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Circulating tumor DNA in the diagnosis of ovarian cancer: a systematic review

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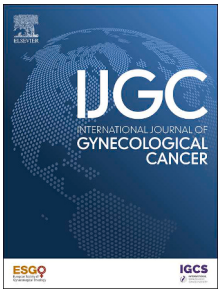
23 April 2026

ORIGINAL RESEARCH

Circulating tumor DNA in the diagnosis of ovarian cancer: a systematic review



Cristina Taliento[a,b], Pantaleo Greco[a], Giulia Bruni[a], Ina Marie Dueholm Hjorth[c,d], An Coosemans[e], Wouter Froyman[b,f], Dirk Timmerman[b,f], John Charles Rotondo[g], Chiara Mazziotta[a,h], Martina Arcieri[i], Stefano Restaino[i], Francesco Multinu[j], Giuseppe Vizzielli[i,k], Carlotta Giorgi[a], Lars Dyrskjøt[c,l], Paolo Pinton[a,*], Giampaolo Morciano[a,m]

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ABSTRACT

Objective: Ovarian cancer remains a leading cause of gynecologic cancer mortality worldwide, largely due to late-stage diagnosis and limited early detection tools. Circulating tumor DNA (ctDNA) has emerged as a promising non-invasive biomarker with the potential to improve diagnostic accuracy through detection of tumor-specific genetic and epigenetic alterations.

Methods: This systematic review aimed to evaluate the diagnostic accuracy of ctDNA in detecting ovarian cancer compared to healthy controls or benign conditions. A comprehensive literature search was conducted across PubMed, Web of Science, and EBSCO databases through April 2024, including studies that assessed sensitivity, specificity of ctDNA assays in plasma or serum samples. Risk of bias was assessed using the Quality Assessment of Diagnostic Accuracy Studies-2 tool. PROSPERO registration number: CRD42024590089.

Results: Nineteen studies met inclusion criteria, employing a variety of molecular techniques including polymerase chain reaction-based methylation assays (73.7%) and sequencing methods (whole genome sequencing/next-generation sequencing) (21%), targeting single genes or multi-gene panels. Diagnostic accuracy of ctDNA varied, with sensitivity (40.6%-94.7%) and specificity (56%-100%) ranging broadly, but often outperforming CA125, particularly in early-stage. Concordance between ctDNA and tumor tissue ranged from moderate ($r = 0.428$) to strong ($r = 0.771$).

Conclusions: Although heterogeneity across studies precluded meta-analysis, narrative synthesis suggests that ctDNA may offer an improved early detection capability over CA125, through methylation and copy number variation analyses. Further controlled prospective studies are needed to validate the clinical utility of ctDNA as a complementary tool in ovarian cancer detection.

Keywords:
Circulating Tumor DNA; Ovarian Cancer; Diagnosis; Early Detection; Diagnostic Accuracy

WHAT IS ALREADY KNOWN ON THIS TOPIC

Circulating tumor DNA is a promising biomarker widely used in various solid tumors. However, its application in ovarian cancer remains limited, primarily due to the high heterogeneity of the disease.

WHAT THIS STUDY ADDS

This study presents a systematic review of the available literature evaluating the diagnostic accuracy of circulating tumor DNA in ovarian cancer. The findings indicate that circulating tumor DNA holds significant potential as a diagnostic tool. Moreover, the review underscores the variety of assessment approaches and emphasizes how evidence synthesis can guide the identification of the most appropriate strategies for its clinical implementation.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

By offering a comprehensive overview of studies assessing circulating tumor DNA's ability to distinguish ovarian cancer from healthy individuals or benign adnexal lesions, this review contributes to a better understanding of its diagnostic performance. It also provides insight into the different diagnostic approaches, including multi-gene and single-gene analyses, as well as the methodologies employed, potentially informing future research directions and clinical practice.

* ***Correspondence to*** *Prof Paolo Pinton, Section of Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara Department of Medical Sciences, Ferrara 44121, Italy; paolo.pinton@unife.it (P. Pinton)*

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INTRODUCTION

Ovarian cancer is the seventh most common cancer among women worldwide, and has the worst prognosis and highest mortality rate among gynecological cancers.[1] Much of the relapse rate and mortality associated with ovarian cancer is due to late-stage diagnosis and the lack of effective screening methods.[2] Therefore, early detection is crucial for reducing mortality and morbidity. Serum CA125 is a circulating biomarker commonly used in clinical practice as diagnostic and prognostic tool for ovarian cancer.[3] However, in approximately 50% of early-stage disease, CA125 levels may not be significantly elevated, resulting in a limited sensitivity, ranging between 50% and 62%.[4,5] In addition, CA125 can also be elevated in other non-malignant conditions, further limiting its specificity, which is relatively low (78%).[6]

In recent decades, extensive efforts have been made to identify biomarkers with improved diagnostic accuracy and higher detection rates. Circulating tumor DNA (ctDNA) has emerged as a promising minimally-invasive biomarker, offering the potential to detect genetic alterations directly from a patient's blood sample.[7] While cell-free DNA (cfDNA) includes all types of circulating DNA from different cell types (eg, white blood cells, epithelial cells, and others), ctDNA analysis qualitatively targets the DNA that carries tumor-specific genetic and epigenetic alterations, such as mutations, methylation patterns, and copy number variations (CNVs).

Both the diagnostic and prognostic roles of ctDNA have been explored in the literature. Evidence suggests that this marker may also be useful for disease surveillance and for predicting platinum resistance.[8] Therefore, using ctDNA to guide therapy decisions (ie, escalation, de-escalation, and treatment change) may by clinically useful.[8] In other tumor types, such as lung and gastrointestinal cancers, the implementation of this marker in clinical practice is more widespread compared to ovarian cancer.[9] In a previous narrative review, Trevisi and colleagues[8] concluded that the published data are not sufficiently consistent to draw definitive conclusions regarding its clinical applicability. However, to our knowledge, there is a lack of systematic reviews that aggregate studies providing data on the sensitivity and specificity of ctDNA, limiting the overall evidence on its diagnostic accuracy.

This systematic review therefore aims to evaluate the available evidence on the diagnostic accuracy of ctDNA for the detection of ovarian cancer. Specifically, we will assess the sensitivity, specificity, and overall diagnostic accuracy of ctDNA, as well as its relationship with CA125, to determine its potential utility as a diagnostic tool.

METHODS

Search Strategy

We conducted a comprehensive search across several databases, including PubMed, Web of Science, and EBSCO, as well as Google Scholar for additional sources. The literature search was conducted on April 9, 2024. No restrictions were applied regarding the start date of publication, and all studies available up to the search date were screened for eligibility. The search terms were "ctDNA", "ovarian cancer" and "diagnosis", with the full search strategy provided in Supplementary Material 1.

This systematic review was conducted following the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines 2020[10] and was prospectively registered in PROSPERO (registration number: CRD42024590089). Two independent reviewers, TC and GB, were responsible for screening articles, extracting data, and assessing the risk of bias. The reviewers initially screened titles and abstracts for relevance, followed by a full-text review. Any disagreements between the 2 reviewers were resolved through discussion with a third reviewer.

Eligibility Criteria

The primary analysis of this systematic review focuses on the diagnostic accuracy of ctDNA to distinguish ovarian cancer from healthy controls and/or benign conditions, providing a qualitative synthesis of the results from the included studies, assessing sensitivity, specificity, and overall diagnostic performance of ctDNA. We included studies that met the following criteria: (1) use of plasma and/or serum samples for ctDNA analysis; (2) inclusion of a control group (either healthy individuals or patients with benign ovarian cysts); (3) reporting of diagnostic accuracy metrics, such as sensitivity, specificity, and area under the receiver operating characteristic curve (AUC). Studies including patients with both epithelial and non-epithelial ovarian cancer were considered. Only studies published in peer-reviewed journals were considered. We excluded studies in which results for ovarian cancer were not reported separately from other cancer types, conference abstracts, animal studies, and case reports.

Data Extraction

Data extraction was performed independently by 2 reviewers (TC and GB) using a standardized form. The following data were collected from each study: study characteristics, sample type and timing of collection, patient characteristics, ctDNA detection method used (eg, polymerase chain reaction [PCR], next-generation sequencing [NGS], or whole genome sequencing [WGS]), and the specific genes or markers targeted. In addition, data on diagnostic accuracy (sensitivity, specificity, AUC), concordance rate between serum/plasma and the tumor tissue, and relationship between ctDNA and CA125 levels were extracted.

Bias Assessment

The risk of bias was evaluated using the QUADAS-2 tool, which is specifically designed for assessing the quality of diagnostic accuracy studies. Each study was evaluated across 4 domains: patient selection, index test, reference standard, flow, and timing. Studies were rated as low, high, or unclear risk of bias for each domain.

Statistical Analysis

The included studies varied significantly in terms of patient populations, sample types, control groups, detection methods, gene targets, and reported outcomes. Sensitivity was defined as the proportion of ovarian cancer patients correctly identified as positive by the ctDNA test, whereas specificity was defined as the proportion of control individuals (healthy or with benign ovarian cysts) correctly identified as negative. These definitions and measures were used consistently across the studies included in this systematic review. Due to this heterogeneity, a meta-analysis was not conducted. Conversely, a narrative synthesis was performed, focusing on the reported sensitivity, specificity, and

diagnostic accuracy of ctDNA for detecting ovarian cancer. Descriptive statistics were used to summarize key findings across the included studies.

RESULTS

A total of 172 records were identified. Following the removal of duplicates and subsequent screening phases, 48 full-text articles were assessed for eligibility. Finally, 19 observational studies were included in this systematic review (Fig. 1). The sample size varied among the included studies. Li and colleagues[11] (2020) reported a study with 17 patients, whereas Liang and colleagues[12] (2022) included 1052 patients. Detection and analysis of ctDNA were primarily conducted using amplification or sequencing techniques such as WGS and PCR. Specifically, PCR was implemented in various formats, including methylation-specific PCR (MSP), multiplex PCR, nested MSP, and quantitative MSP (qMSP). The key parameters evaluated for assessing ctDNA as a diagnostic tool were sensitivity and specificity, which were compared to CA125 in 7 of 19 studies.

The selected studies employed various strategies to identify ctDNA, including the detection of target genes (individual or in multi-gene panels), analysis of methylation status, chromosomal instability assessment, and the identification of somatic mutations, among other molecular features used to trace ctDNA. Amplification techniques (primarily PCR-based) and sequencing methods (WGS and NGS) were used for this purpose. PCR-based methods were applied in 14 of 19 studies (73.7%). These included approaches such as MSP or multiplex PCR aimed at identifying methylation statuses or mutations. In the study by Su and colleagues (2009),[13] MSP was used to detect methylation states in target genes such as *SFRP* (1, 2, 4, 5), *SOX1*, *PAX1*, and *LMX1A*. Singh and colleagues[14] (2021) employed a qMSP technique within the MethyLight approach, which quantitatively measures DNA methylation using quantitative PCR (qPCR).

Sequencing-based methods, particularly low-coverage WGS, were utilized in 4 of 19 studies (21%). Braicu and colleagues[15] (2021) used WGS to investigate copy number instability (CNI), referring to alterations in the copy number of specific ctDNA sequences. Vanderstichele and colleagues[16] (2017) focused on CNV assessment, while Cristiano and colleagues[17] (2019) leveraged the technique to identify ctDNA based on distinct fragmentation patterns.

Among 19 studies focusing on the detection of mutations or methylation patterns in specific genes, 2 (10.5%) focused on a single gene, while 12 (63%) sought to improve diagnostic accuracy

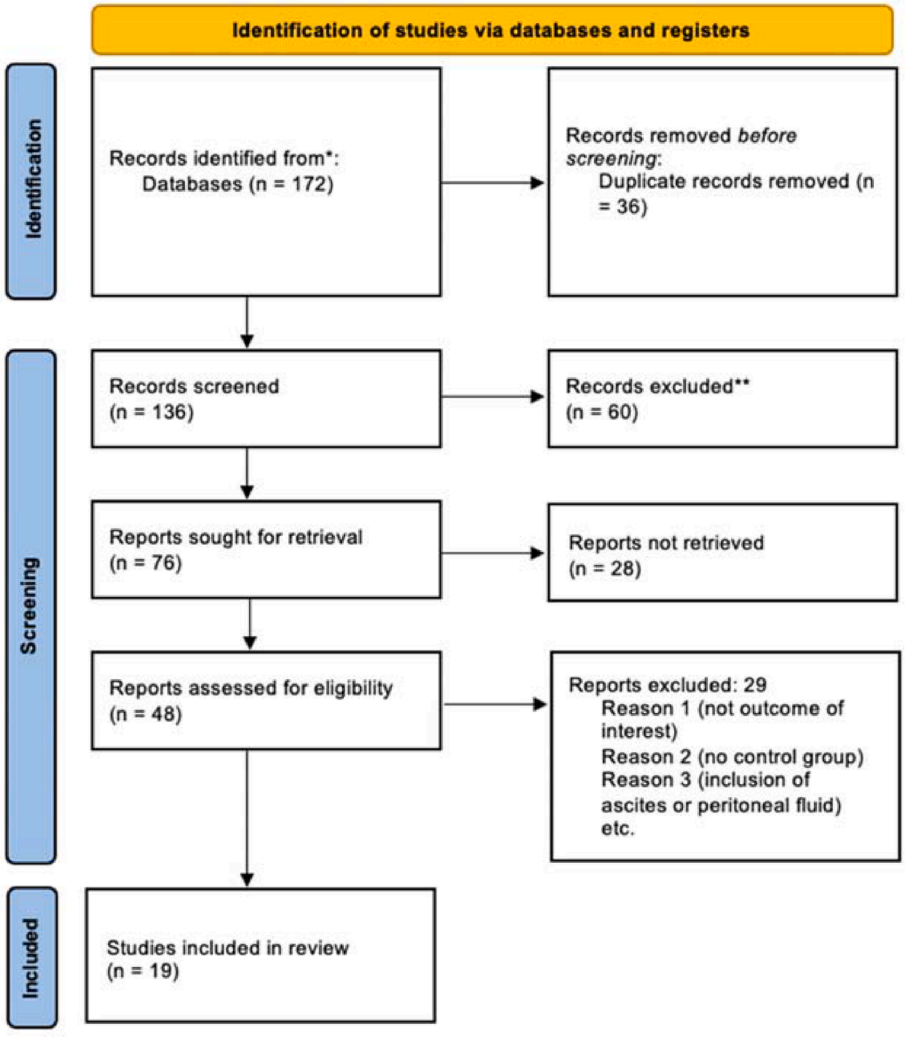

Figure 1 PRISMA flowchart of the included studies. PRISMA, Preferred Reporting Items for Systematic reviews and Meta-Analyses.

by analyzing multi-gene panels. In the former case, Li and colleagues[11] (2020) analyzed the methylation status of the *hTERT* promoter, while Campan and colleagues[18] (2011) examined the methylation status of the *IFFO1* promoter. However, most studies prioritized the analysis of multi-gene panels and their methylation status. For instance, Wang and colleagues[19] (2017) focused on *OPCML*, *RUNX3*, and *TFPI2*, while Dvorská and colleagues[20] (2019) investigated *PAX1, PTEN, CDH1*, and *RASSF1*. The most frequently analyzed target genes in the studies included in this systematic review were *TP53*, *RASSF1A*, *BRCA1*, *HOXA9*, and *OPCML* (Fig. 2).

Methylation status analysis was conducted in 14 of 19 studies (73.7%), employing techniques such as MSP or bisulfite sequencing to identify tumor-specific methylation patterns (Table 1).

Su and colleagues[13] (2009) analyzed the methylation status of *SFRP (1, 2, 4, 5)*, *SOX1*, *PAX1*, and *LMX1A* genes using MSP. By combining the analysis of *SOX1*, *PAX1*, and *SFRP1*, this study achieved 73% sensitivity and 75% specificity. Li and colleagues[11] (2020) applied MSP to evaluate the methylation of the *hTERT* promoter, obtaining 76.9% sensitivity and 50% specificity. Widschwendter and colleagues[21] (2017) employed a bisulfite-treated DNA sequencing approach (targeted bisulfite sequencing) to assess methylation in genes such as *COL23A1*, *C2CD4D*, and *WNT6*, enabling ovarian cancer detection up to 2 years before clinical diagnosis, with 58% sensitivity and 88% specificity.

Wang and colleagues[19] (2017) used nested MSP to evaluate promoter methylation in *OPCML*, *RUNX3*, and *TFPI2*, achieving 90.1% sensitivity and 91.8% specificity. Sensitivity was 87.1% for early-stage disease (International Federation of Gynecology and Obstetrics [FIGO] I-II) and 93.7% for advanced stages (FIGO III-IV). Dvorská and colleagues[20] (2019) reported 91% sensitivity and 56% specificity when studying methylation in *PAX1*, *PTEN*, *CDH1*, and *RASSF1* using MSP. Ibanez de Caceres and colleagues[22] (2004) employed MSP to analyze *BRCA1*, *RASSF1A*, p14, p16, and *DAPK*, achieving 82% sensitivity and 100% specificity. Liggett and colleagues[23] (2011) achieved 90% sensitivity and 86.7% specificity using nested MSP to study *RASSF1A*, *CALCA*, and *EP300*. Melnikov and colleagues[24] (2009) used PCR followed by microarray analysis to assess methylation in a panel of 5 genes (*BRCA1*, *HIC1*, *PAX5*, PGR-PROX, and *THBS1*), reporting 85.1% sensitivity and 61.1% specificity.

Singh and colleagues[14] (2021) used qMSP (MethyLight) to evaluate methylation in 7 tumor suppressor genes (*RASSF1A*, *DAPK1*, *SOX1*, *HOXA9*, *HIC1*, *SPARC*, and *SFRP1*). With a percent of methylated reference cut-off of 4%, they achieved 71% sensitivity and 100% specificity for *HIC1*. The best results, combining *HOXA9* and *HIC1*, yielded 88.8% sensitivity and 100% specificity, confirming the aberrant hypermethylation frequency in epithelial ovarian cancer Swellam and colleagues[25] (2017) evaluated the promoter methylation of *DAPK*, *OPCML*, and *DLEC1* using MSP, reporting sensitivities of 96.7%, 97.8%, and 95.6% and specificities of 75%, 70%, and 75%, respectively. Zhang and colleagues[26] (2013) used Multiplex MSP to evaluate methylation in *APC*, *RASSF1A*, *CDH1*, *RUNX3*, *TFPI2*, *SFRP5*, and *OPCML*, achieving 89.6% sensitivity and 90.5% specificity in retrospective analysis, and 92.3% sensitivity and 82.7% specificity in a screening context. For early-stage (FIGO I), sensitivity was 85.3% and specificity 90.5%.

Marinelli and colleagues[27] (2022) analyzed methylation in *GPRIN1*, *CDO1*, *SRC*, *SIM2*, *AGRN*, *FAIM2*, *CELF2*, *RIPPLY3*, *GYPC*, *CAPN2*, and *BCAT1* using qMSP, reporting 79% sensitivity and 96% specificity. Campan and colleagues[18] (2011) studied *IFFO1* promoter methylation using droplet digital PCR (ddPCR), identifying significantly higher methylation frequencies in patients with cancer compared to healthy controls (AUC = 0.95). Liang and colleagues[12] (2022) used the enhanced linear-splinter amplification sequencing (ELSA-seq) to evaluate methylation in 18 differentially methylated regions specific to ovarian cancer, identified through statistical testing, achieving 94.7% sensitivity and 88.7% specificity.

Chromosomal instability is another hallmark of ovarian cancer, partially attributed to TP53 mutations. This feature was evaluated in 4 of 19 studies (21%). Vanderstichele and colleagues[16] used low-coverage WGS to identify chromosomal instability in cfDNA, achieving a sensitivity of 74% and a specificity of 91% in distinguishing patients from healthy controls (Table 2).

Braicu and colleagues[15] demonstrated that comparing the CNI score between patients with ovarian cancer and controls allowed the identification of the disease with a sensitivity of 91% and a specificity of 95%. They concluded that genomic instability quantification using the CNI score is a highly accurate diagnostic biomarker for high-grade histotype. Cohen and colleagues[28] detected 40.6% of all high-grade serous ovarian cancer, including 38% of early-stage cases, using low-coverage WGS of plasma cfDNA and analysis of chromosomal CNV $\geq$15 Mb.

The analysis of somatic mutations was conducted in the study performed by Wang and colleagues[29] (2018), which employed the multiplex PCR technique to identify mutations in genes such as *AKT1*, *BRAF*, and *EGFR*. Among the 83 women with ovarian cancer included in the study, ctDNA was detected in 43% of cases. The analyses conducted on the samples achieved 100% specificity in disease detection. Regarding sensitivity, a rate of 56% was observed in advanced disease stages, while it dropped to 35% in early-stage cases.

In 4 studies (21%) included in this systematic review, CA125 values were reported, allowing a comparison of its diagnostic accuracy with that of ctDNA. Swellam and colleagues[25] (2017) analyzed *DAPK* gene methylation in ctDNA and reported an AUC of 0.86, while CA125 in the same cohort achieved a slightly higher AUC of 0.92. Sensitivity was 96.7% for the ctDNA marker compared to 94.4% for CA125. Zhang and colleagues[26] (2013) observed higher diagnostic accuracy for ctDNA compared to CA125 in early-stage disease (FIGO I), with a sensitivity of 85.3% for ctDNA versus 56.1% for CA125 ($p = .003$). In the study by Wang and colleagues[19] (2017), methylation analysis of a gene panel (*OPCML*, *RUNX3*, and *TFPI2*) yielded an overall sensitivity of 90.1% and specificity of 91.9%, outperforming CA125, which showed a sensitivity of 67.6% and specificity of 63.41%. This superior performance was especially evident in detecting early-stage disease (FIGO I-II), in which ctDNA achieved a sensitivity of 87.1% versus 53.8% for CA125. In the study by Liang and colleagues[12] (2022), ctDNA detection through the analysis of differentially methylated regions using the ELSA-seq technique achieved a sensitivity of 94.7% and an AUC of 0.96. This marginally outperformed CA125, which had an AUC of 0.90.

Three studies reported concordance rates between cancer-specific genetic alterations detected in blood-derived ctDNA and those identified in tumor tissue. Dvorská and colleagues[20] (2019) investigated the correlation between plasma DNA and tumor tissue DNA.

Table 1 Characteristics of Included Studies Analyzing Circulating Tumor DNA Methylation Status

Study	Country	Sample	Population of interest with primary OC	Sub-type/stage	Method	Genes	Sensitivity	Specificity	AUC (95% CI)
Su and colleagues,[13] 2009	Taiwan	Serum	126 M, 14 BOT 75 B (serum: 26 OC, 20 B)	FIGO I-IV OC[a]	MS-PCR	SFRP1, SFRP2, SFRP4, SFRP5, SOX1, PAX1, LMX1A	73%	75% (SOX1, PAX1, SFRP1)	NR
Li and colleagues,[11] 2020	China	Plasma	N = 17 OC, 15 B, 15 H	I-III OC	MSP	hTERT promoter methylation	76.9%	50%	NR
Widschwendter and colleagues,[21] 2017	UK	Serum (4 mL)	N = 250 (B+M) +25 M +172 (nested case control study of 172 UKCTOCS control arm participants: 43 M, *N* = 129 H)	FIGO I-IV, OC	Targeted bisulphite sequencing	COL23A1, C2CD4D, WNT6,	41.4% (CI 24.1 to 60.9%) (HGSOC vs B/H)	90.7% (CI 84.3 to 94.8%) (HGSOC vs B/H)	NR
Wang and colleagues,[19] 2017	China	Serum (0.2 mL)	71 M, 80 H, 43 B	I-IV (39 I-II, 32 III-IV) EOC	Nested MSP	promoter methylation in OPCML, RUNX3, TFPI2	90.1%; (stage I/II: 87.1%, III/IV: 93.75%)	91.8% (stage I/II: 91.8%, III/IV: 91.8%)	NR
Dvorská and colleagues,[20] 2019	Slovakia	Plasma	49 M, 8 B, 17 H	FIGO I-IV OC	MSP followed by pyrosequencing	PAX1, PTEN, CDH1, RASSF1	91%	56%	AUC 0.82 (controls vs OC), AUC 0.63 (controls vs OC)
Ibanez de Caceres and colleagues,[22] 2004	USA	Serum (1.5 mL)	50 M, 20 H, 10 B	I-IV OC	MSP	BRCA1, RASSF1A, p14, p16, DAPK	82%	100%	NR
Liggett and colleagues,[23] 2011	USA	Plasma	30 M, 30 B, 30 H	III-IV OC	Multiplex nested MSP	RASSF1A, CALCA, EP300	90%	86.7%	NR
Melnikov and colleagues,[24] 2009	USA	Plasma (0.2 mL)	33 M, 33 H	III-IV	PCR, microarray	Meth status of BRCA1, HIC1, PAX5, PGR-PROX, THBS1	85.1%	61.1%	NR
Singh and colleagues,[14] 2021	India	Serum (1 mL)	85 M, 35 H	FIGO I-IV (20 I/II, 65 III/IV)	qMSP (MethyLight)	RASSF1A, DAPK1, SOX1, HOXA9, HIC1, SPARC, SFRP1 promoter methylation	HOXA9 (62.2%) HIC1 (71.1%), SOX1 (53.3%) HOXA9+SOX1 (66.6%), HOXA9+HIC1 (88.8%) SOX1+HIC1(80%)	HOXA9 (100%), HIC1 (100%), SOX1 (96%), HOXA9+SOX1 (96%), HOXA9 +HIC1 (100%), SOX1+HIC1 (96%)	HOXA9 (0.81), HIC1 (0.88), SOX1 (0.77), HOXA9+SOX1 (0.85), HOXA9+HIC1 (0.95), SOX1+HIC1 (0.93)
Swellam and colleagues,[25] 2017	Egypt	Serum	90 M, 50 B, 30 H	FIGO I-IV (42 I/II, 48 III/IV)	MSP	DAPK, OPCML, DLEC1	DAPK (96.7%), OPCML (97.8%), DLEC1 (95.6%)	DAPK: (75%) OPCML: (70%), DLEC1: (75%)	NR
Zhang and colleagues,[26] 2013	China	Plasma (200 μl)	87 M,62 H,53 B	FIGO I-IV (41 I, 46 II-IV) EOC	Multiplex MSP	APC, RASSF1A, CDH1, RUNX3, TFPI2, SFRP5, OPCML	Retrospective study: 89.66%; screening study: 92.31% (stage I: SN 85.3%)	Retrospective study: 90.57%. Screening study: 82.76% (stage I: 90.5%)	NR
Marinelli and colleagues,[27] 2022	USA	Plasma (6 mL)	253 M, 45 B 110 H). plasma testing on 91 M, 91 H	FIGO I-IV (25 I, 8 II, 19 III, 5 IV) EOC	qMSP	GPRIN1, CDO1, SRC, SIM2, AGRN, FAIM2, CELF2, RIPPLY3, GYPC, CAPN2, BCAT1	79%	96%	AUC 0.91 (0.86-0.96) SIM2 0.82, SRC 0.78, RIPPLY3 0.77, AGRN 0.77, CDO1 0.75, BCAT1 0.75, GYPC 0.73, CELF2 0.73, CAPN2 0.72, FAIM2 0.64, GPRIN1 0.56
Campan and colleagues,[18] 2011	USA	Serum	41 + 16 M; 10 + 8 H)	I-IV EOC	MethyLight, digital MethyLight assay (rtPCR)	IFFO1	-NR	NR	AUC 0.95 (0.83 to 1)
Liang and colleagues,[12] 2022	China	Plasma	(MDRs identification, model training, and validation *N* = 165 M, 105 B, *N* = 93 H; prognostic model training and validation *N* = 131 M, *N* = 558 M)	I-IV OC	ELSA-seq	DMRs	94.7%	88.7%	AUC 0.96

Abbreviations: H, healthy controls; B, benign histology; M, malignant histology excluding BOT; BOT, borderline ovarian tumor; FIGO, International Federation of Gynecology and Obstetrics; NR, not reported; MSP, methylation-specific polymerase chain reaction; ELSA-seq, enhanced linear-splinter amplification sequencing; HGCOS, high-grade serous ovarian cancer; UKCTOCS, UK Collaborative Trial of Ovarian Cancer Screening; OC, ovarian cancer.

[a] 'OC' indicates inclusion of both EOC and non-EOC histology; 'EOC' refers to studies including only epithelial ovarian cancers; 'HGSOC' is used when only high-grade serous ovarian cancers were included.

Table 2 Characteristics of Included Studies Analyzing Chromosomal Instability in Circulating Tumor DNA

Study	Country	Sample	Time	Population of interest with primary OC	Sub-type/stage	Method	Genes	Cut-off	Sensitivity	Specificity	AUC (95% CI)
Braicu and colleagues,[15] 2021	Germany	Plasma (2 × 8-10 mL)	BS	109 (23 patients with treatment-naïve advanced disease) 241 H	HGSOC FIGO IIIB-IVB	Low-coverage WGS	[CNI- score]	CNI- score 24,5 (Youden index)	91%	95%	NR
Cristiano and colleagues,[17] 2019	USA	Plasma	NR	N = 236 (*N* = 28 M), *N* = 215 H	NR	WGS	[cfDNA fragmentation patterns]	NR	NR	NR	AUC 0.99 (0.97-1)
Vanderstichele and colleagues,[16] 2017	Belgium	Plasma	BT	54 M, 44 H, 11 B	I-IV, OC (45 HGSOC)	Low-coverage WGS	CNA	Genome-wide z-score = 0.71	74%	91%	AUC 0.89 (B vs BOT/M); AUC 0.94 (B vs HGSOC)
Cohen and colleagues,[28] 2016	Australia	Plasma (1 x 9 mL)	BS	32 M, 32 B	I-IV HGSOC	Low-coverage WGS	CNV	NR	40.6%	93.8%	NR

Abbreviations: B, benign histology; BOT, borderline ovarian tumor; BS, before surgery; BT, before any treatment; cfDNA, cell-free DNA; CNA, copy number alteration; CNI, copy number instability; CNV, copy number variation; FIGO, International Federation of Gynecology and Obstetrics (cancer staging system); H, healthy controls; HGSOC, high-grade serous ovarian cancer; M, malignant histology (ovarian cancer); NR, not reported; OC, ovarian cancer; WGS, whole genome sequencing.

Results showed the highest correlation observed for the *PAX1* gene (r = 0.771) and a moderate correlation for the *CDH1* gene (r = 0.428, Pearson correlation coefficient). In the study performed by Vanderstichele and colleagues[16] (2017), a Spearman correlation coefficient of 0.67 was observed when comparing cfDNA plasma samples and tumor tissue genomes (specifically for high-grade serous epithelial ovarian cancer cases) based CNV, indicating robust concordance between ctDNA and tumor tissue genetic alterations. Similarly, Su and colleagues[13] (2009) reported a significant correlation between serum tumor DNA and tumor tissue DNA, with a minimum kappa (k)-value of 0.332 (for *PAX1*) and a maximum k-value of 0.598 (for *SFRP1*), revealing a significant correlation.

The methodological quality of the included studies was assessed using the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) tool. This evaluation examined risk of bias and applicability across 4 domains: patient selection, index test, reference standard, and flow and timing. All 19 studies included in the review were judged to be at low risk of bias in all assessed domains. A detailed summary of the QUADAS-2 assessment is provided in the Supplementary Material 2.

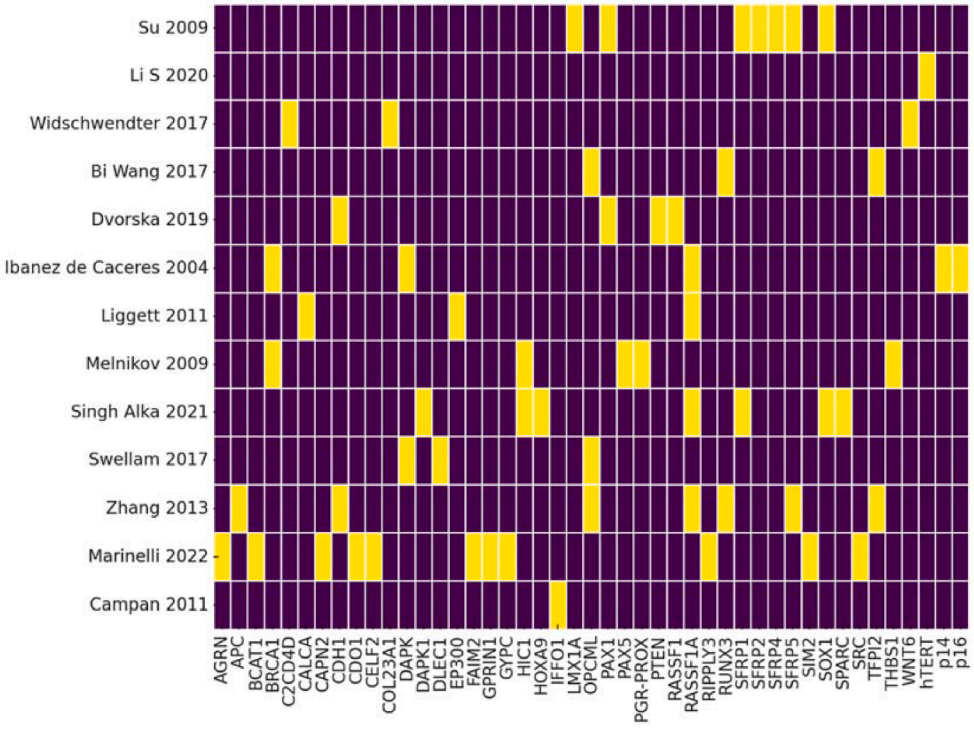

Figure 2 Overview of gene targets across included articles. Heatmap illustrating the genes investigated in each study included in the systematic review. Each row represents a study, and each column represents a gene. Yellow cells indicate that the gene was analyzed in the corresponding study.

DISCUSSION

Summary of the Main Results

In our systematic review, we found that most studies aimed to detect tumor suppressor gene alterations in ctDNA, focusing on both sequence alterations and epigenetic modifications through promoter methylation during carcinogenesis. Multi-gene approach, based on prior knowledge of the target genes, was employed by most included studies. Among the studies included, the most frequently analyzed genes were *TP53*, *RASSF1A*, *BRCA1*, *HOXA9*, and *OPCML*, based on their established association with ovarian cancer.

Results in the Context of Published Literature

The qualitative assessment of the genes studied was conducted using 2 main methods. Primarily, the analysis of the methylation profiles of tumor suppressor gene promoters and the assessment of chromosomal instability using WGS. Epigenetic inactivation of tumor suppressor genes through promoter methylation represents a modification occurring in the early oncogenesis, making this ctDNA feature an excellent target for early diagnosis. However, a clinically validated panel of markers has not yet been developed. ovarian cancer is a highly complex, heterogeneous disease, where no single gene has shown consistent methylation across all histological sub-types. It has been observed that the promoter methylation of *SFN*, *TMS1*, and *WT1* is more frequent in clear cell ovarian carcinomas compared to other histotypes, while in invasive ovarian cancer, methylation at the promoters of *RASSF1A*, *APC*, *GSTP1*, and *MGMT* is more frequent than in low-malignancy tumors (Zhang and colleagues,[26] 2013). This type of ctDNA analysis can be conducted in 2 ways: a tumor-guided approach, where the mutational or methylation profile of a specific tumor is already known, and a tumor-independent approach, where no a priori knowledge of specific mutations is available, and common mutations across different sub-types are investigated.

Differences in diagnostic accuracy have been observed between studies adopting the single-gene approach and studies investigating multi-gene panels. While single-gene analyses (eg, *hTERT*) showed moderate sensitivity and specificity,[11] studies using multi-gene panels consistently reported higher accuracy, with several panels achieving over 90% sensitivity and specificity.[19,23,26]

As illustrated in Figure 3, targeted approaches based on a priori known mutations may fail to detect tumors with atypical or unknown specific mutations. Using approaches such as WGS allows the processing of large amounts of multi-omics data, improving diagnostic accuracy and adapting to the specific genomic landscape of the patient's tumor.

Exploring the entire methylome is particularly useful for identifying new markers or relevant methylated regions. In studies investigating chromosomal abnormalities, WGS has been employed to analyze chromosomal instability.[15-17,28] This method provides a comprehensive view of chromosomal alterations across the entire genome at low depth, detecting large-scale copy number changes and chromosomal rearrangements. WGS offers advantages such as detecting a wide range of chromosomal anomalies, including deletions, amplifications, and rearrangements. It also enables dynamic monitoring of the genomic landscape over time, providing insights into tumor progression and therapy response. However, WGS has limitations, such as reduced

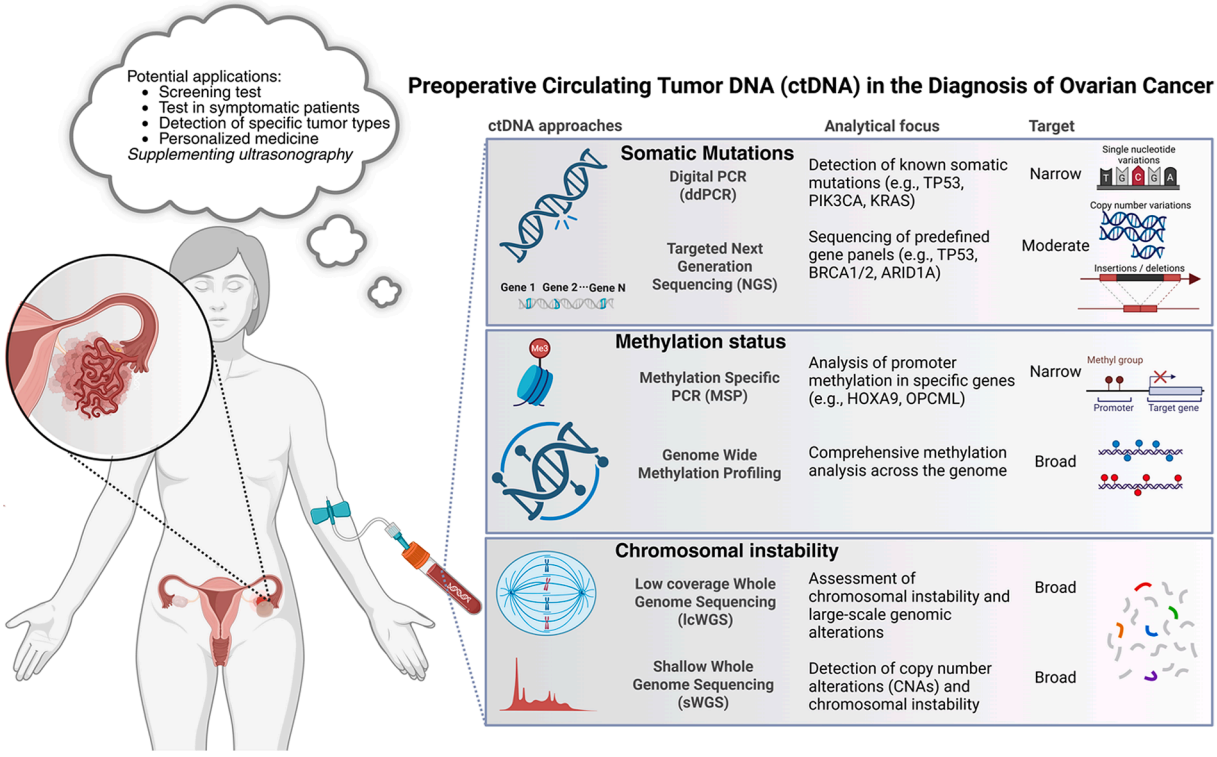

Figure 3 Preoperative circulating tumor DNA in the diagnosis of ovarian cancer. Schematic overview of circulating tumor DNA-based approaches and their potential clinical applications. CAN, copy number alteration; ctDNA, circulating tumor DNA; ddPCR, droplet digital polymerase chain reaction; lcWGS, low-coverage whole genome sequencing; MSP, methylation-specific polymerase chain reaction; NGS, next-generation sequencing; sWGS; shallow whole genome sequencing.

sensitivity for small mutations or focal CNVs, particularly in early-stage with low tumor burden. Additionally, genomic instability may generate complex patterns requiring advanced computational tools and significant bioinformatics expertise for interpretation.

For somatic mutation detection, techniques such as NGS and PCR-based methods (eg, dPCR or ddPCR) are employed. NGS enables the detection of somatic mutations across a broad gene panel or even the entire genome. Its advantages include not requiring prior knowledge of molecular alterations and the ability to analyze multiple alterations across diverse genes simultaneously. Techniques like ddPCR offer high sensitivity for detecting low-frequency mutations in ctDNA, facilitating early diagnosis, minimal residual disease detection, or treatment monitoring. Nevertheless, ddPCR is limited to analyzing specific, known mutations, and the heterogeneous mutational landscape of ovarian malignancy makes a universal mutational panel difficult to create.

Strengths and Weakness

This systematic review highlighted the diverse strategies employed across the included studies and analyzed their diagnostic accuracy results. Plasma or serum ctDNA analysis is a promising tool for diagnosis, outperforming CA125. However, the heterogeneity across studies in terms of methodology, target selection, sample size, and populations examined, limits the applicability of these results. Therefore, it is not yet possible to provide cumulative quantitative data on the diagnostic accuracy of ctDNA.[30] Instead, we are at a stage where the focus remains on identifying what to look for, how to search for it, and which technique offers the best compromise between diagnostic accuracy, applicability, and costs in a highly heterogeneous disease like ovarian cancer.

Implications for Practice and Future Research

ctDNA tests may be relevant in several parts of the diagnostic process - both as a screening tool and for further discriminating adnexal lesions deemed at risk of malignancy by ultrasonography. Although ctDNA offers advantages as a minimally-invasive tool with a short half-life that reflects the real-time mutational profile of cancer, significant limitations remain, particularly the challenge of detecting mutations in ctDNA due to its low plasma concentration, especially in early disease stages with minimal tumor and mutational burden. As highlighted by this systematic review, the primary limitation of liquid biopsy and ctDNA analysis for diagnosis is its low sensitivity, raising the risk of false-negative results in some cases due to low ctDNA levels. However, despite these limitations, ongoing technological and methodological advancements in liquid biopsy are steadily improving the sensitivity of ctDNA testing, paving the way for its integration as a more reliable and valuable diagnostic tool in clinical practice.

CONCLUSIONS

ctDNA is emerging as a highly promising diagnostic biomarker for ovarian cancer. This systematic review highlights the encouraging results, in the wide variety of methodologies and genetic anomalies that can be exploited for qualitative ctDNA analysis. Most studies have focused on the analysis of promoter methylation profiles of various tumor suppressor genes, identifying a strategy that has shown great potential, particularly for the early detection. However, for the clinical implementation of liquid biopsy through ctDNA analysis, continued research is necessary to identify the most effective approach for a tumor as highly heterogeneous as ovarian cancer.

Author Affiliations

[a]University of Ferrara, Department of Medical Sciences, Ferrara, Italy
[b]KU Leuven, Department of Development and Regeneration, Leuven, Belgium
[c]Aarhus University, Department of Clinical Medicine, Aarhus, Denmark
[d]Aarhus University Hospital, Department of Obstetrics and Gynecology, Aarhus, Denmark
[e]KU Leuven Cancer Institute, Department of Oncology, Laboratory for Tumor Immunology and Immunotherapy, Leuven, Belgium
[f]University Hospitals Leuven, Department of Gynaecology and Obstetrics, Leuven, Belgium
[g]IRCCS Ospedale Policlinico San Martino, Genova, Italy
[h]Harvard Medical School, Dana-Farber Cancer Institute, Department of Medical Oncology, Boston, Massachusetts, USA
[i]Azienda Sanitaria Universitaria Friuli Centrale (ASUFC), "S. Maria della Misericordia" University Hospital, Clinic of Obstetrics and Gynecology, Udine, Italy
[j]IRCCS, IEO, Department of Gynecology, European Institute of Oncology, Milan, Italy
[k]University of Udine, Department of Medicine (DMED), Udine, Italy
[l]Aarhus University Hospital, Department of Molecular Medicine (MOMA), Aarhus, Denmark
[m]University of Bari "A. Moro", Department of Biosciences, Biotechnologies and Environment, Bari, Italy

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