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Circulating tumour DNA analysis for early detection of lung cancer: a systematic review

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Background: Circulating tumor DNA (ctDNA) analysis has been applied in cancer diagnostics including lung cancer. Specifically for the early detection purpose, various modalities of ctDNA analysis have demonstrated their potentials. Such analyses have showed diverse performance across different studies.

Methods: We performed a systematic review of original studies published before 1 January 2023. Studies that evaluated ctDNA alone and in combination with other biomarkers for early detection of lung cancer were included.

Results: The systematic review analysis included 56 original studies that were aimed for early detection of lung cancer. There were 39 studies for lung cancer only and 17 for pan-cancer early detection. Cancer and control cases included were heterogenous across studies. Different molecular features of ctDNA have been evaluated, including 7 studies on cell-free DNA concentration, 17 on mutation, 29 on methylation, 5 on hydroxymethylation and 8 on fragmentation patterns. Among these 56 studies, 17 have utilised different combinations of the above-mentioned ctDNA features and/or circulation protein markers. For all the modalities, lower sensitivities were reported for the detection of early-stage cancer.

Conclusions: The systematic review suggested the clinical utility of ctDNA analysis for early detection of lung cancer, alone or in combination with other biomarkers. Future validation with standardised testing protocols would help integration into clinical care.

Keywords: Liquid biopsy; circulating tumor DNA (ctDNA); lung cancer; multi-cancer early detection

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Introduction

Lung cancer is characterised by its high incidence and poor overall prognosis. It ranks among the top cancer types in both incidence and mortality in different parts of the world (1,2). Much effort has been devoted to improving the survival outcome of patients with lung cancer, including development of early detection methods. The basis for early detection of lung cancer, similar to other types of cancer,

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is the better survival noted among early-stage disease. The five-year survival rate for localised lung cancer is about 60%, but only less than 10% for metastatic diseases. Unfortunately, only 40% of lung cancer cases are diagnosed as localised or regional diseases at presentation. This highlights an unmet need for improving early detection of localised diseases (1).

Circulating tumour DNA (ctDNA) refers to DNA released into the circulation/blood from cancer cells and could be used to inform genetic and epigenetic alterations present in the tumour (3). There has been intense research on ctDNA analysis for screening, prognostication and surveillance in cancer diagnostics (4). Specifically for the screening purpose, the minimal invasiveness and simple logistic arrangement of a blood draw required for ctDNA analysis provides a readily available screening biomarker (5). In contrast, for screening of lung cancer, low dose computed tomography (LDCT) is another option but remains underutilised despite evidence suggesting survival benefits through radiological screening among high-risk individuals (6,7). Utility of ctDNA for early detection of lung cancer has been explored in a number of studies, including those which focused on lung cancer only or pan-cancer detection (8,9). Given the highly variable degrees of evidence supporting use and adoption of ctDNA analysis, we have conducted this systematic review to evaluate the available evidence on various methods of ctDNA assessment for screening of lung cancer.

Highlight box

Key findings

- Different molecular features of circulating tumour DNA (ctDNA) have been evaluated for early detection of lung cancer. The diagnostic performance metrics of analyses based on the different molecular features were revealed to be variable in the current systematic review. This could be attributed to the difference in the molecular testing approaches, study design and the heterogeneity of cancer and control samples.

What is known and what is new?

- Various molecular features of ctDNA were utilized for lung cancer detection in liquid biopsy. Some studies evaluated a single molecular feature, while others utilized different combinations of molecular features and/or circulating protein biomarkers. Regarding the study design, some studies were designed for detection of lung cancer only, while others targeted for pan-cancer detection.
- The systematic review revealed the diverse study designs and the high heterogeneity of case and control cohorts included in the different studies.

What is the implication, and what should change now?

- The high heterogeneity may affect the direct comparison of diagnostic performance metrics across studies and the generalizability of the studies' conclusions to routine clinical settings.

Biology of ctDNA

Before the systematic review analysis, the fundamental concepts of ctDNA (and plasma DNA in general) are to be discussed. Plasma DNA is a mixture of DNA fragments released from different organs or cell types [as a result of apoptosis or necrosis (10)]. Therefore, identifying ctDNA at the background of plasma DNA released from other normal cell and tissue types would pose a challenge on sensitivity, especially for small tumours in the early detection setting when the level of ctDNA is expected to be low (11).

Cancer-associated somatic mutation and aberrant methylation are the two most studied molecular features of ctDNA in cancer diagnostics (12,13) (*Figure 1*). Detection of somatic mutations in plasma is aimed at cancer-derived mutation. However, there is a lack of common hotspot mutations for virtually all cancer types (including lung cancer). Therefore, multiple targets (over multiple genes) have been included with an attempt to improve the detection sensitivity (8,14). The specificity of somatic mutations detected in plasma DNA as surrogates of tumour-derived DNA is another issue. These somatic mutations may be derived from conditions other than tumour cells, such as clonal hematopoiesis of indeterminate potential (CHIP) (15). CHIP describes the expansion of a subclone of haemopoietic cells which carry leukemogenic mutations. Though associated with an increased risk of hematological malignancies, it is yet to be defined as malignant. It is common among the elderly population with a prevalence of up to 10% among adults older than 70 years old. Cancer-associated aberrant methylation is another frequently explored ctDNA marker. Compared to somatic mutation analysis, methylation profiling of plasma DNA could yield the tissue-of-origin information (16,17), which would largely facilitate the establishment of a blood-based pan-cancer screening test (18,19). Methylation analysis forms the core technology of the now commercially available multi-cancer early detection (MCED) test branded as Galleri™ test by Grail, Inc.

As mentioned, plasma DNA exists as fragments of DNA in the circulation (20,21). The size distribution of plasma

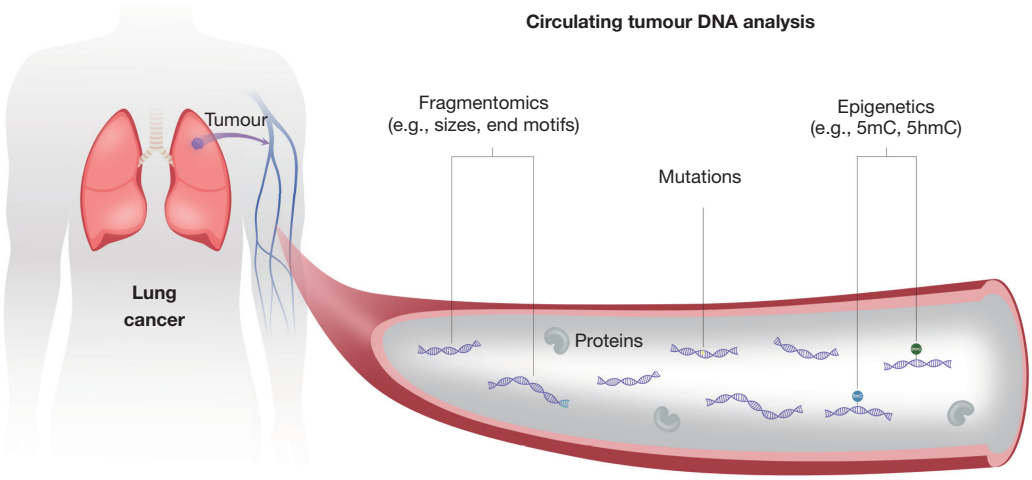

Figure 1 Different molecular analyses of circulating tumour DNA for early detection of lung cancer.

DNA exhibits a modal peak of ~160 bp that corresponds to a mononucleosomal size (21). Specifically, ctDNA is suggested to be shorter than its normal counterparts in plasma (21). The realization of non-random fragmentation has fuelled research on the fragmentation biology and the simultaneous exploration of 'fragmentomics' markers with diagnostics potentials (*Figure 1*). These markers are signatures of differential fragmentation of ctDNA and DNA derived from normal cells possibly because of the underlying pathophysiological process. They include fragment size (22,23), fragment ends (24), end motifs (25) and jagged ends (26) and all of them have demonstrated potential clinical utilities in a cancer model.

Analytical methods and pre-analytical considerations

Differentiating ctDNA from non-tumor circulating DNA requires detection of cancer-associated genetic, epigenetic and/or fragmentomic alterations as discussed above. Digital polymerase chain reaction (dPCR) and massively parallel sequencing (MPS), also known as next generation sequencing (NGS), are the most commonly employed methods (27). Adaptations to the techniques enable detection of the properties of ctDNA.

Each of the molecular techniques, dPCR and NGS, has its own advantages and disadvantages. dPCR offers precise and sensitive detection of variants even at low allelic fractions, and its downstream analysis and interpretation are relatively straightforward. However, it relies on prior knowledge of the presence of variants in the tumor, and it is less efficient in detecting multiple variants in a single assay (27,28). These limitations hinder the utility of dPCR in early cancer detection where the presence of analytical targets is unknown. In contrast, NGS enables multiplexing, allowing the simultaneous detection of multiple variants within or across samples. This enables untargeted or semi-targeted detection of multiple variants at a lower average cost. Nonetheless, NGS requires more complex downstream bioinformatic analysis, longer analysis time, and higher upfront instrumental costs (29).

Pre-analytical factors also impose a significant impact on the results. These factors include types of blood tubes used, post-collection handling of specimens and delay which could affect the degree of *ex-vivo* release of DNA from blood cells into plasma (30). In addition to the above-mentioned factors, method of cfDNA isolation would influence relative abundance of ctDNA within samples, with subsequent impacts on diagnostic performance on the assays (31). We present this article in accordance with the PRISMA reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-23-1572/rc).

Methods

Literature search

We performed a systematic review on publications of PubMed before 1 January 2023. Search terms were "(cell-free DNA (cfDNA) OR circulating tumour DNA (ctDNA)) AND (lung cancer) AND ((screening) OR (detection) OR (diagnosis))". Only publications in English were reviewed.

Titles and abstracts were screened and full-texts were reviewed when applicable. We also screened all referenced articles of the selected studies. Studies which evaluated ctDNA analysis for lung cancer detection in symptomatic/asymptomatic patients with no known previous history of lung cancer (primary but not recurrent cancer) were included. Abstracts were reviewed by the authors independently. Discrepancies were resolved with discussion and reaching a consensus.

Data analysis

Information on the study design and the inclusion and exclusion criteria of the selected studies were reviewed. For lung cancer cases, the cancer stage and histologic subtype information were retrieved. For control cases, the clinical status information including smoking habits, chronic obstructive pulmonary disease (COPD) status and presence of lung nodules were retrieved, if available.

Clinical end points

For evaluation of diagnostic performance, the primary outcome of interest was the ability of ctDNA analysis in differentiating patients with lung cancer from individuals without lung cancer, including the sensitivity, specificity, positive and negative predictive values and area under the curve value in receiver operating characteristics curve analysis. For the majority of diagnostic studies, tissue biopsy served as the reference method for determination of cancer statuses in participants.

Quality assessment

Quality assessment for the identified diagnostic studies was performed with the Quality Assessment of Diagnostic Accuracy Studies-2 tool (32).

Results

Study selection is shown in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flowchart in *Figure 2*. Among the 797 articles identified in the database search, 56 were included in the systematic review. A summary table on the ctDNA properties, study cohort (study size, target population and control group), methodology and findings was provided (*Table 1*).

Study design

A case-control study design was adopted in all except one study in the evaluation of the diagnostic performance of ctDNA analysis for detection of lung cancer. In the remaining study, the group prospectively analyzed ctDNA variants and circulating protein biomarkers [by CancerSEEK (75)] for pan-cancer detection including lung cancer (9). Among these case-control studies, the majority included samples of both early- (stage I–II) and advanced- (stage III–IV) stage cancer, except for a few that targeted early-stage (or stage I) cancer only (44,47,72,80). There were 39 studies that were designed for detection of lung cancer only, and 17 studies for pan-cancer early detection. Different histological subtypes of lung cancer had been included in the cancer cohorts. However, the performance of the various ctDNA analyses was not separately reported for the different lung cancer subtypes in most studies (53,68). The control groups used in the studies were also heterogenous, while some studies included 'healthy' controls (37,66,70,78) and other studies included individuals with a benign lung nodule or other pulmonary diseases, e.g., COPD (56,67,81). Such comparison with benign lung disease cases could be used to address the utility of ctDNA analysis under certain clinical scenarios, such as management of patients with lung nodules of unknown nature (56,67). Given the variability in case selection (distribution of early versus advanced cases) and the control groups used, it would be difficult to directly compare the diagnostic performances of the different ctDNA assays for lung cancer detection.

ctDNA properties and analytical methods

Various molecular features of cell-free DNA (cfDNA) were utilized to differentiate lung cancer and non-cancer samples. Quantification of cfDNA concentration was employed in 7 studies (33,34,56,67,69,73,74); cfDNA mutation was employed in 17 studies (8,9,14,35-43,73-77); cfDNA methylation in 29 (*Table 1*); cfDNA hydroxymethylation in 5 (62-66); and cfDNA fragmentation profiles in 8 (66,70-72,78-80,83). Among the selected studies, 17 studies utilised different combinations of the above cfDNA molecular features and/or cfDNA concentration and/or circulating protein biomarkers (8,9,14,33,34,56,63,66-69,73-78).

For detection of cfDNA mutations (8,9,14,35-43,73-77), most studies have targeted multiple genes (usually driver

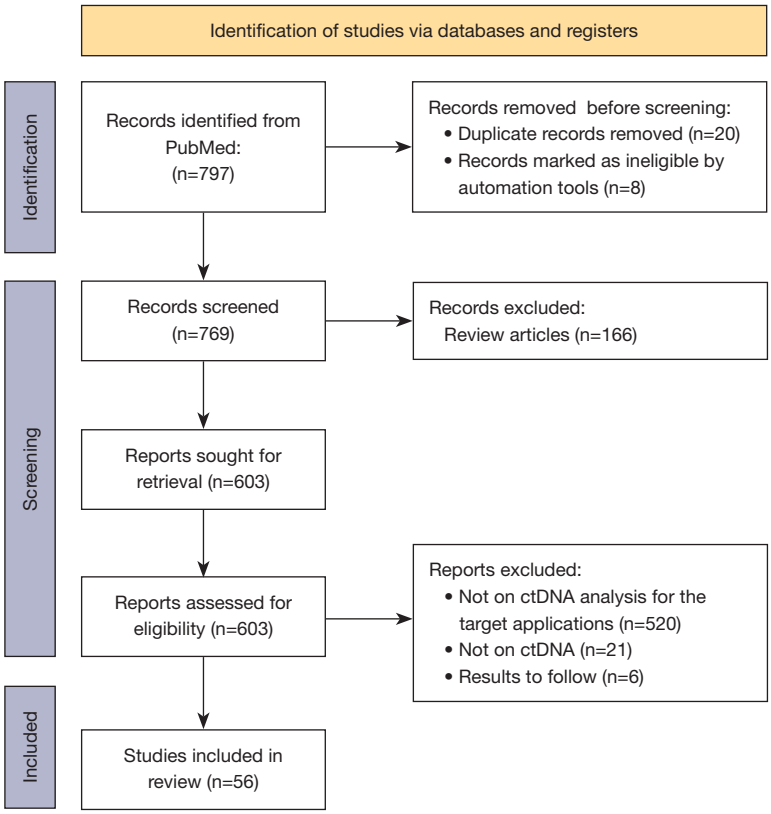

Figure 2 Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram on the study selection. ctDNA, circulating tumor DNA.

genes) in order to enhance sensitivity for lung cancer detection. In contrast to mutation analysis, methylation analysis of cfDNA offers the advantage to inform the tissue of origin based on the cancer type- and/or tissue-specific methylation profile and therefore favours the development of a pan-cancer early detection test (18). The performance of mutation-based or methylation-based analysis have been evaluated in both types of studies specifically designed for lung cancer and for pan-cancer detection. In general, for both modalities, lower sensitivities were reported for detection of early-stage cancer, which were below 50%. In a large-scale, case-control, pan-cancer detection study based on cfDNA methylation analysis, the sensitivities for stage I and II lung cancer were about 20% and 50%, and those for stage III and IV cancer were over 80%, at a specificity of 99.3% (validation data) (19).

As mentioned, 'fragmentomics' analysis of cfDNA is based on the pathophysiologically associated fragmentation patterns of cfDNA and it has been explored for cancer diagnostics (20). Such cfDNA fragmentomics analysis have also been evaluated for lung cancer detection (66,70-72,78-80,83). Cristiano *et al.* (70) and Mathios *et al.* (71). have developed a prediction score, known as the DELFI (DNA evaluation of fragments for early interception) score, based on fragmentation size and coverage characteristics in windows throughout the genome, as well as chromosomal arm and mitochondrial DNA copy numbers. Using the DELFI prediction score, they could achieve a sensitivity of about 60% and a specificity of about 80% for detection of early-stage (stage I and II) lung cancer in an independent validation cohort (70,71).

In order to enhance the diagnostic performance, some researchers have explored the potential synergistic effect of combining the analyses of different molecular features of cfDNA, circulating protein biomarkers and clinical features (8,9,14,33,34,56,63,66-69,73-78). In one study, Liu *et al.* demonstrated that the integrated model which incorporated the analyses of cfDNA mutation, methylation and protein biomarkers achieved the highest diagnostic performance than individual models with a single type of biomarker (8). Similarly, in the prospective liquid biopsy-based pan-cancer screening study, the cancer cases (not only limited to lung

Table 1 Studies on the use of ctDNA analysis for early detection of lung cancer

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
26854716	Szpechcinski *et al.*, 2016 (33)	Lung cancer	cfDNA concentration and integrity	cfDNA concentration and integrity (difference in concentrations in products from PCR with the 100-bp amplicon *vs.* the 400-bp amplicon)	N=65: stage I/II/IIIA =30/23/12, histological subtypes (LUAD/LUSC/others) =28/27/10	Benign lung tumours (N=28); healthy controls (N=16)	NSCLC cases *vs.* patients with benign pulmonary nodules and healthy individuals cfDNA concentration: sen./spec. =86.4%/61.4%, AUC =0.80 cfDNA integrity: sen./spec. =91%/68.2%, AUC not reported
29113814	Leng *et al.*, 2018 (34)	Lung cancer	cfDNA concentration and integrity	cfDNA concentration (ALU115) and integrity (ratio of ALU-115bp to ALU-247bp)	N=106: stage =NA, histological subtypes (NSCLC) =106	Tuberculosis (N=105); healthy controls (N=107)	NSCLC *vs.* healthy controls cfDNA concentration (ALU115): sen./spec. =57.5%/89.7%, AUC =0.747 cfDNA integrity: sen./spec. =79.2%/67.3%, AUC =0.759 NSCLC *vs.* tuberculosis cfDNA concentration (ALU115): sen./spec. =57.5%/64.8%, AUC =0.628 cfDNA integrity: sen./spec. =55.7%/82.9%, AUC =0.722
17449174	Gautschi *et al.*, 2007 (35)	Lung cancer	cfDNA variants	KRAS gene codon 12 mutation	N=180: stage I/II/III/IV =15/11/63/91, histological subtypes (LUAD/LUSC/large cell carcinoma/undifferentiated) =79/47/36/18	No control group	Sen. =9%
27377626	Fernandez-Cuesta *et al.*, 2016 (36)	Lung cancer	cfDNA variants	TP53 gene mutations	N=51: stage I/II/III/IV =7/7/28/9, histological subtype (SCLC) =51	Group 1: non-cancer controls (N=123) Independent control group: non-cancer controls (N=102)	SCLC *vs.* controls in Group 1: sen./spec. = 49%/88.6% SCLC *vs.* controls in independent control group: spec. =89.2%
27018799	Newman *et al.*, 2016 (37)	Lung cancer	cfDNA variants	292 predefined somatic mutation hotspots of 29 genes via iDES-enhanced CAPP-seq	N=24 (pretreatment NSCLC): stage IB/IIA/IIB/IIIA/IIIB/IV =3/2/1/4/2/12, histological subtypes (LUAD/NSCLC/LUSC/other/unknown) =16/1/1/1/5	Healthy controls (N=18)	Sen.: I–II/III/IV =~30%/~50%/~100% (estimated from the manuscript figure); spec. =94.4%
31069172	Tailor *et al.*, 2019 (38)	Lung cancer	cfDNA variants	10 pre-selected mutations	Malignant nodule (N=17): stage I/II/III/IV =8/2/5/2, histological subtypes (LUAD/LUSC/large-cell neuroendocrine carcinoma) =10/6/1	Benign nodule (N=16)	Sen./spec. =82.4%/100%
31100334	Savli *et al.*, 2019 (39)	Pan-cancer	cfDNA variants	Hostspot regions on 11 genes related (*ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, TP53* genes)	N=96: stage (unspecified), histological subtype (unspecified)	No control group	Sen. =84.4%
31739500	Peng *et al.*, 2019 (40)	Lung cancer	cfDNA variants	cfDNA copy number variation detection (*EGFR, ERBB2, MET* genes)	Group 1 (N=48): stage IIIB-IV, histological subtype (NSCLC) Group 2 (N=5980): stage (unspecified), histological subtype (NSCLC)	Group 1: healthy controls (N=10)	EFGR amplification: Group 1: sen./spec. =35%/100%; Group 2: sen. = 6.47% ERBB2 amplification: Group 1: sen./spec. =37.5%/100%; Group 2: sen. =1.56% MET amplification: Group 1: sen./spec. =40%/100%; Group 2: sen. =1.97%

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
32269342	Chabon *et al.*, 2020 (41)	Lung cancer	cfDNA variants	255 genes via CAPP-seq Lung Cancer Likelihood in Plasma (Lung-CLiP) model: an ensemble classification framework integrating the outputs of two constituent SNV and CNV models	Training group (N=104): stage IA/IB/IIA/IIB/IIIA/IIIB =21/28/12/16/17/10, histological subtypes (LUAD/LUSC/not specified/large cell carcinoma) =71/23/7/3	Training group (N=98): risk-matched controls (N=56)/low-risk controls (N=42)	Training group: stage I/II/III sen. (at 0.98 spec.) =0.41/0.54/0.67, stage I/II/III AUC =0.82/0.85/0.87
					Testing group (N=46): stage IA/IIB/IIA/IIB/IIIA/IIIB =22/10/0/9/2/3, histological subtype (LUAD/LUSC) =36/10	Testing group (N=48): risk-matched controls	Testing group: sen. (at 0.98 spec.): stage IA/IB/II/III =0.2/0.50/0.30/0.60 (estimated from the manuscript figure); stage I/II/III AUC =0.69/0.71/0.98
34217228	Qvick *et al.*, 2021 (42)	Lung cancer	cfDNA variants	197 genes (AVENIO ctDNA surveillance kit)	N=60: stage I–IIIa/IIIb–IV =19/41, histological subtype (LUAD/LUSC/SCLC) =37/15/8	Benign lung disease (N=16)	Significantly more variants detected in lung cancer cases compared to controls Significantly more variants were detected in patients with advanced stage disease (stage IIIb–IV)
34439258	Ris *et al.*, 2021 (43)	Pan-cancer	cfDNA variants	3,062 genomic variants	N=30: stage =NA, histological subtype =NA	Controls without known cancer diagnosis (N=415)	Sen. at 95% spec. =67% Sen. at 99% spec. =53%
25922721	Gao *et al.*, 2015 (44)	Lung cancer	cfDNA methylation markers	APC and RASSF1A gene methylation	N=58: stage I =58, histological subtype (LUSC/LUAD/undifferentiated/SCLC) =23/18/15/2	Benign lung disease (N=31); healthy controls (N=23)	cfDNA RASSF1A and/or APC methylation: lung cancer *vs.* benign lung disease: sen./spec. =56.9%/90.3%, AUC =0.81
26311076	Powrózek *et al.*, 2016 (45)	Lung cancer	cfDNA methylation markers	DCLK1 gene promoter methylation	N=65: stage (NSCLC): IIA/IIB/IIIA/IIIB/IV =3/4/12/10/17, stage (SCLC): III/IV =9/10; histological subtype (LUAD/LUSC/Large cell carcinoma/SCLC) =22/20/4/19	Healthy controls (N=95)	Sen./spec.: 49.2%/91.6%
27485611	Powrózek *et al.*, 2016 (46)	Lung cancer	cfDNA methylation markers	RTEL1 and PCDHGB6 gene promoter methylation	N=70: stage (NSCLC): I/II/IIIA/IIIB/IV =8/12/10/9/16, stage (SCLC): IIIB or IV =15; histological subtypes (LUAD/LUSC/SCLC) =25/30/15	Healthy controls (N=80)	Methylation of at least one out of studied genes (*RTEL1* and *PCDHGB6*) Sen./spec. =62.9%/90%, AUC =0.755
28855354	Ooki *et al.*, 2017 (47)	Lung cancer	cfDNA methylation markers	CDO1, HOXA9, AJAP1, PTGDR, UNCX, and MARCH11 genes	N=83: stage I =83, histological subtypes (LUAD/LUSC) =43/40	Healthy controls (N=42)	Sen.: LUAD/LUSC =72.1%/60%; spec.: 71.4%
30429608	Shen *et al.*, 2018 (48)	Pan-cancer	cfDNA methylation markers	Up to 2,100 DMRs selected for model training by cfMeDIP-seq	Training group (N=25): stage (III–IV/unknown =22/3, histological subtype =NA	Training group: healthy controls (N=24)	Training group: all lung cancers *vs.* healthy controls and other cancers: AUC =0.8 (estimated from the manuscript figure)
					Testing group (N=55): stage (I–II/III–IV =32/23, histological subtype =NA	Testing group: healthy controls (N=62)	Testing group: early-stage LCs (N=32): AUC =0.975; late-stage LCs (N=23): AUC =0.966
31436249	Zhao *et al.*, 2019 (49)	Lung cancer	cfDNA methylation markers	RUNX3 and RASSF1A genes	N=58: stage: malignant small SPN ≤10 mm; histological subtype (unspecified)	Benign SPNs (N=89)	Significantly higher rate of serum *RUNX3* and *RASSF1A* gene methylation in malignant SPNs than that in benign SPNs: 65.5% *vs.* 12.3%, and 67.2% *vs.* 10.1%, respectively
30516882	Yang *et al.*, 2019 (50)	Lung cancer	cfDNA methylation markers	8 genes: CDH13, WT1, CDKN2A, HOXA9, PITX2, CALCA, RASSF1A, and DLEC1	N=39: stage: early stage =39, histological subtypes =NA	Non-lung cancer patients (N=11)	Methylation of any of the 8 genes sen./spec. =72%/91%

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
31037156	Liang *et al.*, 2019 (51)	Lung cancer	cfDNA methylation markers	9 DMRs were selected after training	Training group (N=40): stage: unspecified, histological subtypes: NA	Training group: benign samples (N=26)	9 DMRs: training group: AUC =0.839
					Testing group (N=39): stage IA/IB/IIA/ Later stages/unknown =20/7/1/10/1, histological subtypes =NA	Testing group: benign samples (N=27), age and gender matched normal plasma samples (N=118)	Testing group: sen./spec.: 79.9%/85.2%, AUC =0.816 Lung cancer cases *vs.* age and gender matched normal plasma samples (N=118): spec. =93.2%
31888670	Zang *et al.*, 2019 (52)	Lung cancer	cfDNA methylation markers	IDH1, SHOX2 and PTGER4 genes	Training group (N=115): stage =NA, histological subtypes (LUAD/LUSC/ SCLC/others) =90/14/9/2	Training group: healthy controls (N=55)	Training group: sen./spec. =86.1%/67.3%, AUC =0.835
					Testing group (N=35): stage =NA, histological subtypes (LUAD/LUSC/ SCLC/others) =22/5/6/2	Testing group: Healthy controls (N=16)	Testing group: sen./spec. =80.0%/87.5%, AUC =0.905
32138766	Chen *et al.*, 2020 (53)	Lung cancer	cfDNA methylation markers	*CDO1, TAC1, SOX17, HOXA7, HOXA9, GATA4*, *GATA5*, and *PAX5* genes	N=163: stage =NA, histological subtype (LUAD/LUSC/NOS) =139/22/2	Non-cancerous lesions (N=83)	Combination of *CDO1, SOX17*, and *HOXA7* genes methylation: sen./spec. =90%/71%, AUC =0.88
31775567	Vrba *et al.*, 2020 (54)	Lung cancer	cfDNA methylation markers	*MIR129-2, LINC01158, CCDC181, PRKCB, TBR1, ZNF781, MARCH11, VWC2, SLC9A3, HOXA7* genes	N=18: stage I/II/III/IV =5/3/2/8, histological subtype (LUAD/LUSC) =15/3	Healthy controls (N=47)	10 DNA methylation biomarkers: sen. 83% at 95% spec., AUC =0.956; 5 DNA methylation biomarkers subgroup: AUC =0.97
33506766	Liu *et al.*, 2020 (19)	Pan-cancer	cfDNA methylation markers	103,456 distinct regions (17.2 Mb in the genome) covering 1,116,720 CpGs	The CCGA study (training group) (N=260): stage (I/II/III/IV =59/23/72/106, histological subtypes: NA	CCGA study (training group): healthy controls (N=1,521)	CCGA study (training group): sen.: I/II/III/IV =~20%/~80%/~87%/~90% (estimated from the manuscript figure); spec. =99.8%
					Testing group (N=111): stage (I/II/III/IV =27/11/31/42, histological subtypes: NA	Testing group: healthy controls (N=610)	Testing group: sen.: I/II/III/IV =~24%/~48%/~82%/~88% (estimated from the manuscript figure); spec. =99.3%
34176681	Klein *et al.*, 2021 (18)	Pan-cancer	cfDNA methylation markers	103,456 distinct regions (17.2 Mb in the genome) covering 1,116,720 CpGs	N=404: stage I/II/III/IV/NA =96/44/118/145/1, histological subtypes: NA	Non-cancer (N=1,254)	Lung cancer (N=404) *vs.* non-cancer (N=1,254): Sen.: I/II/III/IV/missing =21.9%/79.5%/90.7%/95.2%/100%, spec. =99.5%
							Lung cancer *vs.* other cancer types: sen.: 91.7% (220/240)
34131323	Liang *et al.*, 2021 (55)	Lung cancer	cfDNA methylation markers	80,672 CpG sites which were segregated into 8,312 blocks (2,473 blocks used for ctDNA analysis)	Training-testing group (N=140): stage IA/IB/II/III =74/16/33/17, histological subtypes (LUAD/LUSC/others) =109/22/9	Training-testing group: healthy controls (N=124)	Training-testing group: sen./spec. =69%/96%, AUC =0.93
					Single-blind testing group (N=168): stage IA/IB/II/III =83/26/33/16, histological subtype (LUAD/LUSC/others) =126/34/8	Single-blind testing group: healthy controls (N=122)/benign (N=15)	Single-blind testing group: sen./spec. =58%/ (normal controls: 97% and benign patients: 93%), AUC =0.90
32516173	Ponomaryova *et al.*, 2021 (56)	Lung cancer	cfDNA concentration and cfDNA methylation markers	cfDNA concentration (LINE-1) and cfDNA methylation markers (LINE-1)	N=23: stage I/II/III =1/5/17, histological subtype (LUSC/LUAD) =13/10	Healthy controls/COPD/ bronchitis =16/15/16	LINE-1 index of methylation: lung cancer *vs.* joint control group: sen./spec. =64.3%/94.4%, AUC =0.832

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
34251068	Qi *et al.*, 2021 (57)	Lung cancer	cfDNA methylation markers	Top 300 DMR identified via cfMeDIP-seq	Lung cancers (tumor size >3 cm, N=32): stage: NA (tumor size >3 cm), histological subtype (LUAD/invasive LUAD/LUSC/SCLC) =18/2/9/3	Healthy individuals without pulmonary nodules (N=7)/benign pulmonary nodules (N=23)	Lung cancers (tumor size >3 cm, N=32) *vs.* healthy individuals without pulmonary nodules (N=7): sen./spec. =100%/100%
					Malignant pulmonary nodules detected by CT scan (nodule size <3 cm, N=35): stage: NA; LUAD/adenocarcinoma in situ/Invasive LUAD/micro-invasive LUAD/poor-differential carcinoma/LUSC =9/5/10/7/1/3		All cases of pulmonary malignant tumors (N=67) *vs.* controls (N=30): sen./spec. =91.0%/93.3%, AUC =0.963
35450968	Magenheim *et al.*, 2022 (58)	Lung cancer	cfDNA methylation markers	17 loci with lung-specific methylation patterns	N=26: stage III–IV, histological subtype: NSCLC NOS/LUAD/poorly differentiated carcinoma/LUAC/SCLC =1/15/1/8/1	Healthy controls (N=30)	AUC =0.835
34830765	Huang *et al.*, 2021 (59)	Pan-cancer	cfDNA methylation markers	3 selected DMCGIs	cfMBD-seq group (N=12): stage III–IV =12, histological subtypes: LUAD/Others =9/3	cfMBD-seq group: non-lung cancer (N=16)	cfMeDIP-seq group (3 DMCGIs): lung cancer *vs.* non cancer: AUC =0.949
					cfMeDIP-seq group (N=80): stage I–II/III–IV/unknown =3/22/55, histological subtypes: NA	cfMeDIP-seq group: non-lung cancer (N=86)	
35126793	Zhao *et al.*, 2022 (60)	Lung cancer	cfDNA methylation markers	10-DMR marker panel	CfDNA group1 (MethylationEPIC data) (N=4): stage: unspecified, histological subtype: NSCLC	cfDNA group1 (MethylationEPIC data): healthy controls (N=12);	cfDNA group2 (10-DMR marker panel): sen./spec. =92%/92.3%, AUC =0.922
					CfDNA group2 (RRBS data) (N=29): stage: unspecified, histological subtype: unspecified	CfDNA group2 (RRBS data): healthy controls (N=74)	
35538556	Markou *et al.*, 2022 (61)	Lung cancer	cfDNA methylation markers	5 selected gene promoters (*APC, RASSFIA1, FOXA1, SLFN11, SHOX2* genes)	N=42: stage IA–IIIA, histological subtype (LUAD/LUSC/undfferentiated carcinoma) =14/24/4	Healthy controls (N=12)	Methylation of at least one gene promoter (*APC, RASSFIA1, FOXA1, SLFN11, SHOX2*): sen./spec. =33.3%/83.3%
28820176	Song *et al.*, 2017 (62)	Pan-cancer	cfDNA 5hmC markers	2082 differentially hydroxymethylated genes	N=15: stage I–II/III–IV/NA =3/10/2, histological subtypes (NSCLC/SCLC) =14/1	Healthy controls (N=8)	A progressive global loss of 5hmC enrichment was observed from healthy subjects to early stage non-metastatic lung cancer to late stage metastatic lung cancer
30010036	Zhang *et al.*, 2018 (63)	Lung cancer	cfDNA 5hmC markers	cfDNA 5hmC markers (2459 differential 5hmC genes) (7 serum protein markers (CEA, AFP, CA19-9, CA15-3, CA125, NSE, CYFRA21-1) for comparison)	N=66: stage I/II/III/IV/NA =26/17/18/1/4, histological subtypes (LUAD/LUSC/adenosquamous carcinoma) =46/17/3	Healthy controls (N=67)	Training group (51 tumors and 42 healthy controls): AUC =0.927
							Testing group (17 lung cancers and 24 healthy controls): AUC =0.96
32694610	Chen *et al.*, 2020 (64)	Pan-cancer	cfDNA 5hmC markers	477 cancer-specific DMRs (associated with 657 genes and 10,613 CpG sites) for training	'Pre-diagnosis' lung cancer patients (cancer diagnosis not yet confirmed at the time of blood collection): N=24	Healthy controls (N=207)	Pre-diagnosis lung cancer patients: sen. =96%
					'Post-diagnosis' lung cancer patients (cancer diagnosis already confirmed at the time of blood collection): N=28		Post-diagnosis lung cancer patients: sen. =96%, spec. =96.1%
					Stage: NA		
					Histological subtypes: NA		

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
34689838	Zhou *et al.*, 2021 (65)	Pan-cancer	cfDNA 5hmC markers	39 tissue-shared 5hmC-modified lncRNA gene markers	N=66: stage I/II/III/IV/NA:26/17/18/1/4, LUAD/LUSC/adenosquamous carcinoma =46/17/3	Healthy controls (N=67)	5hmC-LncRNA diagnostic score (5hLD-score) model based on the 39 markers Lung cancer *vs.* healthy controls: AUC =0.851
35073982	Hu *et al.*, 2022 (66)	Lung cancer	cfDNA 5hmC markers and cfDNA fragmentomics patterns	37 5hmC markers and cfDNA fragmentomics patterns	N=157: stage I/II/III/IV/NA: 3/9/49/82/14, histological subtypes: LUAD/LUSC/SCLC/Others =62/48/25/22	Healthy controls (N=189)	37 5hmC biomarkers Testing group (48 lung cancers *vs.* 62 healthy controls): sen./spec. =87.50%/83.87%, AUC =0.89 External testing group (66 lung cancers *vs.* 67 controls from Zhang *et al.*, 2018): sen./spec. =72.7%/80.6%, AUC =0.85 Fragmentation profiles (48-feature fragmentation model) Training group (109 lung cancers *vs.* 127 healthy controls): sen./spec. =91.74%/93.70%, AUC =0.9837 Testing group (48 lung cancers *vs.* 62 healthy controls): sen./spec. =87.50%/80.65%, AUC=0.9257 External testing group (66 lung cancers *vs.* 67 controls from Zhang *et al.*, 2018): sen./spec. =78.79%/76.12%, AUC =0.822 Integration of 5hmC features and fragmentation profiles Training group (109 lung cancers *vs.* 127 healthy controls): AUC =1 Testing group (48 lung cancers *vs.* 62 healthy controls): sen./spec. =87.50%/90.30%, AUC =0.9432 External testing group (66 lung cancers *vs.* 67 controls from Zhang *et al.*, 2018): sen./spec. =83.33%/77.61%, AUC =0.8639
26425700	Wielscher *et al.*, 2015 (67)	Lung cancer	cfDNA methylation markers and cfDNA concentration	63 methylation markers and total cfDNA concentration	N=33: stage I&II/III&IV/unknown =9/15/9, histological subtypes (LUAD/LUSC/SCLC/large cell lung cancer) =11/8/7/7	N=171: healthy control/COPD 0/COPD/ILD =27/34/42/68	Integrated model using methylation of 63 candidate loci and cfDNA amounts Original group: Lung cancer *vs.* healthy controls: sen./spec. =0.82/0.89, AUC =0.91 Lung cancer *vs.* non-cancer and healthy controls: sen./spec. =87.8%/90.2% Lung cancer *vs.* ILD: spec. =88% Lung cancer *vs.* COPD: spec. =88%

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
27544059	Weiss *et al.*, 2017 (68)	Lung cancer	cfDNA methylation markers and protein markers	cfDNA methylation markers [SHOX2 and PTGER4] and protein markers (CYFRA 21-1, CEA, SCC and NSE genes)	Training group (N=117): stage I/II/III/IV/unknown =26/21/42/24/4, histological subtype (LUAD/LUSC/other/SCLC) = 46/58/8/5	Training group: healthy control (N=212)	Methylation of SHOX2 and PTGER4 Training group: AUC =0.93
					Testing group (N=50): stage I/II/III/IV =12/11/16/11, histological subtype (LUAD/LUSC/other/SCLC) =18/25/7/0	Testing group: patients with nonmalignant lung disease (N=50)/healthy patients (N=72)	Testing group: Lung cancer (N=50) *vs.* nonmalignant lung disease (N=50) and healthy controls (N=72): AUC =0.88 Lung cancer (N=50) *vs.* nonmalignant disease (N=50): AUC =0.86 Lung cancer (N=50) *vs.* healthy controls (N=72): AUC =0.91 Protein panel (CYFRA 21-1, CEA, SCC and NSE) Subgroup of training group (59 lung cancer cases *vs.* 92 healthy controls): AUC =0.79
33901468	Park *et al.*, 2021 (69)	Pan-cancer	cfDNA methylation markers, cfDNA concentration and integrity	LINE-1 hypomethylation, Alu cell-free DNA concentration, Alu index	N=64: stage T0/T1/T2/T3 =1/27/31/5, histological subtypes: unspecified	Healthy controls (N=64)	Combination of Alu index, LINE-1 methylation level, and Alu longer and shorter fragments DNA concentrations Lung cancer *vs.* healthy controls: sen./spec. =89.06%/82.81%, AUC =0.895
31142840	Cristiano *et al.*, 2019 (70)	Pan-cancer	cfDNA fragmentomics patterns	cfDNA fragmentomics patterns (DELFI score)	N=12: stage I/II =3/9, histological subtypes: LUAD/adenosquamous carcinoma/LUSC =9/1/2	Healthy controls (N=215)	DNA evaluation of fragments for early interception (DELFI) score utilizing fragmentation patterns Lung cancer *vs.* healthy controls: sen./spec. =100%/98%, AUC =1
34417454	Mathios *et al.*, 2021 (71)	Lung cancer	cfDNA fragmentomics patterns, protein marker and clinical information	cfDNA fragmentomics patterns, CEA, age, smoking history, and presence of COPD	LUCAS group (N=129): stage IA/IB/IIA/IIB/IIIA/IIIB/IIIC/IV =11/4/2/5/17/15/3/72, histological subtypes (LUAD/LUSC/SCLC/adenosquamous/NSCLC, NOS/mixed small cell and NSCLC/mesothelioma/neuroendocrine/metastasis from other organ/unknown) =62/29/11/3/3/1/1/1/15/3	LUCAS group: non-cancer (N=239, including 158 patients with no prior, baseline, or future cancers); independent testing group: non-cancer (N=385)	DNA evaluation of fragments for early interception (DELFI) score utilizing fragmentation profiles LUCAS group: lung cancer (N=129) *vs.* healthy controls (N=158): AUC =0.90 (stage I/II/III/IV =0.76/0.89/0.92/0.92)
					Independent testing group (N=46): LUAD/large cell/LUSC/adenosquamous/small cell/mixed small cell and NSCLC =27/9/7/1/1/1		Independent testing group: lung cancer (N=46) *vs.* healthy controls (N=358): sen. (I/II/III–IV) =0.57/0.6/1 (estimated from manuscript figure) DELFImulti: multimodal model combining genome-wide cfDNA fragmentation features with CEA levels, age, smoking history, and presence of COPD LUCAS group: overall AUC =0.93 (I/II/III/IV =0.78/0.95/0.94/0.95) DELFImulti approach followed by LDCT: lung cancer (N=129) *vs.* healthy controls (N=158): spec. =94% at sen. of 80%

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
35690859	Bao *et al.*, 2022 (72)	Pan-cancer	cfDNA fragmentomics patterns	cfDNA fragmentomics patterns (FSC, FSD, EDM, BPM) and CNV	Training group (N=146): stage I =146, histological subtype: LUAD Testing group (N=146): stage I =146, histological subtype: LUAD	Training group: healthy controls (N=122) Testing group: healthy controls (N=121)	Testing group: model using the 5 features sen./spec. =90.4%/95.0%, AUC =0.973
27555497	Chen *et al.*, 2016 (73)	Lung cancer	cfDNA variants, clinical features, total cfDNA concentration, and serum protein markers	cfDNA variants [50 genes], cfDNA concentration, clinical features and serum protein markers (CA125, CA19-9, CEA, CYFRA21-1, NSE)	N=58: stage IA/IB/IIA =30/16/12, histological subtype (LUAD/LUSC) =51/7	Disease-free individuals (N=4)	cfDNA analysis only: Sen. = 89.7% Serum protein marker only: Sen. = 43.1%
28814544	Phallen *et al.*, 2017 (74)	Pan-cancer	cfDNA variants and cfDNA concentration	cfDNA variants (58 cancer-related genes) and total cfDNA concentration	N=71: stage I/II/III/IV =29/32/4/6, histological subtype (LUAD/adenosquamous carcinoma/carcinoma/large cell carcinoma/small cell-large cell adenocarcinoma/SCLC/LUSC) =38/4/1/7/1/1/19	Healthy controls (N=44)	cfDNA mutation (58 cancer related genes) only: sen. (I/II/III/IV): 44.83%/71.88%/75.00%/83.33% cfDNA concentration only: significantly higher concentration of cfDNA in plasma from 65 lung cancer patients than healthy individuals
29348365	Cohen *et al.*, 2018 (75)	Pan-cancer	cfDNA variants and circulating protein markers	CancerSEEK combining cfDNA variants and circulating protein markers: 16 genes (*NRAS, CTNNB1, PIK3CA, FBXW7, APC, EGFR, BRAF, CDKN2A, PTEN, FGFR2, HRAS, KRAS, AKT1, TP53, PPP2R1A, GNAS* genes), 8 protein biomarkers (CA125, CA19-9, CEA, HGF, myeloperoxidase, OPN, prolactin, TIMP-1)	N=104: stage I/II/III =46/27/31, histological subtype: NSCLC (N=103)/SCLC (N=1)	Healthy controls (N=812)	Performance for lung cancer detection (estimated from the manuscript figure): sen. =~58% (at spec. of 99%)
30981987	Peng *et al.*, 2019 (76)	Lung cancer	cfDNA variants, protein markers and clinical features	cfDNA variants [65 lung cancer-related genes], 6 protein markers (NSE, CYFRA 21–1, CEA, ProGRP, CA-125 and SCC), clinical features and patient age]	N=136: stage I/II/III/IV =87/29/17/4, histological subtype (LUAD/LUSC/SCLC/others) =100/28/1/7	Non-lung cancer patients (N=56)	Combination of cfvariants, serum protein markers and patient age: sen./spec. =80%/99%
32345712	Lennon *et al.*, 2020 (9)	Pan-cancer	cfDNA variants and circulating protein markers	cfDNA variants and circulating protein markers: 16 genes (*AKT1, APC, BRAF, CDKN2A, CTNNB1, EGFR, FBXW7, FGFR2, GNAS, HRAS, KRAS, NRAS, PIK3CA, PPP2R1A, PTEN, TP53* genes); 9 protein biomarkers (AFP, CA125, CA15-3, CA19-9, CEA, HGF, OPN, Prolactin, TIMP-1)	Prospective recruitment of participants with no known history of malignancy (N=9,911)	Non-cancer subjects in the prospective group	Out of 134 screen-positive subjects (defined as either positive for ctDNA or protein biomarkers), 9 lung cancer cases were identified (Stage I/II/III/IV distribution=1/2/2/4) Another 12 lung cancer cases were identified from the group within the same study period Sen. = 42.9% (9/21) Spec. (for detection of all type of cancer) = 98.9%
33514352	Chen *et al.*, 2021 (14)	Lung cancer	cfDNA variants, cfDNA methylation markers and serum protein	cfDNA variants: weighted summation allele fractions of all variants cfDNA methylation markers: regional methylation ratio of 54 selected DMRs serum CEA level	N=128: stage 0/IA/IB/II/III/IV =2/54/29/17/19/7, histological subtype (LUAD/LUSC/large cell carcinoma/SCLC) =97/23/3/5	Benign lung nodule (BLN) patients: (N=94)	Best performance for the combined analysis of cfDNA mutation, methylation and serum CEA: sen. =76.9%, spec. =58.3%, AUC =0.78

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
34258160	Liu *et al.*, 2021 (8)	Lung cancer	cfDNA variants, cfDNA methylation markers, clinical features and protein cancer biomarkers	cfDNA variants [29 genes]; 8 protein markers (CA125, CA 15-3, CEA, CYFRA 21-1, NSE, PROGRP, SCC, and SF) 30 methylation-correlated blocks; 10 clinical features	Discovery group (N=70): stage Ia/Ib/IIb/IIIa/IIIb =19/41/4/4/2, histological subtype: carcinoid/LUSC/large cell carcinoma/LUAD =1/6/2/3/58	Discovery group: benign (N=28)	An integrative multianalytical machine learning model based on patient clinical features, cfDNA mutation, cfDNA methylation, and protein cancer biomarkers:
					Independent testing group (N=15): stage Ia/Ib =6/9, histological subtype: LUAD	Independent testing group: benign (N=14)	Discovery group: AUC =0.85 Testing group: sen./spec. =80%/85.7%, AUC =0.86
34800919	Metzenmacher *et al.*, 2022 (77)	Lung cancer	cfDNA variants and methylation markers	cfDNA methylation markers-methylation of a CpG locus (cg03287111) on the GLI2 gene body (variants of KRAS, BRAF, and EGFR have also been analysed)	N=109: stage I–III/IV = 48/61, histological subtype: NSCLC	Healthy controls (N=39)	cfDNA methylation (only) by AUC =0.94 for early-stage cases; AUC =0.96 for late-stage cases
36379302	Kim *et al.*, 2022 (78)	Lung cancer	cfDNA methylation markers and cfDNA fragmentomics	6,243 lung-tumor-specific CpG markers; Short fragment ratio (100–150 bp/151–220 bp); 5' end-motif profile;	Model training and testing group (N=139): stage I/II/III/IV=19/23/35/62, histological subtype: NSCLC	Healthy controls (N=97)	TOF score which is based on an ensemble learning model using the logistic regression of three features, ctDNA methylation candidate count, end-motif, and short fragment ratio: test group in the model training and testing group: (40 NSCLC *vs.* 30 healthy): sen./spec.=95%/96.7%, AUC =0.98
					Independent testing group (N=62): stage: "limited stage"/"extensive stage" =21/36, histological subtype: SCLC		
36346614	Wang *et al.*, 2023 (79)	Lung cancer	cfDNA fragmentomics patterns	CNV; FSC; FSD; 6bp EDM; 6bp BPM	Training group (N=113): stage I/II/III/IV =66/26/20/1, histological subtype: LUAD/LUSC =96/17	Healthy controls: training group (N=113)	Stacked ensemble model using five features (CNV, FSC, FSD, EDM, and BPM):
					Testing group I (N=81): stage I/II/III/IV =46/16/16/3, histological subtype (LUAD/LUSC) =66/15;	Testing group I (N=47)	Testing group I: sen./spec. =91.4%/95.7%, AUC =0.984
					Testing group II (N=118): stage I/II/III =85/32/1, histological subtype (LUAD) =118	Testing group II (N=70)	Testing group II: sen./spec. =84.7%/98.6%, AUC =0.987
					Additional testing group (N=120): stage I/II/III/IV =35/26/28/31, histological subtype (LUAD/LUSC) =105/15	Additional testing group(N=120)	Additional testing group: sen./spec. =92.5%/94.2%, AUC =0.974
35780566	Guo *et al.*, 2022 (80)	Lung cancer	cfDNA fragmentomics patterns	6bp BPM	Training group (N=150): stage I, histological subtype: LUAD	Healthy controls: training group (N=115)	Logistic regression model of 6bp breakpoint motifs:
					Internal testing group (N=102): stage I, histological subtype: LUAD	Internal testing group (N=75)	Internal testing group: sen./spec. =98.0%/94.7%, AUC =0.985
					External testing group (N=40): stage I, histological subtype: LUAD	External testing group (N=40)	External testing group: sen./spec. =92.5%/90.0%, AUC =0.954

Table 1 (*continued*)

Table 1 (*continued*)

PMID	Study (references)	Cancer detection	cfDNA property and other types of biomarkers	Details of cfDNA properties and other biomarkers	Lung cancer patients	Controls	Finding: sen. (sensitivity), spec. (specificity), AUC, etc.
36175411	Stackpole *et al.*, 2022 (81)	Pan-cancer	cfDNA methylation markers	23,748 cancer-specific hypermethylation markers, 28,197 cancer-specific hypomethylation markers, 7,547 tissue-specific hypermethylation markers, 7,212 tissue-specific hypomethylation markers	Lung cancer (N=126), other cancers (N=150); stage I/II/III/IV =33/23/30/40, histological subtype (LUAD/LUSC) =77/49	Non-cancer (healthy individuals and patients of various noncancer diseases (e.g., cirrhosis, pancreatitis, hepatitis, diabetes, etc.), N=203)	Integrating four marker types (all cancer types (n=217) *vs.* noncancer (n=191): sen./spec.=80.7%/97.9%, AUC =0.974
35804466	Zhang *et al.*, 2022 (82)	Pan-cancer	cfDNA methylation markers	173 pre-selected ribosomal DNA CpG sites	Lung cancer (N=29): stage: NA, histological subtype: NA	Healthy controls (N=75)	173 pre-selected ribosomal DNA CpG sites: AUC =0.84
35361996	Esfahani *et al.*, 2022 (83)	Lung cancer	cfDNA fragmentomics patterns	cfDNA fragmentomics patterns (promoter fragmentation entropy)	Training group (N=67): stage II/III/IV =7/30/30, histological subtype: NSCLC Testing group(N=20): histological subtype: NSCLC	Noncancer: training group (N=71), testing group (N=23)	Predicted RNA expression by the promoter fragmentation entropy of 144 TSS sites from 117 genes: training group: AUC =0.91, testing group: AUC =0.83

ctDNA, circulating tumour DNA; cfDNA, cell-free DNA; PCR, polymerase chain reaction; AUC, area under the curve; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung carcinoma; NA, not applicable; SCLC, small cell lung carcinoma; DMRs, differentially methylated regions; cfMeDIP, cell-free methylated DNA immunoprecipitation sequencing; SPN, solitary pulmonary nodule; LCs, lung cancers; NOS, not otherwise specified; CpGs, cytosine-guanine sites; CCGA, Circulating Cell-free Genome Atlas; LINE-1, long interspersed nuclear elements-1; DMCGIs, differentially methylated CG islands; CEA, carcinoembryonic antigen; AFP, alpha fetoprotein; CA19-9, carbohydrate antigen 19-9; CYFRA21-1, cytokeratin fragment antigen 21-1; ILD, interstitial lung disease; SCC, squamous cell carcinoma; DELFI, DNA evaluation of fragments for early interception; COPD, chronic obstructive pulmonary disease; LUCAS, lung cancer screening study; FSC, fragmentation size coverage; FSD, fragmentation size distribution; EDM, end motif; BPM, breakpoint motif; CNV, copy number variation; HGF, hepatocyte growth factor; OPN, osteopontin; TIMP-1, tissue Inhibitor of metalloproteinase-1; PROGRP, progastrin-releasing peptide; SF, serum ferritin.

cancer) identified were either ctDNA biomarker-positive or protein biomarker-positive except one case (9). This finding might suggest the advantage of a multi-modality testing approach. At the same time, it would also be interesting to study the difference in tumour characteristics (84), if any, between those that are ctDNA biomarker positive versus protein-biomarker positive.

Complementary diagnostic role with low-dose computed tomography (LDCT)

LDCT was proven to be an effective screening tool for lung cancer but remained underutilized (6,7). The clinical role of liquid biopsy to complement LDCT for lung cancer screening might be partly addressed in one recently published study by Mathios *et al.* (71) and another aforementioned prospective pan-cancer screening study (9). In the prospective pan-cancer screening study, 9,911 asymptomatic participants with no known history of malignancies had received blood testing involving 9 protein biomarkers and cfDNA targeted sequencing for mutation analysis. In addition to the 3 cancer cases picked up by LDCT (unknown total number of participants who underwent LDCT), 9 more lung cancer cases (1 at stage I; 2 at stage II; 2 at stage III and 4 at stage IV) were identified by the blood test (9). In another study by Mathios *et al.* (71), the researchers have modelled the performance of incorporating ctDNA analysis (based on plasma DNA fragmentation and also clinical information) to select high-risk subjects for subsequent LDCT. These attempts were aimed to improve the low adherence rate of LDCT. All these would suggest the potential complementary role of ctDNA-based (or liquid biopsy-based) test to the standard modality for cancer screening.

Emerging applications

Leveraging the ability of NGS in untargeted, multiplex detection of ctDNA, multi-cancer detection represents a reasonable and promising next step in the development of the technology. For instance, Klein *et al.* (18) and Liu *et al.* (19) employed detection of methylation markers in cfDNA for detection and differentiation of tissue of origin, with diagnostic performances comparable to single-cancer detection. This represents an attractive approach which improves cost-effectiveness via simultaneous detection of multiple malignancies.

Discussion

In cancer management, applications of ctDNA analysis such as therapeutic prediction and disease monitoring (in cases with known driver mutations) have already been adopted for routine clinical uses (85-87). However, challenges remain in early detection of lung cancer without common driver mutations, in which defining a tumour-specific genetic/epigenetic alteration as a biomarker would be more costly and less readily available. Ascertaining their clinical utilities necessitates large-scale clinical studies and integration with the diagnostic and/or management flow, which require significant time and resources. Nevertheless, ctDNA analysis could potentially address the unmet clinical need of early detection in lung cancer. This has prompted us to conduct this systematic review to evaluate the performance of the different ctDNA analysis techniques reported for lung cancer detection.

As reviewed in this systematic review, the design of studies is very heterogenous. The high heterogeneity may affect the generalizability of the studies' conclusions to routine clinical settings. Although the objectives of most studies were aimed to evaluate ctDNA for early detection of lung cancer, the study populations of some studies were usually made up of large proportions of cases at advanced stages, while some studies did not specify the proportion of cases at each clinical stage. Tests tend to be more accurate at differentiating patients at advanced stages from healthy subjects. Performance metrics calculated from a population with the majority having advanced diseases tend to be an over-estimate when applied to screening populations, where a large proportion of subjects are healthy or at early stages of disease.

Various methodologies have been used for detection and quantitation of specific molecular features of ctDNA. cfDNA methylation/hydroxymethylation profiling and mutation detection in a panel of genes represent approaches unlimited to exclusive detection of cancers with specific driver mutations. The differing approaches confer different sensitivities and specificities to the analyses, compromising comparability between studies and generalizability of their conclusions. As the field evolves and the repertoire of analytical techniques expands, complexity in the issue is expected to further increase and further validation across different populations would be required. In addition, ctDNA analysis is highly influenced by pre-analytical conditions as mentioned, including blood tubes, storage conditions, DNA extraction methods etc. Standardization and cross-method comparisons would be required to confirm the performance as reported (88-90).

Similarly, studies have utilized various bioinformatics tools to detect the mutational status of target genes and the methylation status of target genes or genomic regions. In addition, various machine learning models have been built to differentiate lung cancer patients and healthy subjects based on various cfDNA properties. High sensitivity and specificity were reported in some of the studies. However, the majority of these studies had relatively small training cohorts. A machine learning model generated from training groups of a larger scale with external validation would better reflect the performance in separating lung cancer patients from both healthy subjects and subjects with other cancer types.

Studies have also investigated the utility of cfDNA analysis for detection of multiple types of cancers (9,18,19,39,43,48,53,59,62,65,69,70,72,74,75,81,82). While this could represent an approach that is expected to be more cost-effective, further studies are needed to address the manner in which the testing could be integrated to current workflows.

One study limitation is that only one search engine is included for the search of eligible studies, though most studies that analysed the different ctDNA markers should have been covered.

Conclusions

ctDNA demonstrates great potential for early detection of lung cancer. While various analytical methodologies have shown promise for clinical translation, validation in prospective cohorts is necessary to confirm the performance. This would help defining the target screening population and integration into clinical care.

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Footnote

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