Circulating tumor DNA as a biomarker for predicting progression-free survival and overall survival in patients with epithelial ovarian cancer: a systematic review and meta-analysis

Cristina Taliento ,[1,2] Giampaolo Morciano,[3] Camilla Nero,[4,5] Wouter Froyman,[2,6] Giuseppe Vizzielli ,[7] Matteo Pavone ,[4,8] Stefano Salvioli,[9] Mara Tormen,[1] Francesco Fiorica,[10] Gennaro Scutiero,[1] Giovanni Scambia ,[4] Carlotta Giorgi,[3] Pantaleo Greco,[1] Paolo Pinton[3]

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/ijgc-2024-005313).

For numbered affiliations see end of article.

Correspondence to
Professor 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

Received 21 January 2024
Accepted 9 April 2024

To cite: Taliento C, Morciano G, Nero C, *et al. Int J Gynecol Cancer* Published Online First: [please include Day Month Year]. doi:10.1136/ijgc-2024-005313

ABSTRACT

Objectives Circulating tumor DNA (ctDNA) is emerging as a potential prognostic biomarker in multiple tumor types. However, despite the many studies available on small series of patients with ovarian cancer, a recent systematic review and meta-analysis is lacking. The objective of this study was to determine the association of ctDNA with progression-free-survival and overall survival in patients with epithelial ovarian cancer.

Methods An electronic search was conducted using PubMed (MEDLINE), Embase, CENTRAL (Cochrane Library), and CINAHL-Complete from January 2000 to September 15, 2023. To be included in the analysis the studies had to meet the following pre-specified inclusion criteria: (1) evaluable ctDNA; (2) progression-free-survival and overall survival reported as hazard ratio (HR); and (3) the patient population had epithelial ovarian cancer at the time of ctDNA detection. We evaluated the association of ctDNA with progression-free survival and overall survival. Secondary outcomes focused on sub-group analysis of genomic alterations and international Federation of Gynecology and Obstetrics (FIGO) stage.

Results A total of 26 studies reporting on 1696 patients with epithelial ovarian cancer were included. The overall concordance rate between plasma-based and tissue-based analyses was approximately 62%. We found that a high level of ctDNA in epithelial ovarian cancer was associated with worse progression-free survival (HR 5.31, 95% CI 2.14 to 13.17, p<0.001) and overall survival (HR 2.98, 95% CI 1.86 to 4.76, p<0.0001). The sub-group analysis showed a greater than threefold increase in the risk of relapse in patients with positive HOXA9 meth-ctDNA (HR 3.84, 95% CI 1.57 to 9.41, p=0.003).

Conclusions ctDNA was significantly associated with worse progression-free survival and overall survival in patients with epithelial ovarian cancer. Further prospective studies are needed.

PROSPERO registration number CRD42023469390.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Circulating tumor DNA (ctDNA) has gained particular interest as a biomarker in different tumor types. The studies published in the last 5 years show encouraging results on ctDNA as a prognostic marker in patients with epithelial ovarian cancer. However, there is a lack of substantial evidence to routinely adopt this approach in clinical practice.

WHAT THIS STUDY ADDS

⇒ This study collects the available evidence on the association between detectable or high levels of ctDNA and progression-free survival and overall survival. Our results confirm ctDNA as a valuable prognostic tool. Additionally, sub-group analysis showed that the presence of detectable or high levels of ctDNA in samples collected both before and after surgery, as well as after medical treatment, are correlated with a shorter progression-free survival.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The results of this systematic review and meta-analysis could increase interest in carrying out clinical studies on ctDNA to further elucidate its role as a marker for prognosis, patient surveillance, and drug resistance in patients with epithelial ovarian cancer.

INTRODUCTION

Ovarian cancer remains a leading cause of death among women worldwide. Despite the advances in cytoreductive efforts, frontline platinum-based chemotherapy and maintenance treatments, recurrence is a common event with more than 70% of women with advanced ovarian cancer experiencing a relapse within 2 years of diagnosis.[1] Moreover, even with the availability of effective biological treatments, the lack of predictive biomarkers of response hinders clinicians from accurately identifying patients who are most likely to benefit from these therapies.[2] Unfortunately, target drugs also favor the emergence of drug tolerant or resistant cells, ultimately resulting in tumor relapse. An early prediction of resistance would

support clinicians in treatment modifications, avoiding unnecessary toxicity for the patient.[3]

Currently, tumor tissue remains the main source of clinically relevant molecular information enabling patients' prognostic stratification.[4] The biomarker adopted in routine care for measuring response in patients receiving chemotherapy for high-grade serous ovarian cancer is the serum protein cancer antigen 125 (CA-125). However, in response to chemotherapy, CA-125 levels do not change rapidly enough to reflect the best response after one or two cycles of treatment.[5] In addition, in predicting early recurrence, although having a good specificity in the diagnosis of recurrence (86.79%), CA-125 alone with a threshold of 35 U/mL does not show optimal sensitivity (67.39%) since about 20–30% of patients with ovarian cancer have no expression of this biomarker.[5]

Circulating tumor DNA (ctDNA) is cell-free DNA derived from cancer cells that can be detected in the bloodstream and is used as a highly specific marker since it carries mutations unique to the tumor.[6–12] In ovarian cancer, ctDNA analysis allows comprehensive molecular profiling of the primary, metastatic, and recurrent tumors.[13–19] As shown in several studies, ctDNA also correlates with progression-free survival and overall survival, making it a clinically valuable means of evaluating residual disease following surgery and chemotherapy and monitoring of responses during treatment and follow-up.[20–26]

In the era of precision medicine, ctDNA emerges as one of the most promising tools to enhance more precise treatment decisions, thereby improving patients' outcomes as well as their quality of life.[27–32] However, its prognostic significance in ovarian cancer has not yet been clearly proven and high-quality evidence that summarizes and integrates the results of the available studies is lacking.[32–36]

The main aim of this study was to investigate whether ctDNA was an independent predictor of progression-free survival and overall survival in patients with epithelial ovarian cancer. A secondary aim was to carry out a sub-group analysis based on genomic aberrations and International Federation of Gynecology and Oncology (FIGO) stage.

METHODS

Eligibility Criteria, Information Sources, and Search Strategy

We only included clinical studies reporting survival outcomes (overall survival or progression-free survival) in patients with epithelial ovarian cancer in which ctDNA was detected in the plasma and/or serum. We included studies where sample collections were taken before or after surgery as well as during or after chemotherapy/systemic therapy. We excluded studies reporting data from other types of primary tumors or circulating tumor cells.

A certified statistical methodologist (GIMBE Education) performed a literature search from database inception to September 2023 on the electronic databases PubMed (MEDLINE), Embase, CENTRAL (Cochrane Library) and CINAHL. A detailed report of the search strategy for each database is shown in Online supplemental material 1. The review was registered in PROSPERO (CRD42023469390) and is reported in accordance with the PRISMA statement 2020.[37]

Study Selection and Data Extraction

Two authors (CT and MT) independently screened the records. Any inconsistency in the selection process was resolved by discussion with a third reviewer (MP) until consensus was reached. Data extracted from the eligible articles included the number of participants and type of epithelial ovarian cancer, FIGO stage, therapeutic regimen, detection method used to analyze ctDNA, genomic alterations, ctDNA cut-off levels, overall survival, progression-free survival, and the concordance rate in the mutational profile between ctDNA in the blood sample and tumor specimen.

Assessment of Risk of Bias

The quality of the included studies was assessed using the Newcastle–Ottawa Scale tool[38] by two independent reviewers (CT and MP) using the same set of decision rules. Randomized controlled trials were evaluated using the Risk of Bias Assessment tool for randomized studies – of Interventions (ROB-2).

Data Synthesis

Progression-free survival and overall survival were used as the main evaluation endpoints and sub-group analysis was performed based on the type of genomic aberration of ctDNA, FIGO stages, and ctDNA detection methods. We performed meta-analyses with the generic inverse variance method with a random-effects model considering the heterogeneity between the studies. We used the hazard ratio (HR) with 95% two-sided confidence intervals (CIs) as the principal summary measure and reported heterogeneity within each sub-group with the I^2 statistic. If the findings were heterogeneous ($p<0.1$, $I^2>50\%$), sensitivity analysis or sub-group analysis was used to determine the source of the heterogeneity.

Meta-analyses were conducted with the Review Manager software, version 5.4 and p values <0.05 were considered statistically significant.

RESULTS

Study Selection

We found a total of 359 records and, after removing 147 duplicates, we reviewed titles and abstracts to identify those that met the inclusion criteria. A further 154 studies were excluded because they did not meet the pre-specified criteria. Full-text copies of the remaining 35 articles were obtained and analyzed for eligibility. An additional nine studies were excluded for the following reasons: absence of overall survival and progression-free survival expressed as HR or p value (log-rank test),[39 40] inclusion of other gynecological malignancies,[41 42] considering only cell-free DNA (cfDNA)[26 43] or circulating mitochondrial DNA,[10 18] and not specifying the type of specimen[44] (see Figure 1 and Online supplemental material 2).

Study Characteristics

The details and main characteristics of included studies are summarized in Table 1. Twenty-four observational and two randomized controlled trials involving a total of 1696 patients with epithelial ovarian cancer were finally included in this systematic review. These studies were predominantly published within the last 5 years (Figure 2). Five of the included studies were conducted in Denmark, and the remaining studies were carried out in Denmark (5), Korea (4), Japan (4), USA (3), France (2), Germany (2), Greece

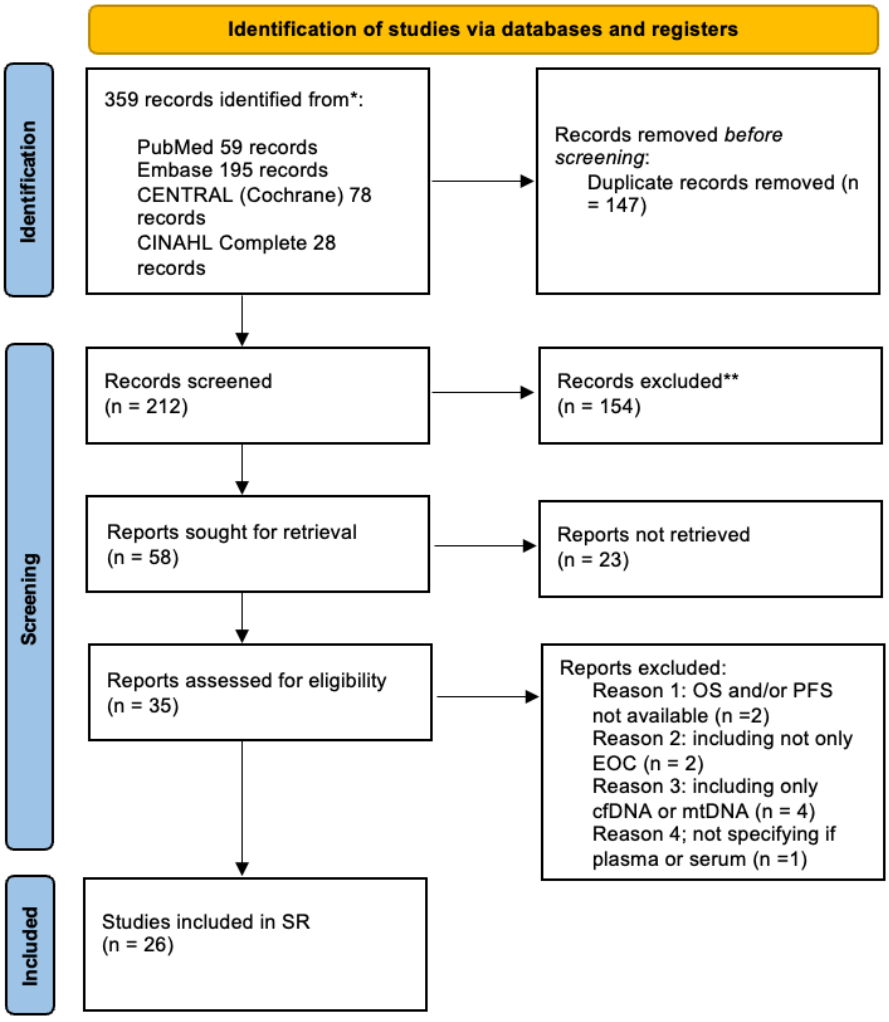

Figure 1 PRISMA 2020 flow diagram. EOC, epithelial ovarian cancer; OS, overall survival; PFS, progression-free survival.

(2), UK (1), Belgium (1), Sweden (1), and Taiwan (1). Of all the studies that included patients diagnosed with epithelial ovarian cancer, 30% (8/26) of the studies included only high-grade serous ovarian cancer.

A total of 37% of studies (11/29) reported that tumor assessment was based on RECIST (Response Evaluation Criteria in Solid Tumors) criteria.

The approaches for ctDNA analysis included digital polymerase chain reaction (n=9) followed by Next Generation Sequencing (n=7), real-timenpolymerase chain reaction (n=2) and Whole Genome Sequencing (2). The limit of detection was the cut-off identified in 17 studies. Ten studies reported the concordance rate between plasma and tumor tissue. The overall concordance rate between plasma- and tissue-based analyses was approximately 62% (223/359) (Online supplemental material 3). The overall survival was used as a clinical endpoint in almost 50% of studies (13/26), whereas 20/26 studies reported progression-free survival. A total of 11 studies reported the HR for the association between survival analysis and ctDNA. Only three of them reported survival analysis in patients with only FIGO advanced stages of the disease. In the remaining studies progression-free survival and overall survival were not stratified for stages. Regarding the time of collecting ctDNA samples, three studies reported progression-free survival or overall survival with HR related to ctDNA immediately after debulking surgery,[6 22 28] two studies before debulking surgery,[15 30] four studies at baseline prior to chemotherapy,[8 21 33 35] and three studies after three cycles of treatment[8 19 28] (Table 2).

Risk of Bias of Included Studies

The study quality scores of the observational studies ranged from 7 to 9 according to the Newcastle–Ottawa Scale, indicating high quality. The two randomized controlled trials were evaluated using ROB-2 and the overall RoB had some concerns. This was predominantly affected by domain 1, bias arising from the randomization process (see Online supplemental material 4). In the bias due to deviations from the intended intervention, bias due to missing outcome data and bias in measurement of the outcome, all studies had a low RoB. The funnel plots showed asymmetries for progression-free survival and overall survival that could suggest the presence of publication bias (see Online supplemental material 5).

Table 1 Characteristics of included studies

Study	Country	Sample	Time	Patients with OC	Sub-type/Stage	Method	Genes	Cut-off	Therapy	Response assessment	Prognostic significance	Median follow-up	OS	PFS
Tp53														
Parkinson 2016[22]	UK 2009–2011	Plasma	BT, DT	40	HGS-EOC, FIGO I–IV	d PCR	Tp53	>20 AC/mL	Platinum and non-platinum-based CTX	RECIST 1.1 criteria	Patients whose ctDNA level had a decrease of >60% after one cycle of CTX had a significantly longer TTP than those whose ctDNA level decreased <60%	59 months (range 43–70)	NA	HR 1.03 (CI 1.01 to 1.05), p=0.019
Swisher 2005[13]	USA	Plasma, serum	BT	137	EOC, FIGO I–IV	RT-PCR	Tp53	Detection	Taxane and platinum	NA	ctDNA was an independent predictor of decreased survival in multivariate analysis. OS was significantly reduced in cases with ctDNA in plasma	NA	Log rank test: p=0.01, median survival 28 vs 56 months	NA
Sabatier 2022[7]	France 2014–2019	Plasma	AT	24 PR	EOC, FIGO III–IV	LC-WGS + WES	Tp53	Detection	Platinum-based CTX, Lapatinib Sorafenib Everolimus	NA	Some ctDNA characteristics such as genome altered fraction and plasma mutation burden showed prognostic value	NA	NA	HR 8.6 (CI 1.4 to 52), p=0.041
Kfoury 2020[23]	France	Plasma	BS, BT, DT, at relapse	38	HGOC, FIGO I–IV	NGS	Tp53	Detection	NA	NA	Detection of TP53m in ctDNA after debulking surgery was significantly associated with poor outcome	NA	HR 1.38 (CI 0.38 to 4.9)	HR 3.71 (CI 1.2 to 11.42), p=0.02
Kim 2019[31]	Korea 2013–2017	Plasma	BS, DT, AT	61	HGSOC- FIGO I–IV	d PCR	Tp53	0.2 copies/µL	Paclitaxel and carboplatin	RECIST 1.1 criteria	Presence of ctDNA was an independent predictor of survival (p=0.02)	48,9 months (range 5.6–50.1)	NA	p=0.038
Chao 2022[6]	Taiwan 2016–2017	Plasma	BS, AS, BT, DT	29	EOC, FIGO I–IV	Ultra-deep NGS	Tp53	Detection	Platinum ± bevacizumab	RECIST 1.1 criteria	Presence of ctDNA mutations immediately after surgery were independent predictors of PFS and OS	33.15 months (range 0.79–46.13)	HR 6.56 (95% CI 1.07 to 40.17), p=0.042	HR 8.41 (CI 2.49 to 28.39)
Minato 2021[11]	Japan	Plasma	AT	11	HGSOC, FIGO III–IV	dd PCR	Tp53, ARID1A, PIK3CA	Detection	PARP inhibitor Paclitaxel+platinum; gemcitabine	NA	6/11 cases showed apparent recurrence during follow-up and all 6 cases were positive in ddPCR analyses	NA	NA	Log-rank test, p=0.0038

Continued

Table 1 Continued

Study	Country	Sample	Time	Patients with OC	Sub-type/Stage	Method	Genes	Cut-off	Therapy	Response assessment	Prognostic significance	Median follow-up	OS	PFS
Vanderstichele 2019[34]	Belgium	Plasma	BT, DT	119	EOC, FIGO I–IV	RT-PCR	Tp53	Detection	Paclitaxel ± ganetespib	RECIST 1.1	Detection of ctDNA at baseline and after second cycle of chemotherapy predicted a worse OS	NA	NA	HR 2.80 (95% CI 1.60 to 4.90)
meth-HOXA9														
Rusan 2020[20]	Denmark	Plasma	BT, DT	24 PR + BRCA mutated	EOC, FIGO I–IV	d PCR	HOXA9 meth-ctDNA	Detection	PARP inhibitor (veliparib)	RECIST 1.1 criteria	After three treatment cycles, patients with detectable HOXA9 meth-ctDNA had a worse PFS and OS compared with patients with non-methylated HOXA9	NA	HR 7.29 (CI 2.46 to 21.58)	HR 6.33 (CI 2.13 to 18.76)
Steffensen 2018[36]	Denmark	Plasma	BT	32	EOC, FIGO I–IV	dd PCR	HOXA9 meth-ctDNA	Detection	PARP inhibitor (veliparib)	RECIST 1.1 criteria	Patients with methylated HOXA9 showed a worse PFS compared with patients with non-methylated HOXA9	NA	NA	p=0.056, HR 2.53 (CI 0.98 to 6.54)
Faboorg 2022[9]	Denmark	Plasma	BT, DT	126	EOC, FIGO I–IV	dd PCR	HOXA9 meth-ctDNA	Detection	Platinum Liposomal Doxorubicin Paclitaxel Topotecan Treosulfan Gemcitabine Vinorelbine Bevacizumab	RECIST criteria	Median OS in patients with an increase in meth-HOXA9 after one treatment cycle was 5.3 months compared with 33 months in patients with undetectable meth-HOXA9 (p<0.001)	NA	HR 2.04 (CI 1.29 to 3.23), log-rank p=0.002	p<0.001
Jakobsen 2021[14]	Denmark	Plasma	BT, DT	48 PR+BRCA mutat	EOC, FIGO I–IV	Bisulphite conversion + dd PCR	HOXA9 meth-ctDNA	Detection	PARP inhibitor (veliparib)	RECIST criteria	ctDNA response rate seems to outperform objective response rate with respect to correlation with survival.	NA	p=0.02	NA
Thomsen 2019[35]	Denmark	Plasma	BS, DT	23 PR	EOC, FIGO I–IV	qPCR	HOXA9 meth-ctDNA	Increase vs decrease*	Bevacizumab + tocotrienol	RECIST 1.1	Patients with an increasing level of HOXA9 meth- ctDNA had a median PFS and OS of 1.4 and 4.3 months, respectively, compared with 7.8 and 12 months in the group with stable or decreasing levels.	NA	p=0.01	p=0.01
CNA profiling														

Continued

Table 1 Continued

Study	Country	Sample	Time	Patients with OC	Sub-type/Stage	Method	Genes	Cut-off	Therapy	Response assessment	Prognostic significance	Median follow-up	OS	PFS
Nakabayashi 2018[15]	Japan	Plasma	BS	36	EOC, FIGO I–IV	Low-coverage WGS	CNA profiling	Gain/loss: 10 Mb	NA	NA	In cases with advanced-stage ovarian cancer, Kaplan–Meier analyses showed shorter PFS but did not find a difference in OS	NA	p=0.234, HR 4.69 (95% CI 0.418 to 52.6)	p=0.043, HR 9.71 (95% CI 1.10 to 85.3)
BRCA reversion mutation														
Lin 2019[32]	USA	Plasma	BS, BT	78	HGS-EOC,	Targeted NGS, hybridization-based	BRCA1–2 reversions mutations	Detection	PARP inhibitor	RECIST 1.1 criteria	Patients with BRCA-mutant cancers but no BRCA reversion mutations detected in cfDNA had significantly longer PFS after rucaparib treatment than those with reversion mutations	NA	NA	HR, 0.12, (CI 0.05 to 0.26), p<0.0001
Kim 2023[33]	Korea 2018–2021	Plasma	BS, DT	54 BRCA-mutated	EOC, FIGO I–IV	NGS	BRCA reversion mutation	Detection	PARP inhibitor	NA	Poor PFS with subsequent CTX in patients with homologous recombination repair restoration and those with the simultaneous involvement of two or more resistance mechanisms.	NA	NA	p=0.003
Miscellaneous														
No 2012[30]	Korea	Serum	BS	36	EOC, FIGO I–IV	RT-PCR	B2M, RAB25, CLDN4, ABCF2	High vs low	Platinum-based adjuvant chemotherapy	NA	A low RAB25 level was an independent prognostic factor for DFS and OS	30.7 months	HR 33.6 (CI 1.8 to 634.8)	HR 18.2 (CI 2.0 to 170.0)
Hou 2022[29]	USA	Plasma	Cohort A BS, Cohort B & C: DT	69	EOC, stage I–IV	Personalized multiplex-PCR NGS assay	TP53, ARID1A KRAS PIK3CA, EPPK1, BRAFATMBRCA1-2 PTEN	High vs low	NA	NA	Presence of ctDNA post-surgically is highly prognostic of reduced RFS	A: 2.7 years (range 0.08–16.7) B & C: 2 years (range 0.3–4.6)	NA	HR 17.6 (95% CI 3.2 to 97.4), p=0.001
Dobilas 2022[27]	Sweden	Plasma	BS	26	EOC, stage I–IV	d PCR	TP53, KRASPIK3CA, PIK3R1, BRAF	10 mutant copies/mL	NA	NA	Patients with >10 ctDNA mutant copies/mL in plasma BS had significantly worse OS	NA	p=0.008	NA

Continued

Table 1 Continued

Study	Country	Sample	Time	Patients with OC	Sub-type/Stage	Method	Genes	Cut-off	Therapy	Response assessment	Prognostic significance	Median follow-up	OS	PFS
Heo 2022[28]	Korea	Plasma	BS, DT	170	EOC, stage I–IV	NGS	TP53, BRCA1-2, ARID1A, CCNE1, KRAS, MYC, PIK3CA, PTEN			CA125, HE4, MRI, and PET-CT	Based on 6 months follow-up ctDNA analysis, the persistently elevated group showed a shorter median survival compared with zero conversion group (7.9 vs 31.2 months; p<0.001)			
Noguchi 2021[25]	Japan 2017–2019	Plasma	BS, BT, DT, AT	51	OC, stage I–IV	NGS	TP53, APC, KRAS, EGFR, MET, and PIK3CA	Detection/ level†	Paclitaxel and carboplatin ± bevacizumab irinotecan	RECIST 1.1 criteria	Higher cfDNA significantly correlated with worse PFS in all patients as well as stage III–IV patients. Further, patients with any pathogenic mutations showed significantly worse PFS	NA	NA	Log-rank test: p=0.048
Elazezy 2021[8]	Germany	Plasma	BT, at relapse, DT	69	HGSOC	Methylation-specific quantitative real-time PCR	BRCA1 promoter meth	Detection	Carboplatin, cisplatin	NA	Patients with methylated BRCA1 promoter detected in cfDNA had a significantly longer survival than patients with unmethylated BRCA1 promoter	39.3 months (range 25.0–49.6)	NA	95% CI 28.0 to 52.3; p=0.0019, log-rank test
Giannopoulou 2018[9]	Greece	Plasma	BS	128	HGSOC	RT-MSP + MS-HRMA	RASSF1A promoter meth	Detection	NA	NA	OS was significantly associated with RASSF1A promoter methylation in primary tumor samples using MS-HRMA	NA	log-rank test, p=0.023	NA
Kuhlmann 2012[27]	Germany	Serum	BS, AT	63	EOC, FIGO I–IV	Fluorescence-labeled PCR for microsatellite	LOH profiling, microsatellite alterations	High vs low	Carboplatinum and paclitaxel	NA	LOH at D6S1581 in both combined fractions was predictive of a reduced OS (p=0.030)	3.04 years (range 0.08–5.83)	OS (p=0.030)	NA
Tserpeli 2021[19]	Greece	Plasma	BS	55	HGSOC, FIGO I–IV	RT-Meth-specific PCR	SLFN11 promoter, meth	Detection	Platinum-based chemotherapy	NA	Methylation of SLFN11 in plasma cfDNA was significantly correlated with worse PFS in advanced stage HGSOC	NA	NA	p=0.045, log-rank test

Continued

Table 1 Continued

Study	Country	Sample	Time	Patients with OC	Sub-type/Stage	Method	Genes	Cut-off	Therapy	Response assessment	Prognostic significance	Median follow-up	OS	PFS
Ogasawara 2020[24]	Japan 2010–2016	Plasma	BS	85	EOC, FIGO I–IV	dd PCR	PIK3A, KRAS	Detection	NA	NA	ctDNA detection was associated with both shorter PFS and OS in EOC patients. Similar trends were observed when the mutations for PIK3CA or KRAS were analyzed separately	NA	PIK3CA p=0.118 KRAS 0.072	PIK3CA p=0.008 KRAS p=0.004

*The group with a level increasing above the 95% CI of baseline was designated 'Increasing Value' patients and compared with the group having stable or decreasing values designated 'Stable Value' patients.
†Authors patients into two groups using the median values (high vs low).
‡CNAs were counted in plasma as a gain or loss if they exceeded 10 Mb from the expected diploid coverage.
AC, amplifiable copies; AS, after surgery; AT, after treatment; BS, before surgery; BT, before treatment; CNA, copy number alterations; CTX, chemotherapy; dd, droplet digital; DT, during treatment; EOC, epithelial ovarian cancer; FIGO, International Federation of Gynecology and Obstetrics; GAF, genome altered fraction; HGSOC, high-grade serous ovarian cancer; LC-WGS, low-coverage whole-genome sequencing; LOH, loss of heterozygosity; MS-HRMA, methylation-sensitive high-resolution melting analysis; NGS, Next Generation Sequencing; OS, overall survival; PFS, progression-free survival; PR, platinum resistant; RT-MSP, real-time methylation-specific polymerase chain reaction ; RT-PCR, real-time polymerase chain reaction; TMB, mutation burden; TTP, time to progression; WES, whole-exome sequencing.

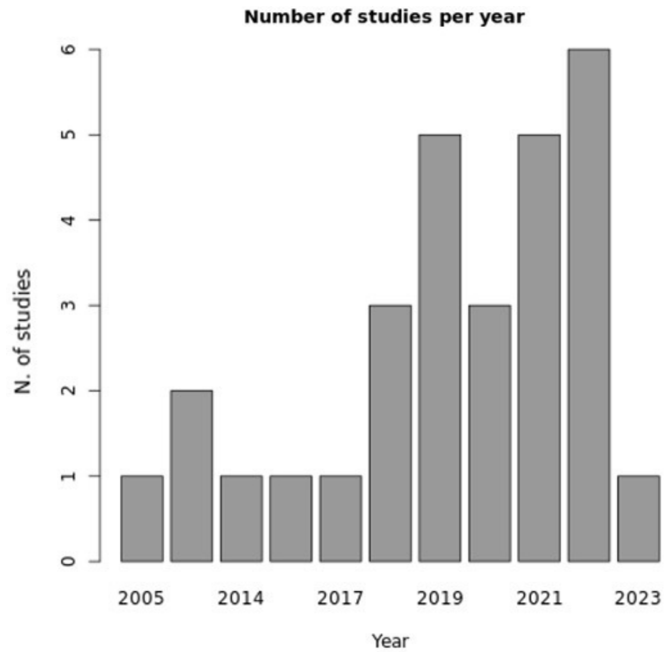

Figure 2 Number of studies per year.

Synthesis of Results

Meta-Analysis

Of the studies that reported HR, nine were pooled for meta-analysis of progression-free survival. The results showed that a high level of ctDNA in epithelial ovarian cancer was associated with worse progression-free survival (HR 5.31, 95% CI 2.14 to 13.17, $p<0.001$). A substantial heterogeneity was observed ($I^2=86.0\%$), so a random-effect model was applied during calculation (Figure 3A).

To investigate whether the timing of collecting ctDNA samples affected its ability to predict outcomes, we conducted a sub-group analysis (see Online supplemental material 6). A shorter progression-free survival associated with ctDNA positivity was consistently observed in samples collected before cytoreductive surgery (HR 13.24, 95% CI 2.81 to 62.40, $p=0.001$, $I^2=0\%$), immediately after surgery (HR 6.97, 95% CI 3.04 to 15.97, $p<0.001$, $I^2=17\%$) and after three cycles of treatment (HR 8.51, 95% CI 3.40 to 21.32, $p<0.001$). The sensitivity analysis was performed by including only those studies that considered the limit of detection as cut-off (HR 2.85, 95% CI 1.29 to 6.33, $p=0.01$). Additionally, we included only the HRs provided for sub-groups of patients with advanced stages FIGO III–IV (HR 5.40, 95% CI 2.13 to 13.68, $p<0.001$).

Seven studies that provided HR were pooled for meta-analysis of overall survival. Similarly, a positive association was found between ctDNA presence and the overall survival of patients with epithelial ovarian cancer (HR 2.98, 95% CI 1.86 to 4.76, $p<0.0001$) with moderate heterogeneity ($I^2=34\%$) (Figure 3B). We conducted a sub-group analysis based on the timing of ctDNA sample collection. A shorter overall survival was observed in association with ctDNA detection before primary cytoreductive surgery, which was found to be statistically significant (HR 10.64, 95% CI 1.59 to 71.27, $p=0.01$, $I^2=2\%$).

Sub-Group Analysis: Tp53

Data on Tp53 as genomic alteration were available in four studies. Due to substantial heterogeneity between the studies ($I^2=77\%$), a random

Table 2 Studies that reported progression-free-survival and overall survival in terms of HR

Study	Time point for blood draw referred to available results	Type of treatment	NACT	PFS (HR)	OS (HR)*	Time ctDNA preceded radiological findings
Chao 2022[6]	Immediately after surgery before adjuvant chemotherapy (AS)	Debulking + platinum + paclitaxel ± bevacizumab	Yes	**AS**: HR 8.41 (95% CI 2.49 to 28.39)	**AS**: HR 6.56 (95% CI 1.07 to 40.17), p=0.042	NR
No 2012[30]	Before surgery (BS)	Debulking + platinum-based adjuvant chemotherapy	NR	**BS**: HR 18.2 (95% CI 2.0 to 170.0)	**BS**: HR 33.6 (95% CI 1.8 to 634.8)	NR
Nakabayashi 2018[15]	Before surgery (BS)	Debulking surgery + chemotherapy	NR	HR 9.71 (95% CI 1.10 to 85.3)	HR 4.69 (95% CI 0.41 to 52.6)	NR
Parkinson 2016[22]	**C1D1**: prior to treatment at baseline (cycle 1 day 1),	Platinum and non-platinum-based chemotherapy	NR	**C1D1**: HR 1.03 (CI 1.01 to 1.0)	NR	NR
Steffensen 2018[36]	C1D1: prior to treatment at baseline	Before initiation of daily oral single agent veliparib	NR	**C1D1**: HR 2.53 (95% CI 0.98 to 6.54)	NR	NR
Sabatier 2022[7]	After initiation of treatment (AT)	Prior line of chemotherapy before the sample: 3 (median)	NR	**AT**: HR 8.6 (95% CI 1.4 to 52)	NR	NR
Kfoury 2020[23]	► Immediately after debulking surgery (AS) ► Longitudinal samples during treatment (DT)	Debulking + chemotherapy	NR	**AS**: HR 2.83 (95% CI 0.6 to 12.9) **DT**: HR 3.71 (95% CI 1.2 to 11.42)	**DT**: HR 1.38 (95% CI 0.38 to 4.9)	NR
Hou 2022[29]	**Cohort A**: before surgery **Cohort B**: following cytoreductive surgery (after 30 days) **Cohort C**: after the completion of adjuvant therapy	► NACT + interval debulking procedure (3%) ► Primary debulking procedure ± adjuvant therapy (64%) ► Unknown (33%)	Yes, (2/69) 3%	**Cohort B and C**: HR 17.6 (95% 3.2 to 97.4), p<0.001	NR	10 months (compared with ~1 month when assessed by CA-125 status)
Rusan 2020[20]	After 3 cycles of treatment (after C3)	Veliparib (300 mg twice daily p.o. on days 1e18)	NR	**After C3**: HR 6.33 (95% CI 2.13 to 18.76)	**After C3**: HR 7.29 (95% CI 2.46 to 21.58)	NR
Vanderstichele 2019[34]	**C1D1**: prior to treatment at baseline (cycle 1 day 1) **C1D2**: 24 hours later (cycle 1 day 2) **C2D1**: at day 1 of cycle 2	Weekly paclitaxel with or without Hsp90-inhibitor ganetespib	NR	NR	**C1D1:** HR 2.3 (95% CI 1.4 to 3.9) **C1D2:** HR 2.2 (95% CI 1.3 to 3.9) **C2D1:** HR 2.8 (95% CI 1.6 to 4.9)	NR
Faboorg 2022[9]	► at baseline ► at the second treatment cycle (C2) ► after 3 cycles of treatment (After C3)	Chemotherapy	NR	NR	**At baseline**: HR 1.89 (95% CI 1.18 to 3.01) **C2:** HR 2.99 (95% CI 1.73 to 5.18) **After C3:** HR 2.17 (95% CI 1.18 to 3.98)	NR

For variables with HR >1, an increase in the value is associated with a higher risk or number of events and a decreased PFS or OS.
*Multivariate Cox regression analyses.
ctDNA, circulating tumor DNA; NACT, neoadjuvant chemotherapy; OS, overall survival; PFS, progression-free survival.

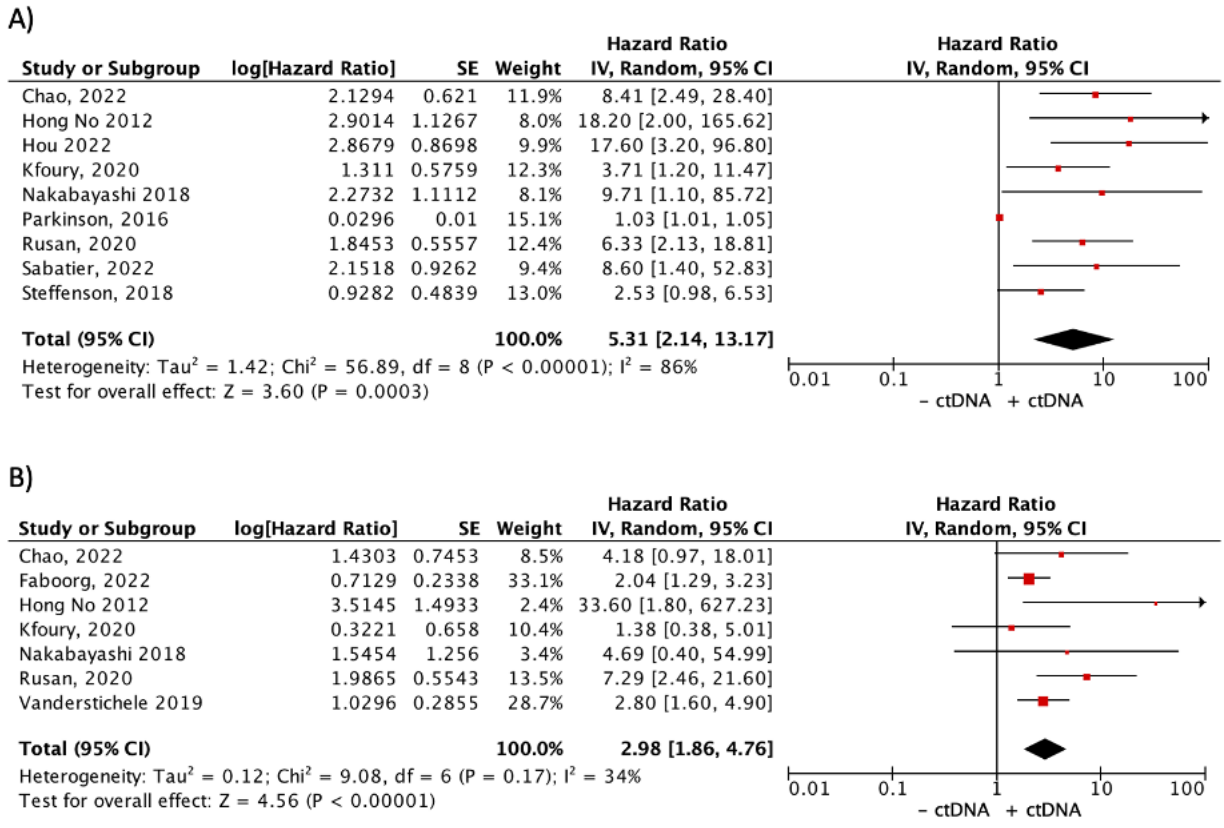

Figure 3 A) Forest plot of comparison of progression-free survival. (B) Forest plot of comparison of overall survival.

effect model was applied. The results showed that ctDNA is associated with decreased progression-free survival in patients with epithelial ovarian cancer but the meta-analytic effect did not reach statistical significance (HR 2.38, 95% CI 0.94 to 6.02, p=0.07). Of these studies, the detection method was Next Generation Sequencing in three studies and in one study a combination of LC-Whole Genome Sequencing and Whole-exome sequencing was used to detect ctDNA. However, the results did not change substantially after the exclusion of this study (Sabatier et al) from the sensitivity analysis (HR 1.81, 95% CI 0.77 to 4.28). Data were insufficient to evaluate overall survival outcome.

Sub-Group Analysis: Meth HOXA-9

In the sub-group analysis for Meth HOXA-9, two studies enrolled patients treated with a PARP inhibitor (veliparib). In all studies Meth-HOXA9 was assessed as a binary variable (detectable vs undetectable).

Two studies reported HR for overall survival and progression-free survival on methylation status in HOXA9 in samples during treatment[9 20] while, in another study, a blood sample was taken only at baseline before chemotherapy.[36] The sub-group analysis showed a greater than threefold increased risk of relapse in patients with positive HOXA9 meth-ctDNA (HR 3.84, 95% CI 1.57 to 9.41, p=0.003).

Moderate heterogeneity was observed (I^2=35%, p=0.111), so a random-effect model was applied during the calculation.

DISCUSSION

Summary of Main Results

In pooled analyses we observed that, irrespective of the genomic alterations, patients with detectable ctDNA/high levels of ctDNA had a significantly worse prognosis than patients with ctDNA undetectable/low levels with lower overall survival and progression-free survival. Moreover, a sub-group analysis of the results of the three studies focusing on ctDNA levels after cytoreductive surgery showed that high levels of ctDNA at that timepoint were correlated with a shorter progression-free survival.

Results in the Context of Published Literature

ctDNA is released into the bloodstream by cancer cells through processes such as cell death and apoptosis in cancer cells. These fragments of DNA contain genetic information specific to the tumor from which they originate.[2 5] Levels of ctDNA can vary according to anatomical proximity to vasculature and cancer type. In a study including 136 metastatic tumors originating from 14 different tissue, Bettegowda et al found that ctDNA was detectable in >75% of patients with advanced ovarian cancer.[45] Moreover, previous studies indicated that quantification of ctDNA correlates well with tumor burden in solid tumors.[46]

Currently, response to treatment and the pertaining clinical decisions are based on imaging and serial CA-125 measurements, with many limitations. First, CT imaging does not fully represent the molecular and pathologic changes that occur within the tumor microenvironment during treatment. Additionally, CT scans can yield different interpretations among radiologists. In a study including

patients with pancreatic cancer, Sausen et al evaluated the time to detection of recurrence comparing ctDNA and CT imaging. The results showed that the average time to recurrence was significantly shorter for patients with detectable ctDNA than for those patients with positive imaging results.[47]

In another study, Pereira et al reported that ctDNA can detect ovarian cancer relapse 7 months earlier than CA-125 and CT scan imaging, while the median time from increased CA-125 to clinical relapse evaluated by CT scan ranged from 2 to 6 months[21] Second, CA-125 is a sensitive biomarker but it lacks specificity because it is widely expressed in tissues other than ovarian cancer and its expression is altered in some benign conditions and other type of malignancies.[48] Moreover, CA-125 does not reflect the present state of ovarian cancer recurrence because it responds to tumor changes with some delays due to its prolonged half-life (9–44 days).[49] On the other hand the half-life of ctDNA in the blood circulation ranges from 16 min to 2.5 hours, representing a real-time perspective picture of tumor burden and a valid tool for real-time monitoring of response to treatment.[22] Furthermore, a large retrospective analysis showed that early change in CA-125 is a poor surrogate for progression-free survival in patients with recurrent ovarian cancer.[50]

Comparing CA-125 elevation and ctDNA detection for recurrence monitoring, Hou et al found that CA-125 was inferior in predicting recurrence when assessed at a single time point after definitive treatment ($p=0.113$ vs $p=0.001$) and longitudinally ($p=0.056$ vs $p<0.0001$).[29] Similarly, Parkinson et al reported that response to chemotherapy was seen earlier with ctDNA, with a median time to nadir of 37 days compared with a median time to nadir of 84 days for CA-125.[22] Consistent with this finding, Minato et al found that droplet digital PCR detected ctDNA signals significantly earlier than increased CA-125 in the detection of ovarian cancer recurrence by imaging (49 days and 7 days before, respectively; $p<0.05$).[25]

In our sub-group analysis we included five studies examining methylation of the HOXA9 gene as alternative targets for epithelial ovarian cancer ctDNA detection (Table 1). Homeobox genes (HOX) constitute a family of transcription factors that are involved in regulating differentiation and are expressed in normal adult reproductive tissue. Methylation of the HOXA9 gene has been observed in 95% of patients with high-grade serous ovarian cancer. Detection of HOXA9 meth-ctDNA was correlated with a worse survival than without non-methylated HOXA9. Three studies included in the present systematic review investigating the role of ctDNA in monitoring the response to veliparib showed that detection of HOXA9 meth-ctDNA longitudinally during PARP inhibitor treatment was feasible and carried prognostic value regarding progression-free survival and overall survival.

Strengths and Weaknesses

In this systematic review and meta-analysis, the strengths include a comprehensive search strategy, a moderate to low risk of bias, and a sub-group analysis based on study methodology and techniques. Nevertheless, our study has several limitations. First, except for three studies, most of the studies included are observational studies. Second, the lack of reporting follow-up periods and HR for both overall survival and progression-free survival resulted in a limited power of analysis. Third, despite our effort to perform a sub-group analysis, the included studies have significant heterogeneity in detection method, ctDNA genomic alterations, cut-off levels of ctDNA, chemotherapy regimens, and time at the sample and timeline of blood draw during patient care. In addition, approximately 30% of the studies included only high-grade serous ovarian cancer. Hence, these results can only be an estimate of the true predictive value of ctDNA in patients with epithelial ovarian cancer.

Implications for Practice and Future Research

Currently, data regarding the prognostic significance of ctDNA in patients with ovarian cancer are limited. Since there is a significant clinical need to develop a non-invasive biomarker to monitor therapeutic responses in real time, the results of this comprehensive analysis shed light on the promising potential of ctDNA to enhance personalized treatment strategies for patients with epithelial ovarian cancer. Identifying patients with an expected poor response to chemotherapy could aid in clinical decision-making regarding alternative treatment options. However, further research efforts are needed to increase the body of evidence on this biomarker and to validate a standardized approach for integrating ctDNA analysis into regular clinical protocols. Furthermore, due to the heterogeneity in ctDNA methodology and target mutations among published studies, we highlight the need for uniform reporting in future studies to better delineate the magnitude of the adverse effect of positive baseline/post-operative ctDNA in the prognosis of epithelial ovarian cancer.

CONCLUSIONS

In patients with epithelial ovarian cancer ctDNA strongly predicts worse progression-free survival and overall survival. The sub-group analysis showed that detectable ctDNA or high levels of ctDNA before and after cytoreductive surgery and after three cycles of treatment are associated with significantly shorter progression-free survival.

Author affiliations
[1]Department of Medical Sciences, Obstetrics and Gynecology Unit, "S. Anna" University Hospital, University of Ferrara, Ferrara, Italy
[2]Department of Development and Regeneration, KU Leuven, Leuven, Belgium
[3]Department of Medical Sciences, Section of Experimental Medicine and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy
[4]Department of Women and Child Health, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy
[5]Università Cattolica del Sacro Cuore Dipartimento Scienze della Vita e Sanità Pubblica, Rome, Italy
[6]Department of Obstetrics and Gynecology, University Hospitals KU Leuven, Leuven, Belgium
[7]Department of Obstetrics and Gynecology, University of Udine Medical Area Department, Udine, Italy
[8]IHU Strasbourg, Institute of Image Guided Surgery, Strasbourg, France
[9]Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy
[10]Azienda ULSS 9 Scaligera, Verona, Veneto, Italy

X Giuseppe Vizzielli @giusvizzielli

Contributors Concept and design: CT, GM, PP, CG, PG. Acquisition, analysis, or interpretation of data: CT, SS, MT, MP. Drafting of the manuscript: CT, GM, CN, WF. Critical revision of the manuscript for important intellectual content: GV, PP, PG, CG, MP, FF, WF. Statistical analysis: CT, SS. Administrative, technical, or material support: GM, PP. Supervision: GM, PP, WF, PG, GScu, GSca, GV. Guarantors: CT, PP.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests CN: travel support from AZ, Illumina. Sponsored talks from Veeva, Illumina, MDS, AZ.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

ORCID iDs
Cristina Taliento http://orcid.org/0000-0003-0278-1202
Giuseppe Vizzielli http://orcid.org/0000-0002-2424-2691
Matteo Pavone http://orcid.org/0000-0002-9791-9464
Giovanni Scambia http://orcid.org/0000-0003-2758-1063

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SUPPLEMENTARY MATERIAL

Supplementary material 1. Search Strategy

CENTRAL (Cohrane)

Ovarian Neoplasms [Mesh]
Ovar* Neoplasm* [TiAbKw]
Ovar* Cancer [TiAbKw]

AND

Circulating Tumor DNA [Mesh]
Circulating Tumor DNA [AllText]
Cell-Free Tumor DNA [AllText]
Cell Free Tumor DNA [AllText]
ctDNA [AllText]

AND

Progression-Free Survival [Mesh]
Prognosis [Mesh]
Disease-Free Survival [Mesh]
Prognosis [TiAbKw]
Prognoses [TiAbKw]
Progression-Free Survival [TiAbKw]
Progression Free Survival [TiAbKw]
Event-Free Survival [TiAbKw]
Event Free Survival [TiAbKw]
Prognostic Factor* [TiAbKw]
Disease-Free Survival [TiAbKw]
Disease Free Survival [TiAbKw]
Overall Survival [TiAbKw]

Complete:

ID	Search	Hits
#1	MeSH descriptor: [Ovarian Neoplasms] explode all trees	
#2	(Ovar* Neoplasm*):ti,ab,kw	
#3	(Ovar* Cancer):ti,ab,kw	

#4	**#1 OR #2 OR #3**
#5	**MeSH descriptor: [Circulating Tumor DNA] explode all trees**
#6	**(Circulating Tumor DNA)**
#7	**(Cell-Free Tumor DNA)**
#8	**(Cell Free Tumor DNA)**
#9	**(ctDNA)**
#10	**#5 OR #6 OR #7 OR #8 OR #9**
#11	**MeSH descriptor: [Progression-Free Survival] explode all trees**
#12	**MeSH descriptor: [Prognosis] explode all trees**
#13	**MeSH descriptor: [Disease-Free Survival] explode all trees**
#14	**(Prognosis):ti,ab,kw**
#15	**(Prognoses):ti,ab,kw**
#16	**(Progression-Free Survival):ti,ab,kw**
#17	**(Progression Free Survival):ti,ab,kw**
#18	**(Event-Free Survival):ti,ab,kw**
#19	**(Event Free Survival):ti,ab,kw**
#20	**(Prognostic Factor*):ti,ab,kw**
#21	**(Disease-Free Survival):ti,ab,kw**
#22	**(Disease Free Survival):ti,ab,kw**
#23	**(Overall Survival):ti,ab,kw**
#24	**#11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20 OR #21 OR #22 OR #23**
#25	**#4 AND #10 AND #24**

15/09/2023 78 risultati (records)

CINAHL Complete

Complete:

AB (ovarian neoplasms or ovarian cancer) AND TX (circulating tumor dna or ctdna) AND TX (prognosis or outcome or recovery or predictor or survival)

Link permanente:

https://search-ebscohostcom.proxy2.biblio.supsi.ch/login.aspx?direct=true&db=ccm&bquery=AB+(+ovarian+neoplasms+or+ovarian+cancer+)+AND+TX+(+circulating+tumor+dna+or+ctdna+)+AND+TX+(+prognosis+or+outcome+or+recovery+or+predictor+or+survival+)&type=1&searchMode=Standard&site=ehost-live

15/09/2023 28 risultati (records)

PUBMED

Ovarian Neoplasms [Mesh]

Ovar* Neoplasm* [TiAb]
Ovar* Cancer [TiAb]

AND

Circulating Tumor DNA [Mesh]
Circulating Tumor DNA [TextWord]
Cell-Free Tumor DNA [TextWord]
Cell Free Tumor DNA [TextWord]
ctDNA [TextWord]

AND

Progression-Free Survival [Mesh]
Prognosis [Mesh]
Disease-Free Survival [Mesh]
Prognosis [TiAb]
Prognoses [TiAb]
Progression-Free Survival [TiAb]
Progression Free Survival [TiAb]
Event-Free Survival [TiAb]
Event Free Survival [TiAb]
Prognostic Factor* [TiAb]
Disease-Free Survival [TiAb]
Disease Free Survival [TiAb]
Overall Survival [TiAb]

Search Strategy:

"ovarian neoplasms"[MeSH Terms] OR ("ovar*"[All Fields] AND "neoplasm*"[Title/Abstract]) OR ("ovar*"[All Fields] AND "Cancer"[Title/Abstract])

AND

"circulating tumor dna"[MeSH Terms] OR "circulating tumor dna"[Text Word] OR "cell free tumor dna"[Text Word] OR "cell free tumor dna"[Text Word] OR "ctDNA"[Text Word]

AND

"progression free survival"[MeSH Terms] OR "Prognosis"[MeSH Terms] OR "disease free survival"[MeSH Terms] OR "Prognosis"[Title/Abstract] OR "Prognoses"[Title/Abstract] OR "progression free survival"[Title/Abstract] OR "progression free survival"[Title/Abstract] OR "event free survival"[Title/Abstract] OR "event free survival"[Title/Abstract] OR "prognostic factor*"[Title/Abstract] OR "disease free survival"[Title/Abstract] OR "disease free survival"[Title/Abstract] OR "overall survival"[Title/Abstract]

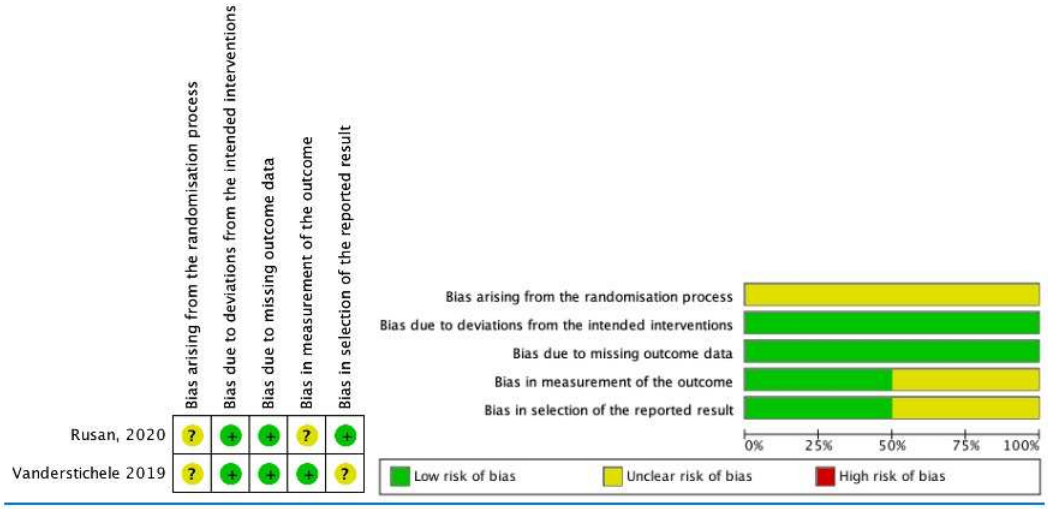
Completa:

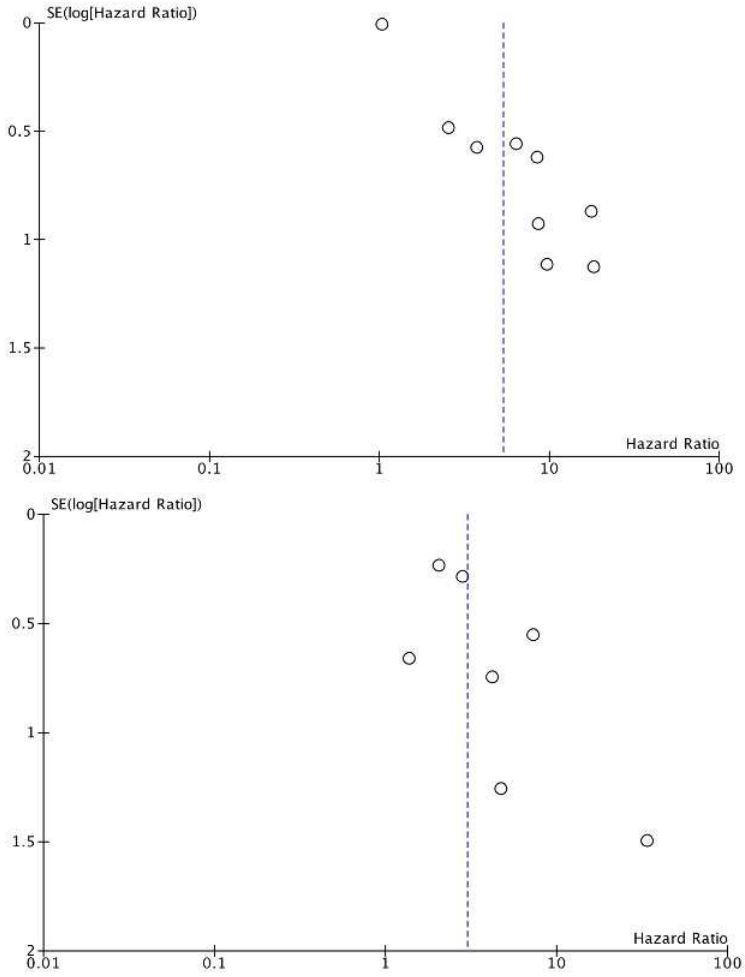
("ovarian neoplasms"[MeSH Terms] OR ("ovar*"[All Fields] AND "neoplasm*"[Title/Abstract]) OR ("ovar*"[All Fields] AND "Cancer"[Title/Abstract])) AND ("circulating tumor dna"[MeSH Terms] OR "circulating tumor dna"[Text Word] OR "cell free tumor dna"[Text Word] OR "cell free tumor dna"[Text Word] OR "ctDNA"[Text Word]) AND ("progression free survival"[MeSH Terms] OR "Prognosis"[MeSH Terms] OR "disease free survival"[MeSH Terms] OR ("Prognosis"[Title/Abstract] OR "Prognoses"[Title/Abstract] OR "progression free survival"[Title/Abstract] OR "progression free survival"[Title/Abstract] OR "event free survival"[Title/Abstract] OR "event free survival"[Title/Abstract] OR "prognostic factor*"[Title/Abstract] OR "disease free survival"[Title/Abstract] OR "disease free survival"[Title/Abstract] OR "overall survival"[Title/Abstract]))

Supplemental Material 2. Table of excluded studies

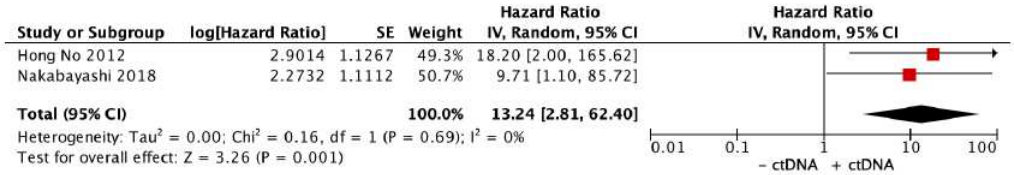
Reason for exclusion	Number of excluded studies	Authors and year
Including only cfDNA or mtDNA	4	Dobrzycka 2010, Meng 2019, Kalavska 2014, Steffensen 2014
OS and/or PSF not available	2	Otsuka 2004, Paracchini 2022
Including not only EOC	2	Charo 2020, Iwahashi 2019
Not specifying if plasma or serum	1	Wimberger 2011

Study	*Patients with OC*	*Detection Method*	*Concordance rate between blood and tumor*
Kim 2019	61	Droplet digital PCR	100% (38/38)
Swisher 2005	137	Real-time PCR	30% (21/69)
Chao 2022	29	Ultra-deep NGS analysis	58.6% (17/29)
Minato 2021	11	Droplet digital PCR	36.6% (4/11)
Dobilas 2022	26	Droplet digital PCR	62,5% (15/24)
Heo 2022	170	NGS	92.9% (39/42)
Noguchi 2021	51	NGS	81% (13/16)
Giannopoulou 2017	128	Real-Time Methylation-Specific PCR	62.3% (33/53)
Tserpeli 2021	209	Real-Time Methylation-Specific PCR	86.5% (32/37)
Ogasawara 2020	85	Droplet digital PCR	PIK3CA 27.5% (11/40), KRAS 25.0% (12/48)

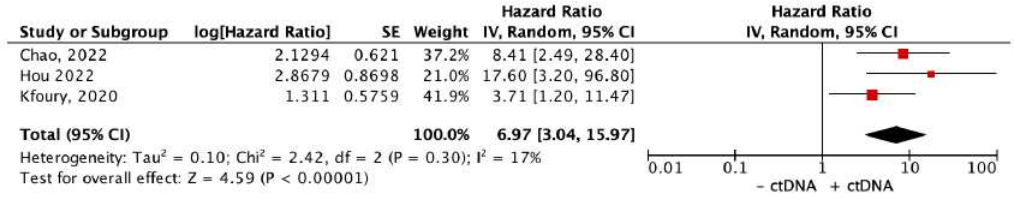

Bias arising from the randomisation process
Bias due to deviations from the intended interventions
Bias due to missing outcome data
Bias in measurement of the outcome
Bias in selection of the reported result
Rusan, 2020
Vanderstichele 2019
Bias arising from the randomisation process
Bias due to deviations from the intended interventions
Bias due to missing outcome data
Bias in measurement of the outcome
Bias in selection of the reported result
0%
25%
50%
75%
100%
Low risk of bias
Unclear risk of bias
High risk of bias


SE(log[Hazard Ratio])
0
0.5
1
1.5
2
0.01
0.1
1
10
100
Hazard Ratio
SE(log[Hazard Ratio])
0
0.5
1
1.5
2
0.01
0.1
1
10
100
Hazard Ratio

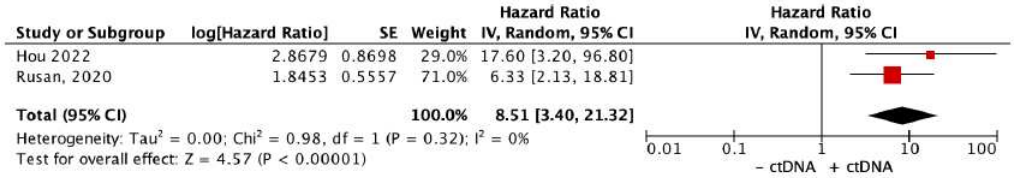
PSF_ Before surgery



PFS_After surgery



PFS_After 3 cycles of chemotherapy



OS_ Before surgery