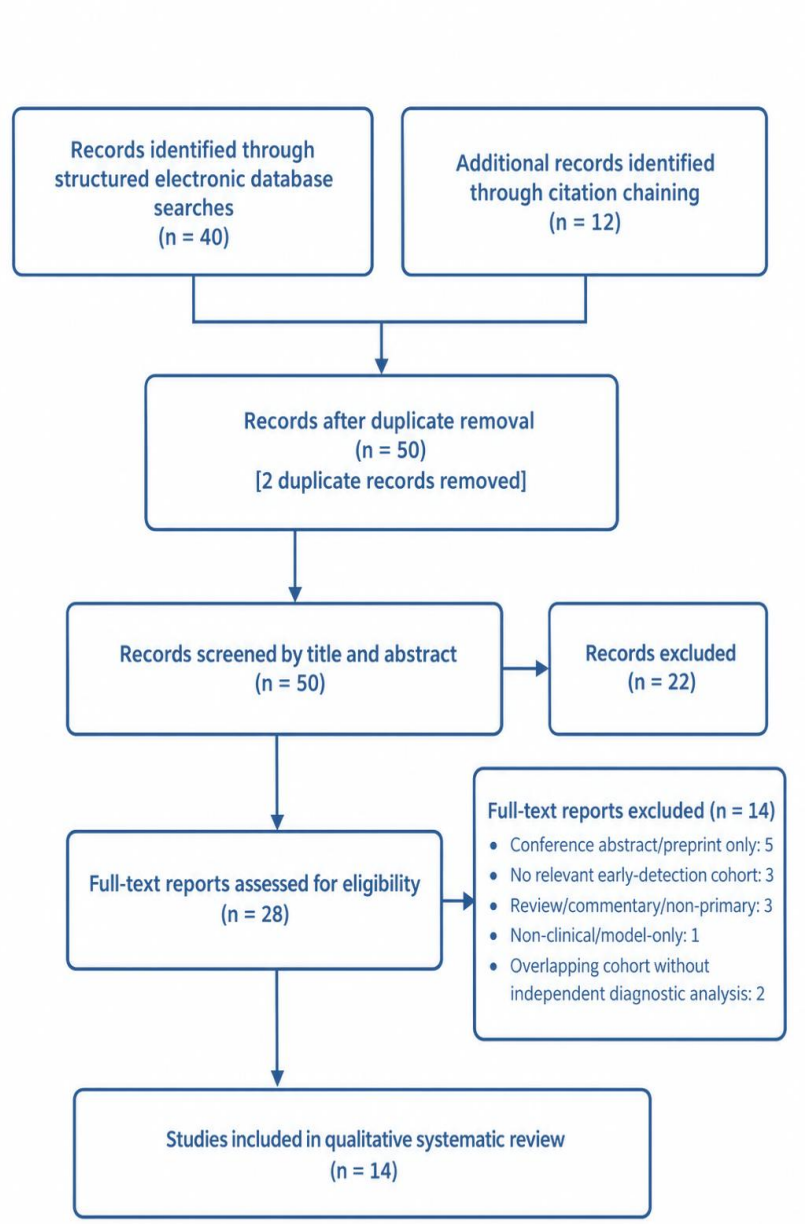

Figure 1. PRISMA 2020-style flow diagram for study identification and selection.

3. RESULTS

3.1 Overview of included studies

Fourteen primary studies published between 2014 and 2025 were included. The evidence base comprised retrospective case-control studies, nested longitudinal analyses, independent validation studies, prospective diagnostic cohorts, and prospective screening implementation studies. Sample sizes ranged from several hundred participants in biomarker-development cohorts to more than 10,000 participants in prospective screening studies. The principal assay classes were mutation-based ctDNA analysis, targeted methylation or hydroxymethylation profiling, genome-wide fragmentomics, and multi-analyte approaches that combined cfDNA with proteins or imaging.

Table 1. Characteristics of Included Studies

Study	Design	Population / sample	cfDNA strategy	Key early-detection findings	Main limitation
Bettegowda et al. 2014 [1]	Cross-sectional multi-cancer cohort	640 patients with localized or metastatic cancers; multiple tumor types	Mutation-based ctDNA; tumor-informed/targeted sequencing	ctDNA detectable in >75% of advanced pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, HCC, and head/neck cancers; localized	Heterogeneous tumors/stages; not a population-screening cohort

				detection varied by tumor type, including ~73% colorectal, 57% gastroesophageal, 48% pancreatic, and 50% breast	
Phallen et al. 2017 [2]	Case-control diagnostic study	200 patients with stage I–II breast, colorectal, lung, or ovarian cancer plus controls	TEC-Seq error-corrected targeted sequencing	Detected somatic mutations in a substantial proportion of stage I–II cancers (roughly 59–71% across evaluated tumor types); high specificity with error correction	Enriched case-control design; limited panel; early-stage tumor fraction remains limiting
Cohen et al. 2018 [3]	Case-control multi-cancer study	1,005 patients with nonmetastatic cancers; 812 healthy controls	CancerSEEK: cfDNA mutations + 8 circulating proteins	Median sensitivity 70% across 8 cancers; specificity >99%; localization to a small number of sites feasible	Clinically detected cases; spectrum bias; multi-analyte rather than cfDNA-only
Cristiano et al. 2019 [4]	Case-control multi-cancer study	236 patients with 7 cancer types; 245 healthy controls	DELFI genome-wide cfDNA fragmentation + machine learning	Overall AUC 0.94; sensitivity 57% to >99% by tumor type at 98% specificity; fragmentation + mutation analysis detected ~91%	Retrospective case-control design; classifier development and evaluation within enriched cohorts
Lennon et al. 2020 [5]	Prospective interventional screening study	10,006 women without a previous cancer diagnosis	Multi-analyte blood test followed by confirmatory PET-CT	26 cancers detected through blood testing; 1.0% underwent PET-CT after false-positive blood tests; 0.22% underwent futile invasive diagnostic procedures	Women only; single health system; imaging-dependent localization
Liu et al. 2020 [6]	Large case-control development/validation study (CCGA)	6,689 participants across >50 cancer types in development and validation sets	Targeted cfDNA methylation sequencing	Specificity ~99.3%; sensitivity increased markedly with stage; cancer signal origin predicted with high accuracy	Case-control enrichment; low stage I sensitivity; performance varies by cancer type
Chen et al. 2020 [7]	Nested retrospective longitudinal study (PanSeer)	605 asymptomatic individuals; 191 developed 5 cancer types within 4 years	Targeted cfDNA methylation profiling	Reported detection of cancer-associated methylation signatures before conventional diagnosis, with high sensitivity in pre-diagnostic samples and ~96% specificity	Retrospective nested design; selected cancer types; requires independent prospective replication
Guler et al. 2020 [8]	Case-control study	64 pancreatic ductal adenocarcinoma cases; 243	Genome-wide 5-hydroxymethylcytosine profiling	Independent model performance approximately AUC 0.92–0.94; signal	Small cancer cohort; case-control design; limited external

		non-cancer controls		present in early-stage PDAC	generalizability
Klein et al. 2021 [9]	Independent validation study (CCGA)	4,077 participants in independent validation	Targeted cfDNA methylation MCED assay	Specificity 99.5%; overall sensitivity 51.5%; stage I 16.8%, stage II 40.4%, stage III 77.0%, stage IV 90.1%; cancer signal origin correct ~88.7% of true positives	Case-control spectrum; stage I sensitivity limited
Mathios et al. 2021 [10]	Prospective diagnostic cohort + independent validation	365 high-risk/symptomatic individuals; validation: 46 lung cancers + 385 non-cancer controls	DELFI fragmentomics + clinical risk factors + CEA	Combined strategy detected 94% of lung cancers overall and 91% of stage I/II cancers at 80% specificity	Validation controls from separate cohorts; symptomatic/high-risk setting differs from population screening
Luo et al. 2022 [11]	Training + single-blind independent validation	Training: 120 HCC, 92 cirrhosis, 290 healthy; validation: 67 HCC, 111 cirrhosis, 242 healthy	Targeted cfDNA methylation model	Validation sensitivity 84%, specificity 96%; early-stage HCC sensitivity remained clinically meaningful	HCC-specific; enriched case-control composition; etiology/geography may affect transportability
Schrag et al. 2023 [12]	Prospective screening implementation (PATHFINDER)	6,662 enrolled adults ≥50 years; 6,621 analyzable results	Targeted methylation MCED assay with predicted cancer signal origin	92 cancer signals detected; 35 participants ultimately diagnosed with cancer; demonstrated real-world diagnostic work-up after positive testing	Convenience sample; predominantly White/educated population; not randomized for mortality benefit
Mazzone et al. 2024 [13]	Prospective case-control clinical validation	958 lung-screening-eligible participants; 576 training, 382 held-out validation	Low-coverage WGS cfDNA fragmentome classifier	Held-out validation showed consistent sensitivity across demographic/comorbidity groups; designed as a blood triage strategy before LDCT	Case-control enrichment despite screening eligibility; outcome modeling not equivalent to mortality trial
Xu et al. 2024/2025 [14]	Prospective cohort	265 participants: 124 lung cancer and 141 non-cancer	Four-feature cfDNA fragmentation model using low-depth WGS	Independent test AUC 0.861; sensitivity 70% at specificity 89.4%; early-stage AUC 0.808	Single-center cohort; suspected lung disease and healthy controls; external validation needed

3.2 Mutation-based ctDNA detection

Mutation-based ctDNA analysis established the molecular feasibility of identifying early-stage malignancy. Bettegowda et al. demonstrated that ctDNA detection was highly dependent on tumor type and disease burden [1]. Phallen et al. subsequently used error-corrected targeted sequencing to detect somatic alterations directly from plasma in stage I–II breast, colorectal, lung, and ovarian cancers [2]. These studies showed that very low

variant allele fractions can be measured, but sensitivity becomes biologically constrained when few or no tumor-derived molecules are present in a blood draw.

Multi-analyte approaches can partially compensate for this limitation. CancerSEEK combined cfDNA mutations with circulating proteins and achieved a median sensitivity of approximately 70% across eight nonmetastatic cancers with specificity >99% [3]. The prospective DETECT-A study then demonstrated that a blood-based multi-cancer assay coupled to PET-CT could be incorporated into clinical care, while also quantifying downstream false-positive imaging and invasive procedures [5].

A major limitation of mutation-only detection is clonal hematopoiesis, in which age-related leukocyte clones carry somatic mutations that may be incorrectly attributed to solid tumors. Matched leukocyte sequencing can reduce this source of false positivity but adds cost and analytical complexity [17].

3.3 DNA methylation and hydroxymethylation

DNA methylation offers a broader cancer-associated signal than individual sequence mutations because malignant transformation alters large numbers of CpG loci. Methylation is also strongly tissue dependent, allowing a single assay to address both whether a cancer signal is present and where the signal most likely originates.

In a large CCGA analysis, targeted methylation sequencing achieved specificity around 99% while detecting signals across more than 50 cancer types [6]. Independent validation by Klein et al. showed 99.5% specificity and 51.5% overall sensitivity, but sensitivity was only 16.8% for stage I cancers and rose progressively to 90.1% for stage IV disease [9]. This stage gradient is central to interpretation of MCED performance: very high specificity does not remove the biological challenge of detecting the smallest tumors.

The PanSeer study provided evidence that cancer-associated methylation patterns may be detectable before conventional diagnosis. In a nested longitudinal cohort, methylation signatures were identified in stored plasma from individuals who were subsequently diagnosed with stomach, esophageal, colorectal, lung, or liver cancer [7]. The result is best interpreted as possible detection of clinically occult disease rather than prediction of future carcinogenesis.

Single-cancer epigenetic applications also showed promise. Luo et al. reported 84% sensitivity and 96% specificity for hepatocellular carcinoma in an independent plasma validation cohort [11]. Guler et al. showed that circulating 5-hydroxymethylcytosine profiles could discriminate pancreatic ductal adenocarcinoma from non-cancer controls, including early-stage disease [8].

3.4 cfDNA fragmentomics

Fragmentomics analyzes the physical organization of cfDNA molecules, including fragment length, short-to-long fragment ratios, genomic coverage, preferred ends, end motifs, and nucleosome-related patterns. Because these features are distributed across millions of circulating DNA fragments, fragmentomics can extract signal even when any particular tumor mutation is extremely rare.

Cristiano et al. introduced a genome-wide fragmentation framework with an overall AUC of approximately 0.94 across seven cancer types and showed that combining fragmentation with mutation analysis improved detection [4]. Mathios et al. extended this approach to a prospective lung-cancer diagnostic cohort and an independent validation set; a strategy combining fragmentomic features, clinical risk factors, CEA, and subsequent CT detected 91% of stage I/II lung cancers at 80% specificity [10].

More recent prospective validation strengthened the clinical evidence. Mazzone et al. evaluated 958 lung-screening-eligible participants, using a held-out validation cohort after classifier locking [13]. Xu et al. developed a four-feature fragmentation model in a prospective cohort of 265 participants; the independent test AUC was 0.861, with an early-stage AUC of 0.808 [14]. These studies support low-coverage whole-genome sequencing as a scalable route to structural cfDNA biomarkers, although screening-population validation remains necessary.

3.5 Prospective MCED implementation

Prospective implementation studies clarify that diagnostic accuracy is only one component of screening utility. In PATHFINDER, 6,621 participants had analyzable MCED results; a cancer signal was detected in 92 individuals and 35 were ultimately diagnosed with cancer [12]. The study showed that predicted cancer signal origin can guide diagnostic evaluation, while also demonstrating that even highly specific assays generate false-positive work-ups in low-prevalence populations.

3.6 QUADAS-2 risk-of-bias assessment

The dominant methodological concern was patient selection. Ten of the fourteen studies were judged at high risk in this domain because they used case-control enrichment, clinically diagnosed cancer cohorts, or controls that did not fully represent the intended screening population. Index-test risk was generally low when a locked classifier or independent validation cohort was used, but was judged unclear for earlier development studies in which model selection and threshold determination were closely linked to the analyzed dataset. Reference standards were generally robust because cancer cases were pathologically or clinically confirmed. PATHFINDER was judged unclear for reference standard and flow/timing because participants with negative MCED tests did not all undergo an equivalent definitive diagnostic reference procedure, although clinical follow-up was incorporated. Figure 2 and Table 2 summarize the judgments.

Figure 2. QUADAS-2 risk-of-bias judgments for the 14 included studies.

Table 2. QUADAS-2 Domain Judgments

Study	Patient selection	Index test	Reference standard	Flow/timing	Overall
Bettegowda 2014	High	Unclear	Low	Low	High
Phallen 2017	High	Unclear	Low	Low	High
Cohen 2018	High	Unclear	Low	Low	High
Cristiano 2019	High	Unclear	Low	Low	High
Lennon 2020	Low	Low	Low	Low	Low
Liu 2020	High	Low	Low	Low	High
Chen 2020	Low	Low	Low	Low	Low
Guler 2020	High	Unclear	Low	Low	High
Klein 2021	High	Low	Low	Low	High
Mathios 2021	High	Low	Low	Low	High
Luo 2022	High	Low	Low	Low	High
Schrag 2023	High	Low	Unclear	Unclear	High
Mazzone 2024	High	Low	Low	Low	High
Xu 2024/25	High	Low	Low	Low	High

4. DISCUSSION

This systematic review demonstrates that cfDNA contains several complementary layers of cancer-associated information. The earliest liquid-biopsy paradigm treated cfDNA mainly as a source of tumor mutations. That approach remains highly valuable for molecular profiling, but early detection is a more difficult biological problem because the relevant signal may be represented by only a handful of tumor-derived molecules.

4.1 Why mutation-only assays struggle in stage I disease

The principal limitation is not sequencing depth alone. When the tumor-derived fraction is extremely low, deeper sequencing cannot recover molecules that were never present in the blood sample. Tumor heterogeneity

further reduces the universality of any finite mutation panel, and clonal hematopoiesis can mimic tumor-associated mutations [17]. Mutation analysis therefore offers excellent molecular specificity when a genuine tumor-derived variant is found, but limited sensitivity as a stand-alone pan-cancer strategy for very small tumors.

4.2 Why methylation is attractive for MCED

Epigenetic abnormalities affect far more genomic loci than typical driver-mutation panels and are strongly tissue-specific. A single tumor-derived molecule may therefore contribute multiple informative features, and a classifier may simultaneously estimate cancer presence and likely tissue of origin. The CCGA studies demonstrate this advantage but also reveal the persistent stage I challenge [6,9].

4.3 Why fragmentomics is attractive

Fragmentomics converts the physical structure of cfDNA into a biomarker. Genome-wide fragmentation reflects nucleosome positioning, chromatin accessibility, nuclease activity, and cell of origin. Because the signal is distributed across millions of fragments, useful discrimination can be achieved at comparatively low whole-genome sequencing depth. This may have important implications for cost and scalability [4,10,13,14].

4.4 Complementarity of cfDNA signals

Mutation, methylation, and fragmentomic signals should not be viewed as mutually exclusive technologies. Mutations provide high sequence-level specificity; methylation provides abundant tissue-associated epigenetic information; and fragmentomics provides genome-wide structural information. Combining orthogonal signals may improve sensitivity while preserving the extremely high specificity required for screening. Cristiano et al. provided an early proof of this principle by showing improved detection when fragmentation and mutation information were combined [4].

4.5 Specificity, positive predictive value, and diagnostic harm

Specificity is particularly important in asymptomatic screening because disease prevalence is low. Even a seemingly strong specificity of 95% would generate an unacceptable number of false-positive results when applied to millions of healthy individuals. Modern MCED platforms therefore generally target specificity around 99% or higher. Prospective studies such as DETECT-A and PATHFINDER are important because they quantify the downstream imaging, invasive procedures, and time to diagnostic resolution associated with positive results [5,12].

4.6 The stage I challenge

The most clinically important unresolved problem remains stage I sensitivity. In the independent methylation validation study, specificity reached 99.5% but stage I sensitivity was 16.8% across the overall cancer spectrum [9]. Aggregate sensitivity can therefore be misleading when driven by stage III–IV disease. Future assay development and trial reporting should prioritize stage-specific performance and the proportion of cancers shifted from advanced to potentially curable stages.

4.7 Clinical utility versus diagnostic accuracy

A screening assay can show excellent diagnostic accuracy yet fail to reduce mortality. Clinical benefit depends on whether a positive molecular signal leads to timely localization of a clinically significant tumor and effective earlier treatment. Overdiagnosis, incidental findings, invasive follow-up, patient anxiety, and unequal access to confirmatory care must be measured alongside sensitivity and specificity.

5. Limitations of the Evidence and of This Review

The evidence base is heterogeneous in cancer spectrum, stage distribution, assay technology, sequencing depth, classifier design, and threshold selection. Many studies remain case-control investigations with substantial spectrum bias, as reflected in the QUADAS-2 assessment. Some studies evaluated clinically detected cancers rather than true screening populations. Furthermore, several technologies are evolving rapidly, so the exact implementation studied may be superseded before long-term clinical-outcome trials are completed.

6. FUTURE DIRECTIONS

The next generation of blood-based cancer screening is likely to integrate mutations, methylation, hydroxymethylation, copy-number alterations, fragment size, fragment-end motifs, nucleosome footprints, circulating proteins, and clinical risk factors. Longitudinal analysis may also allow algorithms to detect deviation from an individual baseline rather than relying exclusively on population-level thresholds.

Future studies should emphasize prospective prediagnostic recruitment, representative screening populations, benign-disease controls, locked thresholds, blinded independent validation, long-term follow-up of test-negative participants, stage-specific sensitivity, diagnostic harms, resource utilization, stage shift, and ultimately randomized assessment of cancer-specific and all-cancer mortality.

7. CONCLUSION

cfDNA-based early cancer detection has evolved from simple mutation testing into multidimensional genomic, epigenomic, and fragmentomic profiling. Mutation-based ctDNA assays established the feasibility of blood-based detection but remain constrained by low tumor fraction and clonal hematopoiesis in early disease. Methylation assays provide broad tumor-associated and tissue-of-origin information with very high specificity, while fragmentomics can extract genome-wide structural signal using comparatively low sequencing depth. The

most promising future approach is likely to integrate these orthogonal biomarkers rather than rely on any single signal. The decisive evidence, however, will come from large prospective and randomized studies showing that cfDNA-based screening reduces late-stage cancer incidence and mortality without unacceptable diagnostic harm.

REFERENCES

1. 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.
2. Phallen J, Sausen M, Adleff V, et al. Direct detection of early-stage cancers using circulating tumor DNA. Sci Transl Med. 2017;9(403):eaan2415. doi:10.1126/scitranslmed.aan2415.
3. Cohen JD, Li L, Wang Y, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359(6378):926-930. doi:10.1126/science.aar3247.
4. Cristiano S, Leal A, Phallen J, et al. Genome-wide cell-free DNA fragmentation in patients with cancer. Nature. 2019;570(7761):385-389. doi:10.1038/s41586-019-1272-6.
5. Lennon AM, Buchanan AH, Kinde I, et al. Feasibility of blood testing combined with PET-CT to screen for cancer and guide intervention. Science. 2020;369(6499):eabb9601. doi:10.1126/science.abb9601.
6. Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV; CCGA Consortium. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol. 2020;31(6):745-759. doi:10.1016/j.annonc.2020.02.011.
7. Chen X, Gole J, Gore A, et al. Non-invasive early detection of cancer four years before conventional diagnosis using a blood test. Nat Commun. 2020;11:3475. doi:10.1038/s41467-020-17316-z.
8. Guler GD, Ning Y, Ku CJ, et al. Detection of early stage pancreatic cancer using 5-hydroxymethylcytosine signatures in circulating cell free DNA. Nat Commun. 2020;11:5270. doi:10.1038/s41467-020-18965-w.
9. Klein EA, Richards D, Cohn A, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. Ann Oncol. 2021;32(9):1167-1177. doi:10.1016/j.annonc.2021.05.806.
10. Mathios D, Johansen JS, Cristiano S, et al. Detection and characterization of lung cancer using cell-free DNA fragmentomes. Nat Commun. 2021;12:5060. doi:10.1038/s41467-021-24994-w.
11. Luo B, Ma F, Liu H, et al. Cell-free DNA methylation markers for differential diagnosis of hepatocellular carcinoma. BMC Med. 2022;20(1):8. doi:10.1186/s12916-021-02201-3.
12. Schrag D, Beer TM, McDonnell CH III, et al. Blood-based tests for multicancer early detection (PATHFINDER): a prospective cohort study. Lancet. 2023;402(10409):1251-1260. doi:10.1016/S0140-6736(23)01700-2.
13. Mazzone PJ, Bach PB, Carey J, et al. Clinical Validation of a Cell-Free DNA Fragmentome Assay for Augmentation of Lung Cancer Early Detection. Cancer Discov. 2024;14(11):2224-2242. doi:10.1158/2159-8290.CD-24-0519.
14. Xu F, Wang C, Li H, et al. Evaluation of cfDNA fragmentation characteristics in plasma for the diagnosis of lung cancer: a prospective cohort study. Cancer Sci. 2025;116(1):248-256. doi:10.1111/cas.16360.
15. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;372:n71. doi:10.1136/bmj.n71.
16. Whiting PF, Rutjes AWS, Westwood ME, et al. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529-536. doi:10.7326/0003-4819-155-8-201110180-00009.
17. Razavi P, Li BT, Brown DN, et al. High-intensity sequencing reveals the sources of plasma circulating cell-free DNA variants. Nat Med. 2019;25(12):1928-1937. doi:10.1038/s41591-019-0652-7.
18. Wan JCM, Massie C, Garcia-Corbacho J, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nat Rev Cancer. 2017;17(4):223-238. doi:10.1038/nrc.2017.7.