Journal of Ovarian Research

RESEARCH

Open Access

Risk-stratified CA125 screening integrating CA125 trajectories, trajectory-specific progression and transvaginal ultrasound for ovarian cancer

Hongyuan Duan[1], Xiaomin Liu[1], Yu Zhang[1], Ya Liu[1], Yuting Ji[1], Yunmeng Zhang[1], Zeyu Fan[1], Siwen Liu[1], Lei Yang[2], Tingting Xu[3], Jing Tian[4], Weiqin Li[5], Zhangyan Lyu[1], Fangfang Song[1], Fengju Song[1] and Yubei Huang[1*]

Abstract

Backgrounds Cancer antigen 125 (CA125) is widely used for screening ovarian cancer (OC), yet its effectiveness remains debated. Potential factors may include ineffective cut-off value for CA125 in screening, as well as a lack of consideration for CA125 trajectories and trajectory-specific progression.

Methods Based on data from multiple rounds of CA125 tests and transvaginal ultrasound (TVU) examinations conducted on 28,456 women in the PLCO Trial, time-dependent receiver-operating-characteristic curves (ROCs) and area-under-the-curves (tdAUCs) analyses were employed to identify the optimal CA125 cut-off values for OC screening. Participants were categorized into four CA125 trajectories: stable negative CA125 ($CA125_{SN}$), loss of positive CA125 ($CA125_{LP}$), stable positive CA125 ($CA125_{SP}$), and gain of positive CA125 ($CA125_{GP}$). The associations between different CA125 trajectories, trajectory-specific progression indicators, and OC risk were explored. The effectiveness of risk-stratified CA125 screening, incorporating CA125 trajectories, trajectory-specific progression, and TVU, was evaluated using hazard ratio and 95% confidence intervals [HR (95%CIs)], with adjustments for potential confounders.

Results After a median follow-up of 14.8 years for OC incidence and 23.8 years for OC mortality, 250 OC cases and 218 OC deaths were identified. The tdAUC for 10-year OC incidence with CA125 was 0.663, with an optimal cut-off value of 13.00 U/ml. Trajectory analyses showed that both $CA125_{SP}$ and $CA125_{GP}$ were significantly associated with increased risks of OC incidence [HRs (95%CIs): 2.00(1.47–2.73) and 3.06(2.25–4.16)] and mortality [HRs (95%CIs):1.58(1.13–2.21) and 2.60(1.87–3.62)] compared to $CA125_{SN}$. Trajectory-specific progression analyses identified relative velocity as the optimal progression indicators for both $CA125_{SP}$ and $CA125_{GP}$ (tdAUCs: 0.712 and 0.767), with optimal cut-off values of 9% and 32% per year, respectively. Positive progression was associated with significantly increased risks of OC incidence [HRs (95%CI): 7.26(4.00-13.17) and 3.83(1.96–7.51) $CA125_{GP}$ and $CA125_{SP}$] and mortality [HRs (95%CI): 8.03(4.15–15.56) and 6.04(2.78–13.14)] compared to negative progression. Optimized risk-stratified

*Correspondence:
Yubei Huang
yubei_huang@163.com

Full list of author information is available at the end of the article

CA125 screening, which integrated CA125 trajectories, trajectory-specific progression, and TVU, reduced missed OC by 3.6% and improved accuracy compared to traditional screening methods.

Conclusions Incorporating CA125 trajectories and trajectory-specific progression into screening protocols enhances the identification of the population at high-risk of OC. An optimized screening strategy, which includes these factors along with TVU, is recommended to improve the effectiveness of OC screening.

Keywords Ovarian cancer; CA125, Trajectory, Screening, CA125 progression

Introduction

Ovarian cancer (OC) is a prevalent malignancy and a leading cause of cancer-related mortality among women worldwide. According to GLOBOCAN 2020, approximately 314,000 new cases and nearly 207,300 deaths from OC in 2020 [1]. Over 60% of OC cases are diagnosed at an advanced stage, resulting in 5-year survival rate of less than 30%, compared to 90% for localized disease [2–4]. Current OC screening methods, including serum cancer antigen 125 (CA125) testing, transvaginal ultrasound (TVU), or a combination of both, aim to detect the disease at an earlier stage and reduce OC mortality. However, significant reductions in OC mortality have not been observed in current randomized controlled trials (RCTs) [5–8], despite a notable shift toward earlier-stage disease in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) [6, 9].

Several factors may conjointly contributed to the lack of significant reduction in OC mortality, such as insufficient increases in early-stage cases and decreases in late-stage cases, low incidence rate resulting in low positive predictive value of screening methods, and the dilution effect of including diagnosed OC cases after screening ends [5, 6, 10]. One major factor could be the ineffective cut-off value of CA125 used in screening. Since most participants are healthy women, only a minority have CA125 level above the clinical diagnostic threshold of 35 U/ml. Consequently, relying on this cut-off in population-based screening may lead to missed diagnosis of preclinical OC. For low-incidence cancers, significant increases in early-stage cases and reductions in late-stage cases are necessary to demonstrate meaningful mortality reduction; otherwise, the impact of screening may remain marginal [10]. Additionally, previous studies indicated that approximately 20% of patients with OC have CA125 levels below 35 U/ml [11, 12], with even lower levels expected in asymptomatic women. This underscores the need to redefine the optimal CA125 cut-off value for effective screening.

Furthermore, studies have shown that CA125 levels increase rapidly over time in patients with preclinical OC, while levels remain relatively stable in women without OC, even if initial CA125 levels are elevated [10, 13–15]. Various indices and algorithms, such as the CA125 velocity [16], the empirical Bayesian longitudinal algorithm [17], and the widely used Risk of Ovarian Cancer Algorithm (ROCA) from the UKCTOCS trial [10, 13, 14], have been developed to assess CA125 progression and its association with OC risk. However, few studies have investigated the associations between CA125 trajectories and OC risk, and even fewer have examined optimal CA125 progression indicators within the same trajectory. Therefore, it is crucial to explore the associations and identify the most effective progression indicators. Additionally, while TVU is also recommended for OC screening [18], it remains unclear whether integrating CA125 trajectories, trajectory-specific progression indicators, and TVU into a combined screening approach would provide better outcomes compared to traditional strategy.

Therefore, based on data of multiple rounds of CA125 and TVU screening from the Prostate, Lung, Colorectal and Ovarian Cancer (PLCO) Screening Trial, this study aims to determine the optimal CA125 cut-off value for OC screening and examine the associations between different CA125 trajectories and OC risk. Then this study aimed to identify the optimal trajectory-specific progression indicators for women with elevated CA125 and explore their associations with OC risk. Finally, this study aimed to develop and compare optimized joint screening strategies that integrate CA125 trajectories, trajectory-specific progression indicators, and TVU, with traditional screening methods to assess potential improvements in screening performance.

Materials and methods

Study population

The PLCO Cancer Screening Trial was a multicenter randomized controlled trial (RCT) designed to investigate whether screening could reduce mortality from prostate, lung, colorectal and ovarian cancers. Details information about the trial have been provided elsewhere [19]. Briefly, from November 1993 to July 2001, 78,209 women aged 55 to 74 were recruited across ten PLCO screening centers. Eligible participants were randomly assigned to either the intervention or control groups in a 1:1 ratio. After providing informed consent, all participants completed a baseline questionnaire (BQ) that collected information on demographics, disease history, and lifestyle factors. For OC screening, the intervention group

received annual CA125 tests for 6 years and annual TVU for 3 years, while the control group received usual care [5, 19, 20].

Selection of participants

This study initially included 39,103 female participants from the screening arm. We excluded 1,095 women who did not have an eligible baseline questionnaire after informed consent, 4,833 women who had undergone bilateral oophorectomies, and 2,831 women who did not receive any CA125 test. This left 30,344 women who were initially eligible for this study. After further excluding 1,842 participants who had only one CA125 test before the end of screening and 46 women who had only one CA125 test prior to the diagnosis of OC, 28,456 women with at least two CA125 tests were finally included. A flowchart of participant selection was shown in Supplementary Figure S1.

Outcome measures

The definition of a positive OC screening result has been described in previous studies [5]. Screening results were communicated to participants and their healthcare providers by mail, typically within three weeks. Participants with positive screening results are encouraged to undergo diagnostic assessments. Cancer cases and deaths were identified through the Annual Study Update (ASU) questionnaire and supplemented by an annual search of National Death Index. When the ASU indicated a probable death, the PLCO Center obtained and reviewed the death certificate, and the Data Compilation Center coded the cause of death using ICD-9. The death review committee then determined whether ovarian cancer was the cause of death [5, 19]. Active follow-up for cancer diagnoses continued until December 2009, and extended follow-up data for deaths were updated through 2018. Therefore, the primary outcomes were censored at the date of the OC diagnosis (for OC incidence), death, loss of follow-up, or the end of the follow-up, whichever occurred first.

Statistical analysis

To determine the optimal screening cut-off value of CA125 in OC, time-dependent receiver operating characteristic curve (tdROC) and the area under the curve (tdAUC) were calculated using a COX regression model. Bootstrap resampling with 2000 iterations was employed to internally validate the stability of these results, with the median cut-off value of CA125 and its 95% confidence intervals (CIs). Based on the optimal screening CA125 cut-off value, four CA125 trajectories were defined: stable negative CA125 ($CA125_{SN}$) with CA125 below the cut-off in both first-round (FR) and last-round (LR) tests; loss of positive CA125 ($CA125_{LP}$) with FR positive and LR negative CA125; stable positive CA125 ($CA125_{SP}$) with positive CA125 in both FR and LR tests; and gain of positive CA125 ($CA125_{GP}$) with FR negative and LR positive CA125. Chi-square tests were used to assess significant differences in baseline characteristics across CA125 trajectories.

Among women with elevated CA125 within $CA125_{SP}$ and $CA125_{GP}$ categories, eight progression indicators were calculated based on baseline CA125 (i.e., FR CA125), CA125 increment, CA125 maximum, and time interval between CA125 tests. These indicators include: absolute increment (AbsInc) defined as the difference between FR and LR CA125 tests; maximum absolute increment (MaxAbsInc) as the difference between FR and the maximum CA125 tests; relative increment (RelInc) as AbsInc divided by FR CA125; maximum relative increment (MaxRelInc) as MaxAbsInc divided by FR CA125; absolute velocity (AbsVel) as AbsInc divided by the time between FR and LR CA125 tests; maximum absolute velocity (MaxAbsVel) as MaxAbsInc divided by the time between FR and maximum CA125 tests; relative velocity (RelVel) as AbsVel divided by FR CA125, and maximum relative velocity (MaxRelVel) as MaxAbsVel divided by FR CA125.

To identify the optimal trajectory-specific progression indicators, tdAUCs for these eight progression indicators were calculated using COX regression models and compared pairwise with Delong's test. The indicators with the highest tdAUC were selected as the optimal trajectory-specific progression indicators for $CA125_{SP}$ and $CA125_{GP}$. Time-dependent receiver operating characteristic curves (tdROCs) were used to determine the optimal cut-off values for these indicators, and bootstrap resampling was performed to internally validate the stability of these cut-off values. Based on these cut-off values, trajectory-specific progressions were further reclassified into positive and negative progressions.

The risks of OC incidence and mortality across different CA125 trajectories and trajectory-specific progressions were analyzed using Kaplan-Meier survival curves and compared with the log-rank test. Univariate COX regression models evaluated crude associations between CA125 trajectories, trajectory-specific progression indicators, and OC risk. To investigate the independent associations of CA125 trajectories, trajectory-specific progression indicators, and OC risk, Multivariable COX regression models, adjusting for factors associated with CA125 trajectories as detailed in Supplementary Table S1, were used to investigate independent associations. These factors included age at recruitment (<60 years, 60–70 years, ≥70 years), race(white, non-white), body mass index (0–25, 25–30, >30 kg/m^2), smoking status (never, current, previous), previous oral contraceptives(none, ≤5 years, >5 years), previous hormone replacement therapy

(none, ≤5 years, >5 years), age at menopause (<55 years, ≥55 years), previous hysterectomy(none, yes), live births (0, 1–2, ≥3 times), and time intervals between CA125 tests(1 year, 2 years, 3 years, 4 years, 5 years). Missing data were categorized as an independent group. Associations were measured with hazard ratios and 95% confidence intervals [HR (95% CIs)].

Based on the CA125 trajectories, trajectory-specific progression indicators, and TVU, three optimized joint screening strategies we proposed alongside two traditional strategies. The traditional strategy with TVU screening alone served as the reference. The other strategies explored whether adding CA125 test to TVU screening improved the OC detection accuracy. For the traditional strategy two, a positive screen was defined as any positive TVU and CA125 above diagnostic criteria. In optimized strategy one, a positive screen was defined as any positive TVU and CA125 above the optimal screening cut-off value. To reduce potential false positives associated with CA125 levels below diagnostic criteria, optimized strategy two excluded definite regression of CA125 (namely $CA125_{LP}$). Further reducing potential false positive and focusing on detecting potentially progressive or lethal cancer, optimized strategy three further excluded negative progression in either $CA125_{SP}$ or $CA125_{GP}$. Screening performances metrics including sensitivity, specificity, positivity, Youden index, positive prediction value (PPV), and negative prediction value (NPV) as well as their corresponding confidence intervals were calculated for each screening strategy. When comparing sensitivity (or specificity) between two screening methods, only individuals with cases (or non-cases) were considered. McNemar's test was employed in this context to evaluate whether the number of discordant pairs (where one test is positive and the other is negative) for detecting cases (or non-cases) is significantly differs from what would be expected by chance. In contrast, when comparing positive rates (defined as the proportion of individuals who receive a positive results, including true positives and negative positives, out of the total number of tests conducted) between two screening methods, all individuals (both cases and non-cases) are included. The Pearson chi-square test was used to determine whether the proportion of individuals receiving a positive result from one screening method significantly differs from that of another screening method.

All data analyses were conducted using R (version R 4.2.2) and SPSS software (version R26.0.0.0). Statistical significance was defined as a P value of less than 0.05.

Results

Determination of the optimal screening cut-off value for CA125 and comparison of baseline characteristics across different CA125 trajectories

After a median follow-up of 14.8 years for OC incidence and 23.8 years for OC mortality, a total of 250 OC cases and 218 OC deaths were documented among 28,456 participants included in this study. The tdAUC for 10-year OC incidence risk based on baseline CA125 was 0.663. The optimal cut-off value of baseline CA125 for predicting 10-year OC risk was determined to be 13.00 U/ml (Fig. 1A). Bootstrap resampling with 2000 iterations confirmed this cut-off value (Supplementary Table S2). Participants, as well as incident OC cases and non-cases, were categorized by CA125 levels of <13 U/ml, 13–<35 U/ml, and ≥35 U/ml at different measurement times (Supplementary Table S3). The proportion of women with CA125 levels≥35 U/ml increased from 1.2% in the first-round CA125 test to a peak of 1.8% in the third round, and then stabilized at 1.6% through the sixth round. Furthermore, in rounds two through six, the proportion of newly diagnosed OC patients with CA125 levels above 35 U/ml, as well as those with levels between 13 and 35 U/ml, was significantly higher compared to the non-cancer group.

Based on the optimal cut-off value, participants were categorized into four groups based on their CA125 trajectories: 17,099(60.1%) women with $CA125_{SN}$, 1,740(6.1%) women with $CA125_{LP}$, 6,069(21.3%) women with $CA125_{SP}$, and 3,548(12.5%) women with $CA125_{GP}$. As detailed in Supplementary Table S1, compared to $CA125_{SN}$, the $CA125_{GP}$ group exhibited elder age at

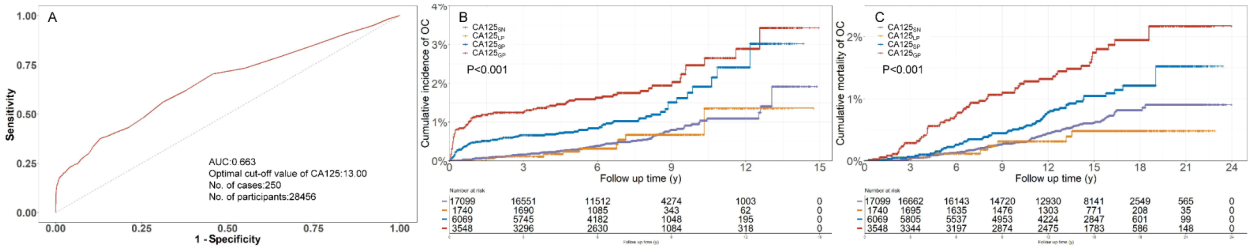

Fig. 1 Time-dependent receiver operating characteristic curves of 10-year OC risk with CA125 (**A**) and Kaplan-Meier curves of OC incidence (**B**) and mortality (**C**) under different CA125 trajectories

Note: $CA125_{SN}$, stable negative CA125; $CA125_{LP}$, loss of positive CA125; $CA125_{SP}$, stable positive CA125; $CA125_{GP}$, gain of positive CA125

Table 1 Associations of CA125 trajectories with OC incidence and mortality

CA125 trajectories	Participants, *N* (%)	Events, *N* (%)	Follow-up, 10,000 PYs	Rates of events, per 10,000 PYs	Unadjusted HR (95%CI)	*P* value*	Adjusted HR (95%CI)†	*P* value†
OC incidence	28,456(100.0)	250(100.0)	20.82	12.01				
$CA125_{SN}$	17,099(60.1)	101(40.4)	12.62	8.00	Ref.	< 0.001	Ref.	
$CA125_{LP}$	1740(6.1)	9(3.6)	1.22	7.35	0.93(0.47–1.85)		0.92(0.46–1.81)	0.799
$CA125_{SP}$	6069(21.3)	70(28.0)	4.28	16.35	2.09(1.54–2.84)		2.00(1.47–2.73)	< 0.001
$CA125_{GP}$	3548(12.5)	70(28.0)	2.70	25.92	3.17(2.34–4.30)		3.06(2.25–4.16)	< 0.001
OC mortality	28,456(100.0)	218(100.0)	39.53	5.52				
$CA125_{SN}$	17,099(60.1)	102(46.8)	24.13	4.23	Ref.	< 0.001	Ref.	
$CA125_{LP}$	1740(6.1)	7(3.2)	2.41	2.91	0.69(0.32–1.48)		0.72(0.34–1.55)	0.404
$CA125_{SP}$	6069(21.3)	54(24.8)	8.14	6.63	1.58(1.13–2.19)		1.58(1.13–2.21)	0.007
$CA125_{GP}$	3548(12.5)	55(25.2)	4.84	11.36	2.69(1.94–3.74)		2.60(1.87–3.62)	< 0.001

Note: PY, person-year; IR, incidence rate; HR (95%CI), hazard ratio (95% confidential interval); *,log-rank test; †, adjusted available factors associated with CA125 trajectories as observed in Supplementary Table S1

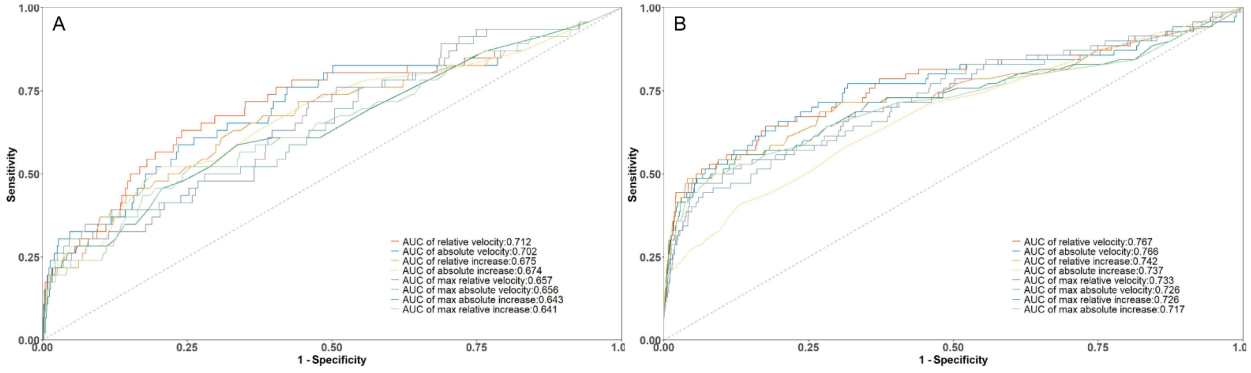

Fig. 2 Time-dependent receiver operating characteristic curves of 10-year incidence of ovarian cancer with trajectory-specific progression indicators among women with stable positive CA125 (**A**) and gain of positive CA125 (**B**)

recruitment, a higher proportion of white American, lower BMI, more smokers, fewer previous users of oral contraceptive, more previous users of hormone replacement therapy, younger age at menopause, fewer prior hysterectomy, more live births, and shorter time interval between CA125 tests (all P values < 0.05).

Associations of CA125 trajectories with OC risk

As shown in Fig. 1B and C and detailed in Table 1, Kaplan-Meier life table analyses revealed that women with $CA125_{GP}$ had the highest crude rates of OC incidence and mortality (25.92 and 11.36 per 10,000 person-years).This was following by women with $CA125_{SP}$ (16.35 and 6.63 per 10,000 person-years), $CA125_{LP}$ (7.35 and 2.91 per 10,000 person-years), and $CA125_{SN}$ (8.00 and 4.23 per 10,000 person-years). Both incidence and mortality rates differed significantly among groups, with all p-values for the log-rank test being < 0.001.

After adjusting for factors associated with CA125 trajectories, as detailed in Supplementary Table S1, the HRs (95%CI) for OC incidence were 3.06(2.25–4.16) for $CA125_{GP}$, 2.00(1.47–2.73) for $CA125_{SP}$, and 0.92(0.46–1.81) for $CA125_{LP}$ compared to $CA125_{SN}$. For OC mortality, the HRs (95%CI) were 2.60(1.87–3.62) for $CA125_{GP}$, 1.58(1.13–2.21) for $CA125_{SP}$, 0.72(0.34–1.55) for $CA125_{LP}$ (Table 1).

Determination of optimal trajectory-specific progression indicators and their corresponding optimal cut-off values for OC risk

As depicted in Fig. 2, among women with $CA125_{SP}$, the tdAUC for RelVel of 10-year OC incidence risk (0.712) was significantly higher than that for AbsInc, MaxAbsInc and MaxRelInc (all P-values < 0.05), though it was comparable to other trajectory-specific progression indicators. Similarly, for women with elevated CA125 in $CA125_{GP}$, the tdAUC for RelVel (0.767) was significantly greater than that for MaxAbsVel and MaxAbsInc (all P value < 0.05), while remaining comparable to other indicators. Thus, RelVel was selected as the optimal trajectory-specific progression indicators for both $CA125_{SP}$ (Fig. 2A) and $CA125_{GP}$ (Fig. 2B). Based on tdROC, the optimal cut-off values for RelVel were determined to be a 9% annual increment for $CA125_{SP}$ and a 32% annual increment for $CA125_{GP}$. Bootstrap resampling confirmed these results (Supplementary Table S2).

Based on these cut-off values, participants with either $CA125_{SP}$ or $CA125_{GP}$ were reclassified into negative and positive progression groups. As shown in Supplementary Figure S2, cumulative OC incidence and mortality were significantly higher in the positive progression group compared to the negative progression group within both $CA125_{SP}$ and $CA125_{GP}$ categories. After adjusting for confounder variables, positive progression remained significantly associated with increased risk of OC incidence [3.83(1.96–7.51) for $CA125_{SP}$ and 7.26(4.00-13.17) for $CA125_{GP}$] and mortality [6.04(2.78–13.14) for $CA125_{SP}$ and 8.03(4.15–15.56) $CA125_{GP}$] (Table 2). Additionally, compared to women with $CA125_{SN}$, both $CA125_{LP}$ and trajectory-specific negative progressions [including RelVel(-) within $CA125_{SP}$ and RelVel(-) within $CA125_{GP}$] were associated with similar risk of OC(Supplementary Figure S3, Table S4).

Comparisons of screening performances across different joint screening strategies

Table 3 summarized the performance metrics of various joint screening strategies. The sensitivity, specificity and positivity for TVU alone were 55.6%, 51.4% and 48.7%, respectively. Compared to traditional strategy one, which used TVU alone, traditional strategy two, which combined TVU and CA125 testing, demonstrated significant higher sensitivity (60.8%) and positivity (50.0%), but lower specificity (50.1%). When the positive criterion for CA125 was adjusted to the optimal cut-off value, optimized strategy one achieved increased sensitivity (79.6%) and positivity (67.9%), though the specificity decreased to 32.2%. To address potential false positive, optimized strategy two excluding $CA125_{LP}$ as a positive screen, resulting in a decrease in both sensitivity (76.0%) and positivity (61.8%), while specificity increased to 38.3%. Further refining the strategy to exclude trajectory-specific negative progression, optimized strategy three led to a reduction in sensitivity (64.4%) and positivity (51.3%), but an increase in specificity to 48.8%. Compared to traditional strategy one, optimized strategy three showed significantly higher sensitivity and positivity, although with lower specificity (both P values < 0.001), and improved Youden, PPV and NPV.

Based on the above results, optimized joint screening strategy three is recommended as the most effective approach for integrating CA125 trajectories, CA125 progression indicators, and TVU for asymptomatic women. As illustrated in Fig. 3, the proposed screening protocol begins an initial round of TVU and CA125 testing. Woman with any positive TVU was advised to undergo further examinations. Those with negative TVU results proceed to a second round of TVU and CA125 testing. In this second round, women with any positive TVU were again referred for further evaluation, while those with positive progression indicators within either $CA125_{SP}$ or $CA125_{GP}$ [namely RelVel(+)] are recommended for further examination. Women with RelVel(-) within both $CA125_{SP}$ and $CA125_{GP}$, as well as those with $CA125_{LP}$, are advised to undergo re-evaluation of TVU and CA125.

Discussion

This study is the first to determine the optimal screening cut-off value for CA125 in OC and to investigate the association between CA125 trajectory, trajectory-specific progression, and OC risk. Our findings reveal that a gain of positive CA125 is associated with a higher OC risk compared to other CA125 trajectories. Additionally, within the same trajectory, positive progression is associate with a significantly higher risk of OC than negative progression. This study also proposes and evaluates a novel screening strategy integrating CA125 trajectories, optimal screening cut-off value, trajectory-specific progression indicators, and TVU. The optimized screening strategy not only significantly reduced the number of missed OC by 3.6%, but also improved over accuracy. In summary, the strategy can effectively refine the identification of high-risk population and is recommended as a

Table 2 Associations of trajectory-specific progression indexes with OC incidence and mortality

CA125 trajectories	Trajectory-specific progression	Participants, *N* (%)	Event, *N*	Follow-up, 10,000 PYs	Event rate, per 10,000 PYs	Adjusted HR (95%CI)†	*P* value†
OC incidence							
$CA125_{SP}$	RelVel (-)	2860(74.8)	17	1.98	8.56	Ref.	
	RelVel (+)	965(25.2)	29	0.72	40.08	3.83(1.96–7.51)	< 0.001
$CA125_{GP}$	RelVel (-)	2891(81.5)	25	2.18	11.46	Ref.	
	RelVel (+)	657(18.5)	45	0.52	86.54	7.26(4.00-13.17)	< 0.001
OC mortality							
$CA125_{SP}$	RelVel (-)	2860(74.8)	11	3.85	2.86	Ref.	
	RelVel (+)	965(25.2)	24	1.24	19.36	6.04(2.78–13.14)	< 0.001
$CA125_{GP}$	RelVel (-)	2891(81.5)	20	4.03	4.97	Ref.	
	RelVel (+)	657(18.5)	35	0.81	43.00	8.03(4.15–15.56)	< 0.001

Note: PY, person-year; HR (95%CI), hazard ratio (95% confidential interval); -, negative; +, positive. RelVel, relative velocity; †, adjusted available factors associated with CA125 trajectories as observed in Supplementary Table S1

Table 3 Comparisons of screening performances between traditional and optimized joint screening strategies for ovarian cancer

Methods	Cases	Non-cases	Total	Sensitivity*		Specificity*		Positivity†		Youden	PPV	NPV
				%(95%CI)	*P*	%(95%CI)	*P*	%(95%CI)	*P*	%	%(95%CI)	%(95%CI)
Traditional strategy 1 (positive screen defined as only any positive TVU)												
Positive	139	13,720	13,859	55.6	Ref.	51.4	Ref.	48.7	Ref.	7.0	1.0	99.2
Negative	111	14,486	14,597	(49.2–61.8)		(50.8–51.9)		(48.1–49.3)			(0.85–1.19)	(99.1–99.4)
Total	250	28,206	28,456									
Traditional strategy 2 (positive screen defined as any positive TVU and CA125 above diagnostic criteria)												
Positive	152	14,074	14,226	60.8	< 0.001	50.1	< 0.001	50.0	< 0.001	10.9	1.1	99.3
Negative	98	14,132	14,230	(54.4–66.8)		(49.5–50.7)		(49.4–50.6)			(0.91–1.25)	(99.2–99.4)
Total	250	28,206	28,456									
Optimized strategy 1 (positive screen defined as any positive TVU and CA125 above optimal cut-off value)												
Positive	199	19,133	19,332	79.6	< 0.001	32.2	< 0.001	67.9	< 0.001	11.8	1.0	99.4
Negative	51	9073	9124	(74.0-84.3)		(31.6–32.7)		(67.4–68.5)			(0.89–1.18)	(99.3–99.6)
Total	250	28,206	28,456									
Optimized strategy 2 (based on strategy 1 and excluding $CA125_{LP}$ as positive screen)												
Positive	190	17,402	17,592	76.0	< 0.001	38.3	< 0.001	61.8	< 0.001	14.3	1.1	99.4
Negative	60	10,804	10,864	(70.1–81.1)		(37.7–38.9)		(61.3–62.4)			(0.93–1.25)	(99.3–99.6)
Total	250	28,206	28,456									
Optimized strategy 3 (based on strategy 2 and excluding negative RelVel in either $CA125_{SP}$ or $CA125_{GP}$ as positive screen)												
Positive	161	14,447	14,608	64.4	< 0.001	48.8	< 0.001	51.3	< 0.001	13.2	1.1	99.4
Negative	89	13,759	13,848	(58.1–70.3)		(48.2–49.4)		(50.8–51.9)			(0.94–1.29)	(99.2–99.5)
Total	250	28,206	28,456									

Note: $CA125_{LP}$, loss of positive CA125; $CA125_{SP}$, stable positive CA125; $CA125_{GP}$, gain of positive CA125; RelVel, relative velocity; PPV and NPV, positive and negative prediction value. *, compared with McNemer tests; †, compared with Pearson chi-square tests

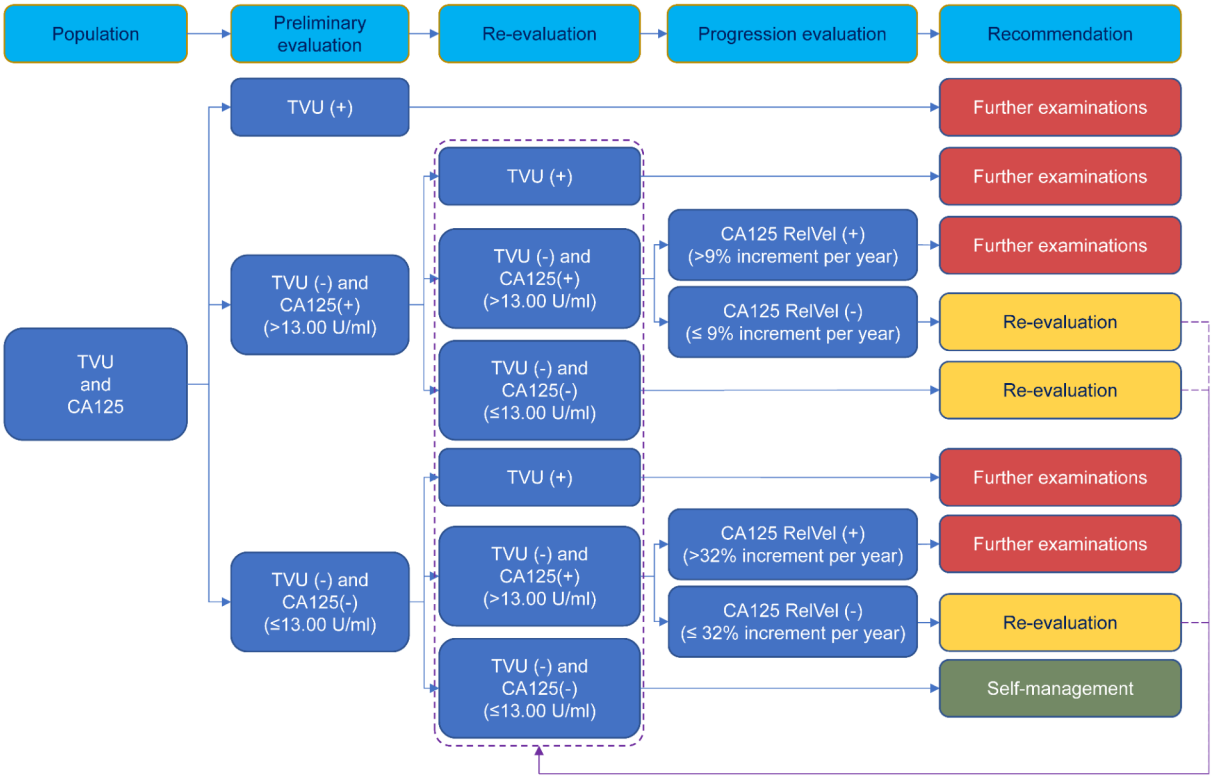

Fig. 3 The recommended flowchart of joint screening with TVU and CA125 for ovarian cancer
Note: -, negative; +, positive. RelVel, relative velocity

fundamental strategy to enhance OC screening performance and reduce missed diagnoses.

Several previous studies have suggested that serial CA125 measurements and longitudinal algorithms, such as ROCA, could improve OC screening effectiveness under single-threshold rules [14, 17, 21–23]. However, some study have indicated that the adoption of ROCA in PLCO did not result in a significant reduction in OC mortality [23]. One potential reason for the limited benefit of ROCA-based two-stage multimodal screening (MMS) in both the PLCO and UKCTOCS might be the underestimated role of ultrasound in OC screening. ROCA is designed to support decisions following a positive CA125 test [24], but in the MMS approach, a second-line TVU examination is only conducted if their first-line CA125 test indicates increased risk [6]. In the UKCTOCS, only 41% of OC cases had a positive screen in the MMS arm, while TVU alone had a sensitivity of 32% [6]. Thus, the performance of MMS based on ROCA is limited by the effectiveness of longitudinal CA125 tests alone. Integrating CA125 and TVU screening in parallel would offer better performance than CA125 alone or CA125 and TVU in series.

Secondly, the ROCA may struggle to differentiate OC risks associated with identical changes in CA125 levels but different time intervals, as it relies solely on current and past CA125 values, along with age, to estimate risk using a Bayesian change-point model. This approach does not incorporate the time intervals between longitudinal CA125 tests, unlike COX models or other discriminant models [10, 14]. Consequently, ROCA might not detect time-dependent changes in CA125 that reflect early progression of OC [24]. In contrast, the four selected screening strategies in this study demonstrated significantly higher sensitivities compared to the 41% observed in the UKCTOCS [6]. These suggest that combining CA125 and TVU in parallel is likely to yield a lower missed diagnostic rate compared to using CA125 and TVU in series, which is comparable to the ROCA-based two-stage MMS in UKCTOCS. Furthermore, to address potential false positive associated with CA125 cut-off value below the diagnostic criteria of OC [2], the optimized screening strategy excluded women with definitive CA125 regression and those with negative progression within either $CA125_{SP}$ or $CA125_{GP}$, even if they had elevated CA125 from baseline. Therefore, the redefined approach is expected to enhance detection of progressive OCs compared to traditional methods and improve screening performance over extended periods.

Another key finding is that the OC risk associated with $CA125_{GP}$ was significantly higher than that with $CA125_{SP}$. Although elevated CA125 levels can occur in other malignancies(e.g., breast and pancreatic cancer) [25–28] and various non-cancerous conditions(e.g., endometriosis, liver cirrhosis, pregnancy) [29–33], CA125 is typically elevated in approximately 50% of early-stage OC, 65% of mucinous OC, and 80–85% of advanced epithelial OC [34–36]. In OC screening, a stable positive CA125 may suggest non-cancerous conditions or relatively stable cancer, potentially reducing the need for aggressive intervention due to lower lethality [13, 14]. Conversely, a significant increase or gain of positive CA125 from a baseline negative level, after excluding noncancerous conditions, likely indicates cancer incidence or progression [13, 14]. Kobayashi et al. found that non-serous cancers showed mildly elevated CA125 levels before diagnosis, while serous ovarian cancers often develop suddenly from normal CA125 levels, with a mean interval of only 1.4 years [37]. This aspect is often overlooked in single-test practice, potentially missing high-risk population suitable for OC screening. Additionally, $CA125_{GP}$ was associated with smoking, previous use of hormone replacement therapy, and older age at menopause—all factors linked to increased risk of OC [38]. These findings warrant further investigation to understand why $CA125_{GP}$ associates with lower BMI and less previous use of oral contraceptive.

Additionally, while distinct optimal trajectory-specific progression indicators were anticipated for $CA125_{SP}$ and $CA125_{GP}$, the same indicators were observed for both trajectories. Furthermore, there was no significant difference between most indicators within the same trajectory. This may be attributed to the long-time dilution effect of including OCs diagnosed clinically after the end of screening period, as different optimal indicators emerged when analyses were censored at an earlier time (results not shown). Nevertheless, trajectory-specific progression indicators are believed to reflect different pathways for OC incidence and/or different OC subtypes associated with varying CA125 changes [35, 39]. Importantly, these findings suggest that different monitoring and intervention strategies may be needed for women with varying CA125 trajectories in the future. For instance, for women with stable positive CA125, intervention should focus on identifying or treating potential non-cancerous condition and controlling CA125 levels. Conversely, for women with a rapid gain of positive CA125, the primary interventional should target reducing exposure to risk factors. For BRCA mutation carriers, bilateral salpingo-oophorectomy remains effective strategy to lower OC risk [40, 41]. Future research with improved study designs and larger sample is needed to validate these results and assumptions.

Despite the lower specificity of the third revised strategy, which is the recommended optimized strategy, it may still be clinically appropriate for the following three points: First, higher sensitivity facilitates early detection of cases, which is crucial for improving patient outcomes. Identifying more true positives may outweigh the concern of increased false positives, particularly in serious conditions where early intervention can significantly alter prognosis. Second, to mitigate the impact of false positives, including the need for confirmatory testing and follow-up procedures, our optimized screening strategy initially recommended an additional round of CA125 only after a previous positive result. Following comparisons between different rounds of CA125 suggest no additional procedures are needed for women with a definitive regression of CA125 or negative progression, even if their CA125 levels were elevated from the baseline. Third, we did not recommend the third revised strategy based solely on higher sensitivity or specificity. Instead, we considered the higher Youden index, which integrates both sensitivity and specificity. Since the third revised strategy demonstrated a higher Youden index compared to the traditional strategy, we recommend it as the optimized screening strategy. To address the trade-offs between sensitivity and specificity more critically, a more comprehensive assessment of the proposed screening strategy is needed, taking into account its potential impact on patients' well-being and overall cost-effectiveness. Additionally, this is not a study where a predefined sample was collected to test a specific hypothesis but rather a post-hoc analysis based on the PLCO study. Due to the relatively small number of ovarian cancer cases, this limited case number is likely to constrain the statistical power of the current analyses.

In addition to the notable findings and the advantages of long-term follow-up, large sample size, and sophisticated analyses, several limitations must be addressed. First, the absence of an independent external validation population may limit the generalizability of these results to other populations. However, bootstrap resampling with 2000 iterations produced similar results, supporting the stability of the current findings. Second, different time intervals within the same CA125 trajectory could introduce bias into the results. Nonetheless, multivariable analyses that adjusted for these time intervals persistently showed significant associations, reinforcing the stability of the findings. Third, while the optimized strategy—after excluding definite regressive trajectory and negative trajectory-specific progression—was anticipated to detect significantly more early-stage OCs compared to traditional strategy, this was affected by the low incidence of OC and limited numbers of OC cases with clear stage information (data not shown). Nevertheless, the selected optimized screening strategy, which included several

improved screening indexes, is expected to offer better long-term benefits compared to traditional methods.

Conclusions

In summary, OC risk varies across different CA125 trajectories and trajectory-specific progression. Future monitoring or intervention strategies should be tailored to these factors. Integrating CA125 trajectories, trajectory-specific progression indicators, and TVU could refine the identification of high-risk population and enhance the performances of OC screening. Future research with more sophisticated design and larger sample is needed to validate the current findings.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13048-024-01535-9.

Supplementary Material 1

Acknowledgements
This research was conducted using the PLCO resource under application number 1107. The authors thank the National Cancer Institute for access to NCI's data collected by the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. The statements contained herein are solely those of the authors and do not represent or imply concurrence or endorsement by NCI.

Author contributions
Dr Huang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.Concept and design: Huang, Yang, Song.Acquisition, analysis, or interpretation of data: Duan, Liu, Zhang, Liu, Ji, Zhang, Fan, Liu, Yang, Lyu, Huang.Drafting of the manuscript: Duan, Liu, Huang.Critical revision of the manuscript for important intellectual content: Yang, Xu, Tian, Li, Lyu, Song, Song, Huang.Statistical analysis: Duan, Liu.Obtained funding: Song, HuangAdministrative, technical, or material support: HuangSupervision: Song, SongAll authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by the Chinese National Key Research and Development Project, grant number 2021YFC2500400, Tianjin Health Committee Foundation, grant number TJWJ2021MS008, and Tianjin Key Medical Discipline (Specialty) Construction Project, grant number TJYXZDXK-009A.

Data availability
All the dataset is available on the PLCO website (https://cdas.cancer.gov/plco/).

Declarations

Ethical approval
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Epidemiology and Biostatistics, Key Laboratory of Molecular Cancer Epidemiology, Tianjin's Clinical Research Center for Cancer, National Clinical Research Center for Cancer, Tianjin Medical University Cancer Institute and Hospital, Tianjin Medical University, Tianjin, Tianjin 300060, China
[2]Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Beijing Office for Cancer Prevention and Control, Peking University Cancer Hospital & Institute, Beijing 100142, China
[3]Department of International Affairs, Tianjin Medical University Cancer Institute and Hospital, Tianjin Medical University, Tianjin 300060, China
[4]Department of Gynecologic Oncology, Tianjin Medical University Cancer Institute and Hospital, Tianjin Medical University, Tianjin 300060, China
[5]Project Office, Tianjin Women and Children's Health Center, Tianjin 300070, China

Received: 11 March 2024 / Accepted: 9 October 2024
Published online: 26 October 2024

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer statistics 2020: GLOBOCAN estimates of incidence and Mortality Worldwide for 36 cancers in 185 countries. Cancer J Clin. 2021;71(3):209–49.
2. Henderson JT, Webber EM, Sawaya GF. Screening for ovarian Cancer: updated evidence report and systematic review for the US Preventive Services Task Force. JAMA. 2018;319(6):595–606.
3. Yousefi M, Dehghani S, Nosrati R, Ghanei M, Salmaninejad A, Rajaie S, et al. Current insights into the metastasis of epithelial ovarian cancer - hopes and hurdles. Cell Oncol (Dordrecht). 2020;43(4):515–38.
4. Terry KL, Schock H, Fortner RT, Hüsing A, Fichorova RN, Yamamoto HS, et al. A prospective evaluation of early detection biomarkers for ovarian Cancer in the European EPIC cohort. Clin cancer Research: Official J Am Association Cancer Res. 2016;22(18):4664–75.
5. Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the prostate, lung, colorectal and ovarian (PLCO) Cancer Screening Randomized Controlled Trial. JAMA. 2011;305(22):2295–303.
6. Menon U, Gentry-Maharaj A, Burnell M, Singh N, Ryan A, Karpinskyj C, et al. Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet (London England). 2021;397(10290):2182–93.
7. Grossman DC, Curry SJ, Owens DK, Barry MJ, Davidson KW, Doubeni CA, et al. Screening for ovarian Cancer: US Preventive Services Task Force Recommendation Statement. JAMA. 2018;319(6):588–94.
8. Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, Davies AP, et al. Screening for ovarian cancer: a pilot randomised controlled trial. Lancet (London England). 1999;353(9160):1207–10.
9. Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, et al. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet (London England). 2016;387(10022):945–56.
10. Skates SJ, Pauler DK, Jacobs IJ. Screening based on the risk of Cancer calculation from bayesian hierarchical changepoint and mixture models of longitudinal markers. J Am Stat Assoc. 2001;96(454):429–39.
11. Cramer DW, O'Rourke DJ, Vitonis AF, Matulonis UA, Dijohnson DA, Sluss PM, et al. CA125 immune complexes in ovarian cancer patients with low CA125 concentrations. Clin Chem. 2010;56(12):1889–92.
12. Høgdall EV, Christensen L, Kjaer SK, Blaakaer J, Kjaerbye-Thygesen A, Gayther S, et al. CA125 expression pattern, prognosis and correlation with serum CA125 in ovarian tumor patients. From the Danish MALOVA Ovarian Cancer Study. Gynecol Oncol. 2007;104(3):508–15.
13. Menon U, Skates SJ, Lewis S, Rosenthal AN, Rufford B, Sibley K, et al. Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer. J Clin Oncology: Official J Am Soc Clin Oncol. 2005;23(31):7919–26.
14. Skates SJ, Menon U, MacDonald N, Rosenthal AN, Oram DH, Knapp RC, et al. Calculation of the risk of ovarian cancer from serial CA-125 values for preclinical detection in postmenopausal women. J Clin Oncology: Official J Am Soc Clin Oncol. 2003;21(10 Suppl):s206–10.
15. Jacobs IJ, Skates S, Davies AP, Woolas RP, Jeyerajah A, Weidemann P, et al. Risk of diagnosis of ovarian cancer after raised serum CA 125 concentration: a prospective cohort study. BMJ. 1996;313(7069):1355–8.
16. Xu JL, Commins J, Partridge E, Riley TL, Prorok PC, Johnson CC, et al. Longitudinal evaluation of CA-125 velocity and prediction of ovarian cancer. Gynecol Oncol. 2012;125(1):70–4.

17. Drescher CW, Shah C, Thorpe J, O'Briant K, Anderson GL, Berg CD, et al. Longitudinal screening algorithm that incorporates change over time in CA125 levels identifies ovarian cancer earlier than a single-threshold rule. J Clin Oncology: Official J Am Soc Clin Oncol. 2013;31(3):387–92.
18. Forstner R. Early detection of ovarian cancer. Eur Radiol. 2020;30(10):5370–3.
19. Prorok PC, Andriole GL, Bresalier RS, Buys SS, Chia D, Crawford ED, et al. Design of the prostate, lung, colorectal and ovarian (PLCO) Cancer Screening Trial. Control Clin Trials. 2000;21(6 Suppl):s273–309.
20. Duffy MJ, Bonfrer JM, Kulpa J, Rustin GJ, Soletormos G, Torre GC, et al. CA125 in ovarian cancer: European Group on Tumor markers guidelines for clinical use. Int J Gynecol cancer: Official J Int Gynecol Cancer Soc. 2005;15(5):679–91.
21. Blyuss O, Burnell M, Ryan A, Gentry-Maharaj A, Mariño IP, Kalsi J, et al. Comparison of longitudinal CA125 algorithms as a first-line screen for ovarian Cancer in the General Population. Clin cancer Research: Official J Am Association Cancer Res. 2018;24(19):4726–33.
22. Menon U, Ryan A, Kalsi J, Gentry-Maharaj A, Dawnay A, Habib M, et al. Risk algorithm using serial biomarker measurements Doubles the number of screen-detected cancers compared with a single-threshold rule in the United Kingdom Collaborative Trial of Ovarian Cancer Screening. J Clin Oncology: Official J Am Soc Clin Oncol. 2015;33(18):2062–71.
23. Pinsky PF, Zhu C, Skates SJ, Black A, Partridge E, Buys SS, et al. Potential effect of the risk of ovarian cancer algorithm (ROCA) on the mortality outcome of the prostate, lung, colorectal and ovarian (PLCO) trial. Int J Cancer. 2013;132(9):2127–33.
24. McIntosh MW, Drescher C, Fitzgibbon MM. Ovarian Cancer Early Detection needs better imaging, not better algorithms or biomarkers. J Clin Oncology: Official J Am Soc Clin Oncol. 2016;34(2):199–200.
25. Einama T, Yamagishi Y, Takihata Y, Suzuki T, Yamasaki T, Hirose Y, et al. Co-expression of mesothelin and CA125/MUC16 is a prognostic factor for breast cancer, especially in luminal-type breast cancer patients. Biomark Res. 2021;9(1):78.
26. Zhang J, Wei Q, Dong D, Ren L. The role of TPS, CA125, CA15-3 and CEA in prediction of distant metastasis of breast cancer. Clin Chim Acta. 2021;523:19–25.
27. Yerushalmi R, Tyldesley S, Kennecke H, Speers C, Woods R, Knight B, et al. Tumor markers in metastatic breast cancer subtypes: frequency of elevation and correlation with outcome. Ann Oncol. 2012;23(2):338–45.
28. Einama T, Kamachi H, Nishihara H, Homma S, Kanno H, Takahashi K, et al. Co-expression of mesothelin and CA125 correlates with unfavorable patient outcome in pancreatic ductal adenocarcinoma. Pancreas. 2011;40(8):1276–82.
29. Bagan P, Berna P, Assouad J, Hupertan V, Le Pimpec Barthes F, Riquet M. Value of cancer antigen 125 for diagnosis of pleural endometriosis in females with recurrent pneumothorax. Eur Respir J. 2008;31(1):140–2.
30. Xiao WB, Liu YL. Elevation of serum and ascites cancer antigen 125 levels in patients with liver cirrhosis. J Gastroenterol Hepatol. 2003;18(11):1315–6.
31. Zuckerman E, Lanir A, Sabo E, Rosenvald-Zuckerman T, Matter I, Yeshurun D, et al. Cancer antigen 125: a sensitive marker of ascites in patients with liver cirrhosis. Am J Gastroenterol. 1999;94(6):1613–8.
32. Tyler C, Kapur A, Felder M, Belisle JA, Trautman C, Gubbels JA, et al. The mucin MUC16 (CA125) binds to NK cells and monocytes from peripheral blood of women with healthy pregnancy and preeclampsia. Am J Reprod Immunol. 2012;68(1):28–37.
33. Quirk JG Jr., Brunson GL, Long CA, Bannon GA, Sanders MM, O'Brien TJ. CA 125 in tissues and amniotic fluid during pregnancy. Am J Obstet Gynecol. 1988;159(3):644–9.
34. Karam AK, Karlan BY. Ovarian cancer: the duplicity of CA125 measurement. Nat Rev Clin Oncol. 2010;7(6):335–9.
35. Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. CA125 and ovarian Cancer: a Comprehensive Review. Cancers. 2020;12(12).
36. Medeiros LR, Rosa DD, da Rosa MI, Bozzetti MC. Accuracy of CA 125 in the diagnosis of ovarian tumors: a quantitative systematic review. Eur J Obstet Gynecol Reprod Biol. 2009;142(2):99–105.
37. Kobayashi H, Ooi H, Yamada Y, Sakata M, Kawaguchi R, Kanayama S, et al. Serum CA125 level before the development of ovarian cancer. Int J Gynaecol Obstet. 2007;99(2):95–9.
38. Tanha K, Mottaghi A, Nojomi M, Moradi M, Rajabzadeh R, Lotfi S, et al. Investigation on factors associated with ovarian cancer: an umbrella review of systematic review and meta-analyses. J Ovarian Res. 2021;14(1):153.
39. Tothill RW, Tinker AV, George J, Brown R, Fox SB, Lade S, et al. Novel molecular subtypes of serous and endometrioid ovarian cancer linked to clinical outcome. Clin cancer Research: Official J Am Association Cancer Res. 2008;14(16):5198–208.
40. Kotsopoulos J, Narod SA. Prophylactic salpingectomy for the prevention of ovarian cancer: who should we target? Int J Cancer. 2020;147(5):1245–51.
41. Mannis GN, Fehniger JE, Creasman JS, Jacoby VL, Beattie MS. Risk-reducing salpingo-oophorectomy and ovarian cancer screening in 1077 women after BRCA testing. JAMA Intern Med. 2013;173(2):96–103.

Publisher's note