Estimating the ovarian cancer CA-125 preclinical detectable phase, *in-vivo* tumour doubling time, and window for detection in early stage: an exploratory analysis of UKCTOCS

Jacob S. Bedia,[a] *Ian J. Jacobs,*[b] *Andy Ryan,*[g] *Aleksandra Gentry-Maharaj,*[b,g] *Matthew Burnell,*[g] *Naveena Singh,*[c,o] *Ranjit Manchanda,*[d,e,f,g] *Jatinderpal K. Kalsi,*[h] *Anne Dawnay,*[i] *Lesley Fallowfield,*[j] *Alistair J. McGuire,*[k] *Stuart Campbell,*[l] *Mahesh K. B. Parmar,*[g] *Usha Menon,*[g,n] *and Steven J. Skates*[a,m,n,*]

[a]MGH Biostatistics Center, Massachusetts General Hospital, Boston, MA, USA
[b]Department of Women's Cancer, Elizabeth Garrett Anderson Institute for Women's Health, UCL, London, UK
[c]Department of Cellular Pathology, Barts Health NHS Trust, London, UK
[d]Centre for Cancer Screening, Prevention & Early Diagnosis, Wolfson Institute of Population Health, Queen Mary University of London, London, UK
[e]Department of Health Services Research, Faculty of Public Health & Policy, London School of Hygiene & Tropical Medicine, London, UK
[f]Department of Gynaecological Oncology, Barts Health NHS Trust, London, UK
[g]MRC Clinical Trials Unit at UCL, Institute of Clinical Trials and Methodology, UCL, London, UK
[h]AGE Research Unit, School of Public Health, Imperial College London, London, UK
[i]Department of Clinical Biochemistry, Barts Health NHS Trust, London, UK
[j]Sussex Health Outcomes Research and Education in Cancer (SHORE-C), Brighton and Sussex Medical School, University of Sussex, Brighton, UK
[k]London School of Economics and Political Science, London, UK
[l]Create Health, London, UK
[m]Harvard Medical School, Boston, MA, USA

eBioMedicine
2025;112: 105554
Published Online xxx
https://doi.org/10.1016/j.ebiom.2024.105554

Summary

Background The ovarian cancer (OC) preclinical detectable phase (PCDP), defined as the interval during which cancer is detectable prior to clinical diagnosis, remains poorly characterised. We report exploratory analyses from the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS).

Methods In UKCTOCS between Apr-2001 and Sep-2005, 101,314 postmenopausal women were randomised to no screening (NS) and 50,625 to annual multimodal screening (MMS) (until Dec-2011) using serum CA-125 interpreted by the Risk of Ovarian Cancer Algorithm (ROCA). All provided a baseline blood sample. Women with invasive epithelial OC diagnosed between randomisation and trial censorship (Dec-2014) in the MMS and NS arms with two or more CA-125 measurements, including one within two years of diagnosis were included. OC-free women (2:1 to cases) from the MMS arm provided information on baseline CA-125 distribution. CA-125 measurements were obtained from MMS results, secondary analysis of baseline samples, and medical records. PCDP duration and *in-vivo* tumour doubling time were estimated using the change-point model underlying ROCA. Early-stage (Stage I and II) PCDP was estimated from a Bayesian model for the probability of early stage given a CA-125 measurement.

Findings Of 541 women (2371 CA-125 measurements) with high-grade serous cancer (HGSC), 93% (504/541) secreted CA-125 into the circulation. Median CA-125 PCDP duration for clinically-diagnosed HGSC was 15.2 (IQR 13.1–16.9, 95% IPR 9.6–21.8) months, of which 11.9 (IQR 10.5–13.1, 95% IPR 7.5–16.5) months was in early stage. The median HGSC *in-vivo* tumour doubling time for cancers secreting CA-125 was 2.9 (IQR 2.3–3.7, 95% IPR 1.5–7.6) months.

Interpretation We report a comprehensive characterisation of the OC CA-125 PCDP. The 12-month window for early-stage detection and short tumour doubling time of HGSC provide a benchmark for researchers evaluating novel screening approaches including need to reduce diagnostic workup interval. Equally the findings provide urgent impetus for clinicians to reduce intervals from presentation to treatment onset.

*Corresponding author. MGH Biostatistics Center, Massachusetts General Hospital, Boston, MA, USA.
E-mail address: sskates@mgh.harvard.edu (S.J. Skates).
[n]Equal contribution.
[o]Deceased.

Funding NCI Early Detection Research Network, Concord (MA) Detect Ovarian Cancer Early Fund, MRC Clinical Trials Unit at UCL Core Funding.

Keywords: CA-125 antigen; Blood; Carcinoma; Ovarian epithelial*; Early detection of cancer*; Longitudinal studies; Ovarian neoplasms*; Epidemiology; UKCTOCS; Mass screening; Biomarker; Preclinical history of cancer; Preclinical detectable phase; Natural history of cancer; Early diagnosis

Research in context

Evidence before this study

We searched PubMed for publications from Jan 1, 2004 to Aug 1, 2024 including ("preclinical" OR "precursor" OR "early detection" OR "screening") AND ("evolution" OR "detection window" OR "preclinical detectable phase" OR "sojourn time" OR "natural history") AND ("ovarian cancer" OR "ovarian carcinoma"). We found six publications on high grade serous cancer estimating either the overall preclinical duration (from precancerous neoplasm to invasive cancer diagnosis), *in-vivo* doubling time, or window for detection in early stage. A study published in 2009 estimated all three parameters by modelling published data on size of occult serous cancers identified at risk-reducing bilateral salpingo-oopherectomy in *BRCA1* mutation carriers. Using data from 68 cases, the authors estimated an overall median preclinical duration from serous tubal intraepithelial carcinoma (STIC) to clinical diagnosis of 5.1 years, with a median of 4.3 years for detection of carcinoma in situ, stage I, or stage II disease. They also estimated tumour doubling times using measured tumour sizes from 63 cases and an exponential growth model with rates of 4 and 2.5 months for early and late-stage disease, respectively. A study published in 2017 modelled tumour dynamics based on whole exome sequencing and copy number analyses of fallopian tube lesions (p53 signatures, STICs, and tubal carcinomas), ovarian cancers, and metastases from five patients with sporadic high grade serous cancers (HGSC) and four with STICs. The researchers estimated a duration of six years for a STIC lesion to progress to invasive ovarian carcinoma and an average of two years between the initiation of the ovarian carcinoma and development of metastasis. A study published in 2019 analysed the genomic landscape using whole-exome and amplicon sequencing of incidental tubal precursor lesions including p53 signature, serous tubal intraepithelial lesion (STIL) and proliferative STIC in women with HGSC (four) or no cancer (seven). The authors reported STIC development from p53 signature and STIL takes several decades, while the overall preclinical duration from STIC to ovarian carcinoma is 7 years. A study published in 2016 modelled high grade serous cancer growth rates *in-silico* using 58,673 preclinical transvaginal ultrasound (TVS) ovarian volume measurements from 13,963 patients who underwent annual TVS for 1–11 years in the University of Kentucky Ovarian Cancer Screening Program. The authors estimated a 1.76 year window for detection by TVS. However, almost 50% of the simulated HGSC growth curves never reached the minimum detection threshold. The authors concluded that TVS screening would not reduce mortality and even a semiannual TVS screening would miss detectable HGSC. A study published in 2007 analysed single timepoint preclinical CA-125 samples from 168 women with serous and 228 with non-serous OC cases and reported the average interval from sample to diagnosis from The Shizuoka Cohort Study on Ovarian Cancer Screening (SCSOCS). The authors found 42 CA-125 samples in serous tumours and 153 CA-125 samples in non-serous tumours had level >35 U/ml, with an average time from sample to diagnosis of 1.4 years and 3.8 years, respectively. A study published in 2024 modelled OC progression using a continuous time Markov chain using Prostate, Lung, Colorectal and Ovarian screening trial data and published summary UKCTOCS results. The estimated sojourn time (which is similar to the PCDP) using a Bayesian approach for HGSC (type II OC) was 1.7–1.8 years.

Added value of this study

We provide a comprehensive report of the OC CA-125 preclinical detectable phase duration, *in-vivo* tumour doubling time, and window for detection in early stage in average risk women. This study uses longitudinal individual data from the largest prospective OC screening trial to date. We find over 90% of HGSCs secrete CA-125 into the circulation. Unlike previous studies which estimate overall duration of the preclinical phase from STIC to clinical diagnosis of ovarian cancer, we estimate the duration of the CA-125 preclinical detectable phase starting from a significant rise in an individual's CA-125 over their baseline CA-125 to clinical diagnosis. For HGSC, this was 15 months, with a duration of 12 months in early stage. The median HGSC *in-vivo* tumour doubling time was 3 months. We also provide estimates of the CA-125 preclinical detectable phase duration and tumour doubling times for low-grade serous, endometrioid, clear cell, and mucinous histotypes.

Implications of all the available evidence

The findings have far reaching implications for both early detection and routine clinical care of women with HGSC. For early detection, the 12-month window for early-stage detection and short tumour doubling time of HGSC provide a benchmark against which to evaluate new screening approaches and highlight the need to reduce the diagnostic workup interval. For routine care, the three month doubling time provides urgent impetus to reduce intervals from clinical presentation to treatment. Additionally, these results provide insights into the preclinical course of individual OC histotypes.

Introduction

Ovarian cancer (OC) remains the deadliest gynecologic malignancy, with a five year survival rate of under 50%.[1] This has motivated numerous OC screening trials aimed at early detection.[2–4] While some approaches have resulted in significant downstaging,[2,3] currently, general population OC screening is not recommended as a mortality benefit has not been demonstrated. The interval prior to clinical diagnosis, during which OC could be detected by screening is the preclinical detectable phase (PCDP).[5] Characterising the PCDP would provide key insights for future screening strategies and inform policy to reduce intervals to diagnostic workup and treatment in routine care.

Characterising the PCDP requires longitudinal monitoring of tumour development. Serum biomarker measurements from screening trials can provide insight into tumour development over time in a large population.[2] Longitudinal biomarker measurements in a cancer case, consisting of a baseline level followed by a rise in biomarker level corresponding to tumour growth, can be analysed retrospectively to estimate the time when the tumour was first detectable, corresponding to the start of the PCDP. CA-125 is the most measured OC serum biomarker and annual CA-125 screening has been utilised in multiple longitudinal screening trials.[2,3,6–8] In these trials, cases are likely to have multiple CA-125 measurements before diagnosis, enabling estimation of dynamic parameters such as PCDP duration and *in-vivo* tumour doubling time.[2,3]

To understand the CA-125 PCDP of HGSC in average risk women, we undertook an exploratory analysis of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS).[2,3] In the UKCTOCS multimodal screening (MMS) arm, women underwent annual serum CA-125 testing, with longitudinal CA-125 measurements interpreted by the Risk of Ovarian Cancer Algorithm (ROCA) to guide screening decisions and follow-up with trans-vaginal sonography (TVS). We used the CA-125 data to estimate the PCDP duration, *in-vivo* preclinical tumour doubling times, and window for detection of early stage (Stage I and II) high-grade serous cancers (HGSC). We also report these parameters for non-HGSCs.

Methods

Study design and data sources

UKCTOCS trial design has been reported elsewhere.[2,3] UKCTOCS sample size was determined to obtain 80% power to detect a 30% mortality reduction in the MMS arm compared to the no screening arm.[2] Detailed inclusion/exclusion criteria have been previously reported.[2] 202,638 post-menopausal, normal risk women were recruited between April 2001 and September 2005 through 13 UK trial centres and randomly assigned 2:1:1 to no screening (NS), multimodal screening (MMS), or ultrasound screening. All participants provided a blood sample at recruitment. Participants in the MMS arm underwent screening using annual serum CA-125 measurements interpreted by the Risk of Ovarian Cancer Algorithm (ROCA) until 31-Dec-2011. ROCA uses longitudinal CA-125 to calculate the probability of having OC to update risk following each blood test. Participants with a normal risk (flat CA-125 profile) had a repeat CA-125 screen in one-year, intermediate risk (a small rise above the woman's previous CA-125 baseline) in three months, and those at elevated risk (a significant rise above the previous CA-125 baseline) a TVS and CA-125 measurement in six weeks. Follow-up was through linkage to electronic national health records and questionnaires till 30-June-2020. A majority (90%) of the clinically diagnosed women, were symptomatic at time of diagnosis and 3% were diagnosed incidentally in the course of clinical investigations for another disease.[9,10] Of the screen detected women, while all were apparently asymptomatic and had not visited a healthcare physician, 48% had some non-specific symptoms when questioned. More details on symptoms and routes to diagnosis are reported in a previous publication.[9,10] Diagnosis of ovarian or tubal cancers, histotype, stage (FIGO 2014) and cause of death were determined by an outcomes review committee.[2,3]

Ethics

The trial was approved by the UK North West Multicentre Research Ethics Committee (00/8/34) on June 23, 2000. All women provided written informed consent.

Procedures

We undertook an exploratory analysis nested within UKCTOCS, of women with invasive epithelial OC diagnosed between randomisation and censorship for primary outcome (31-Dec-2014) who had at least two CA-125 measurements, including at least one measurement within two years of diagnosis (Fig. 1). Cases were grouped into HGSC and non-HGSC (low-grade serous, endometrioid, clear cell, mucinous) as detailed previously.[11] We used data from women with no OC during the entire follow-up to 30-June-2020 (2:1 ratio to cases) chosen randomly from the MMS arm to provide information on the distribution of baseline CA-125 levels within and between normal postmenopausal women.

CA-125 measurements were extracted from (1) the screening measurements undertaken at the central UKCTOCS laboratory in the MMS arm for cases and all OC-free women (2) analysis of the recruitment blood sample in the NS arm at the central UKCTOCS laboratory as part of a secondary study and (3) pre-treatment CA-125 measurements where available from medical notes review.

In the present analysis, all cases of invasive epithelial ovarian cancer meeting the sampling criteria above were analysed. No randomisation occurred in the present analysis. Researchers were not blinded to case/control

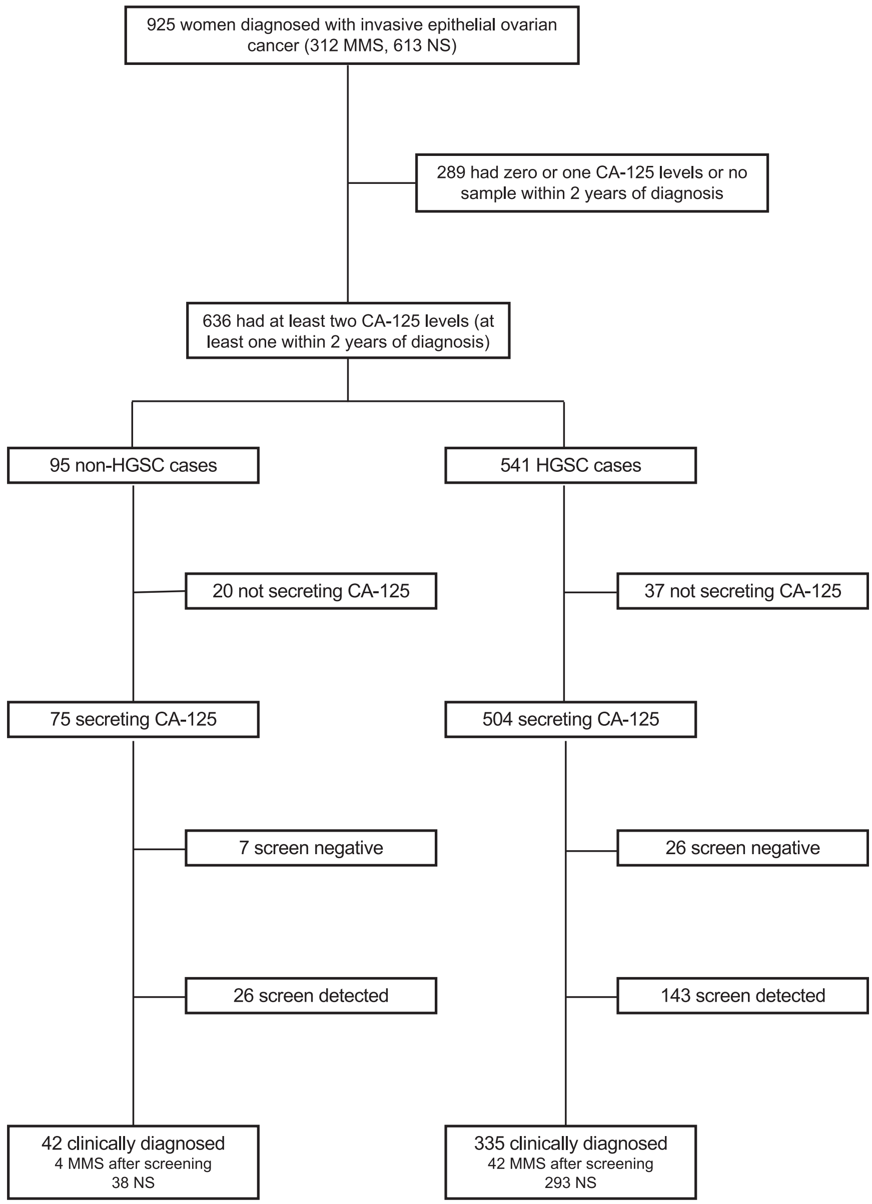

Fig. 1: **Diagram of cases diagnosed with invasive epithelial ovarian cancer in the multimodal and no screening arms of UKCTOCS and selection for analysis.** Participants are grouped based on their estimated Risk of Ovarian Cancer as CA-125 secreting if risk is greater than 1 in 2000 and non-CA-125 secreting if their risk is less than 1 in 2000. Risk of ovarian cancer is computed using all available CA-125 levels. Cases diagnosed in the multimodal screening arm after screening concluded on 31-Dec-2011 are defined as clinically diagnosed. MMS = multimodal screening, NS = no screening, HGSC = high grade serous cancer.

or screen detection status. All CA-125 measurements from UKCTOCS were blinded to case/control and screen detection status as they were obtained prior to diagnosis of ovarian cancer.

Outcomes

We report proportion of cases secreting CA-125 into the circulation, PCDP duration distribution, and *in-vivo* tumour doubling time distribution by histotype. For HGSC, we estimate the window for detection in early stage (stage I/II) as the proportion of the overall CA-125 PCDP and in months. We report the median PCDP duration and *in-vivo* tumour doubling time, along with the IQR and the 95% interpercentile range (IPR), which is the interval from the 2.5th to the 97.5th percentile of the distribution.

Statistics

The details of ROCA have been previously reported.[12] The model underlying ROCA computes Risk of Ovarian Cancer (ROC) using two possible profiles for longitudinal CA-125 (1) a change-point profile consisting of a flat baseline followed by rising CA-125 levels with a fixed doubling time and a change-point corresponding to the start of CA-125 secretion into the circulation by the tumour (Fig. 2a) and (2) a flat profile consisting of a baseline CA-125 level and variation around the baseline.

The ROC for each participant was computed using all available CA-125 measurements and is presented as a probability. The average probability/risk of OC (ROC)

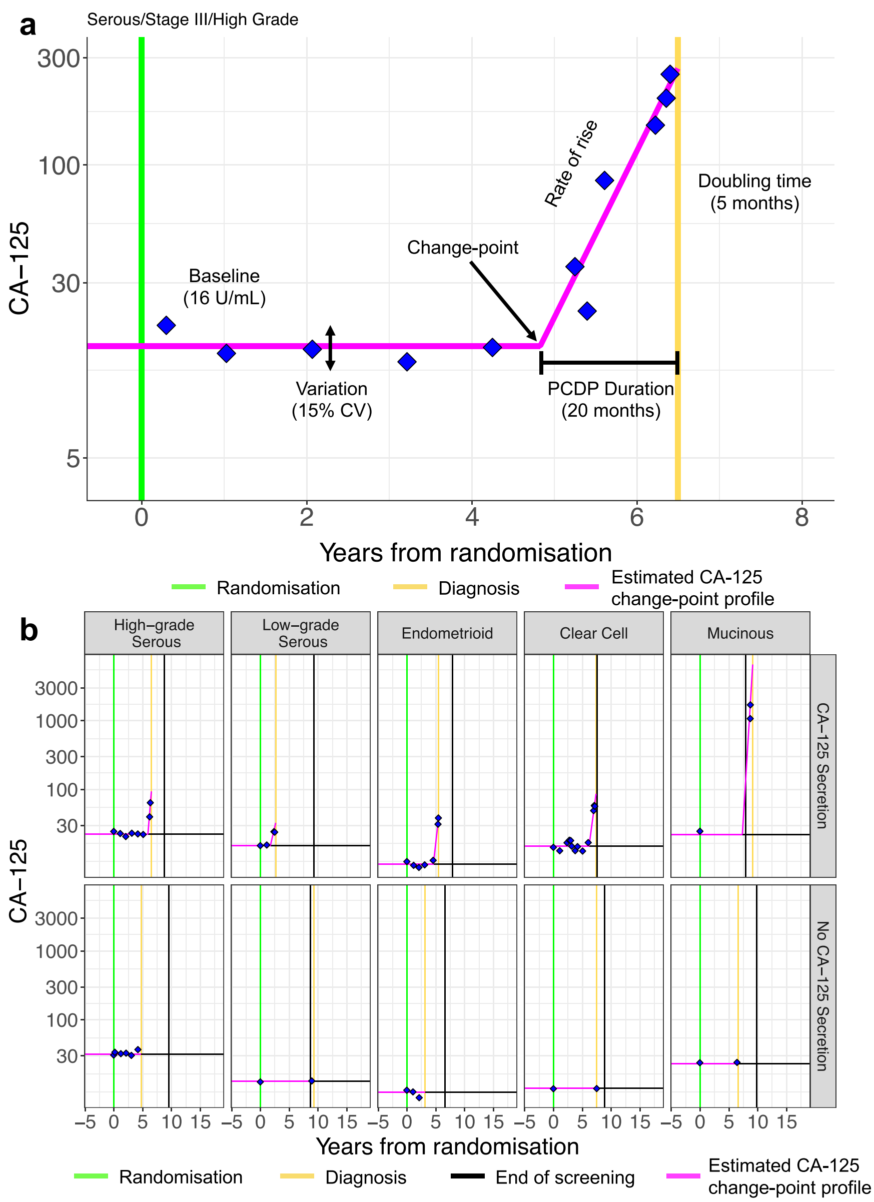

Fig. 2: **Observed and estimated CA-125 secretion profiles for five ovarian cancer histotypes.** a) Schematic of CA-125 secretion showing parameters estimated by the model underlying the Risk of Ovarian Cancer Algorithm (ROCA). Each blue diamond is a CA-125 measurement for that participant. The CA-125 scale is logarithmic, with a linear increase in the graph corresponding to an exponential increase in CA-125, equivalent to CA-125 doubling in a fixed period. The time for CA-125 to double estimates the tumour doubling time. The preclinical detectable phase duration starts when CA-125 levels increase above the participant's baseline and ends at diagnosis. b) Examples of CA-125 secreting and non-CA-125 secreting tumours from the multimodal screening arm for five ovarian cancer histotypes, with an overlay of the CA-125 profile estimated by the model underlying ROCA. PCDP = preclinical detectable phase.

for a woman aged 50–75 in the general population is 1 in 2000.[12] Based on this, a ROC of 1 in 2000 or below is defined as normal risk. Cases were grouped into CA-125 secreting and non-CA-125 secreting based on whether their CA-125 levels had increased significantly above their baseline levels during the preclinical detectable phase. Rather than defining a range for normal and abnormal absolute CA-125 levels, the ROC value was used to determine significant CA-125 rises above the baseline. Those at normal risk (ROC <1 in 2000) were grouped as non-CA-125 secreting, and those with an estimated ROC of ≥1 in 2000 were classified as CA-125 secreting. For individual histotypes, we calculated the proportion of all cases that secreted CA-125. For clarification, non-CA-125 secreting cases include invasive epithelial ovarian cancers that have low expression of CA-125,[13,14] as well as those cancers where at diagnosis serum CA-125 levels have not increased significantly above their baseline levels.

For cases secreting CA-125, we estimated the PCDP and *in-vivo* tumour doubling time using the change-point model underlying ROCA. We defined the PCDP as the interval from the CA-125 change-point to date of diagnosis. The cases include women diagnosed in the MMS arm during screening (screen detected and screen-negative) and those diagnosed after the end of screening and those clinically-diagnosed in the NS arm as previously detailed.[3] It is likely that the screen-detected cases have shorter PCDPs compared to cases who were clinically diagnosed. We therefore estimated separate PCDP distributions for screen-detected and clinically-diagnosed cases. In cases secreting CA-125, we calculated the *in-vivo* tumour doubling time which we defined as the interval in months for the serum CA-125 to double after the change-point.

CA-125 levels were measured during the pre-operative work up of the cases which occurred usually a few days prior to surgery/biopsy. We estimated the CA-125 value in all CA-125-secreting, clinically-diagnosed cases on the date of histopathological diagnosis from the individual case's fitted change point model in early (I/II) and late stage (III/IV/unable to stage) (Supplemental Data p3). The distributions of CA-125 levels for clinically diagnosed cases in early stage versus late stage were compared using the Wilcoxon rank-sum test.

Utilising the knowledge that most late-stage HGSCs have higher CA-125 values than most early-stage HGSCs at diagnosis, we estimated a probability curve that reports the probability a cancer is in early stage at CA-125 values ranging from 10 to 100,000 U/ml (Supplemental Data p3). We reasoned that CA-125 at diagnosis accounts for differences between tumours in their doubling times across a population, unlike PCDP duration. Thus, a late-stage, rapid growing tumour (short PCDP duration) and a late-stage, slower growing tumour (long PCDP duration) would have comparable levels of CA-125 at diagnosis. Based on the probability curve, we defined the CA-125 level at the transition point from early to late stage as the level where the probability of early-stage equals the probability of late-stage disease.

We defined early stage as Stages I and II and late stage as Stages III, IV, or unable to stage. We estimated the window for detection in early stage as the fraction of time from change-point to diagnosis corresponding to the log(CA-125) value at the transition point divided by the estimated average log(CA-125) value at diagnosis in late-stage tumours. To convert this percentage to months, we multiplied the percentage by the PCDP observed in late-stage, clinically diagnosed HGSCs. Details of this probability calculation are available in the Supplemental Data. Histotype frequency and stage distribution between analysed and excluded cases were compared using Pearson's Chi-squared test. All analyses were performed in R version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

Role of funders

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

In UKCTOCS, of 202,562 eligible participants, 50,625 were in the MMS arm and 101,314 were in the NS arm. 925 women (312 MMS, 613 NS) were diagnosed with invasive epithelial OC between randomisation and initial censorship (31-Dec-2014). Of them, 636 (541 HGSC, 20 low grade serous, 33 endometrioid, 23 clear cell, and 19 mucinous tumours) were eligible for inclusion in the analysis (Fig. 1, Table 1).

The median age at diagnosis of the included women with invasive epithelial OC was 68.1 (IQR 63.2–72.9) years, majority were white (98%, 622/636), and 2% (14/636) had a maternal history of OC (Web Table 1, Supplemental Data p5). Analysed and excluded invasive epithelial OC cases were comparable in observed histotype frequency (p = 0.52, Pearson's Chi-squared test) (Web Table 2, Supplemental Data p6). Analysed and excluded clinically-diagnosed cases were comparable in their stage distribution (p = 0.78, Pearson's Chi-squared test) (Web Table 3, Supplemental Data p7). 2730 measurements were available for the 636 cases (median 2 per woman, IQR 2–6), including 2371 measurements in HGSCs and 359 measurements in non-HGSCs. Additionally, 10,322 CA-125 measurements from 1090 OC-free women (median 9 measurements per woman, IQR 7–10) were analysed to estimate the CA-125 baseline distribution for the cases.

Of the analysed HGSC cases, 93% (504/541) secreted CA-125 (Fig. 1, Table 1). Of these CA-125-secreting HGSCs, 143 were screen detected, 26 were screen

	Number analysed	Percentage secreting CA-125	Median tumour doubling time in months	Median preclinical detectable phase in months
High-grade serous	541	93% (504/541)	2.9 (1.5–7.6)	15.2 (9.6–21.8)
Low-grade serous	20	90% (18/20)	6.0 (4.9–10.1)	33.6 (14.9–41.2)
Endometrioid	33	79% (26/33)	3.3 (2.0–9.4)	15.7 (11.2–17.3)
Clear Cell	23	87% (20/23)	5.7 (2.3–7.8)	10.1 (8.0–14.1)
Mucinous	19	58% (11/19)	4.7 (2.6–6.3)	14.0 (10.2–21.5)

Results for CA-125 secretion depicted as % (n/N). The median, 2.5th, and 97.5th percentiles for preclinical detectable phase duration and tumour doubling times in the analysed population are reported as median (95% inter-percentile range). Tumour doubling times computed using all cases secreting CA-125. Preclinical detectable phase duration computed using CA-125 secreting cases that were clinically diagnosed.

***Table 1:* Percentage of tumours secreting CA-125, *in-vivo* tumour doubling time, and preclinical detectable phase duration for five ovarian cancer histotypes.**

negative, and 335 (42 MMS after end of screening and 293 NS) were clinically diagnosed (Fig. 1). CA-125 profiles estimated by the model underlying ROCA matched longitudinal CA-125 values for all five histotypes in both CA-125-secreting and non-secreting tumours (Fig. 2). For the 504 CA-125-secreting HGSCs, the median tumour doubling time was 2.9 months (IQR 2.3–3.7, 95% IPR 1.5–7.6) months (Fig. 3a, Table 1).

For the 335 clinically-diagnosed HGSCs, the median PCDP was 15.2 (IQR 13.1–16.9, 95% IPR 9.6–21.8) months (Fig. 3b, Table 1). The PCDP duration was under one year in 14% (46/335) of the clinically-diagnosed cases. For the 143 screen-detected HGSCs, the median PCDP was 11.6 (IQR 9.2–14.0, 95% IPR 6.3–23.9) months.

To determine the correspondence between CA-125 levels and tumour stage, we assessed CA-125 at diagnosis for clinically diagnosed HGSC in early (stages I/II) and late stage (stages III/IV/unable to stage). Of 335 clinically-diagnosed HGSCs, 38 were diagnosed in early stage and 297 were diagnosed in late stage. CA-125 at diagnosis was lower in early-stage disease compared to late-stage (Fig. 4a, $p < 0.0001$, two-sided Wilcoxson rank sum test). For early-stage HGSC, the median CA-125 level at time of diagnosis was 134 (IQR 76–233, 95% IPR 35–1270) U/ml. 82% (31/38) of early-stage HGSCs had a measurement of <500 U/ml. For late-stage disease, the median CA-125 was 767 (IQR 277–2090, 95% IPR 58–22701) U/ml.

We next used time of diagnosis CA-125 and tumour stage to estimate the probability that a tumour is early stage given a particular CA-125 level, ranging from 10 to 100,000 U/ml (Fig. 4b). Probability of early-stage disease decreased with increasing CA-125 levels. The median percentage of the preclinical CA-125 PCDP spent in early stage was 77% (IQR 76–78, 95% posterior interval 73–80). This point estimate was applied across the PCDP for all 297 clinically-diagnosed, late stage HGSCs, resulting in a median window for detection in early stage of 11.9 (IQR 10.5–13.1, 95% IPR 7.5–16.5) months. 99.7% of cases (296/297) had a window for diagnosis in early-stage greater than six months and 46% (137/297) had a window greater than one year.

For non-high grade serous histotypes, 90% (18/20) of low-grade serous, 79% (26/33) of endometrioid, 87% (20/23) of clear cell, and 58% (11/19) of mucinous tumours secreted CA-125. Across the five histotypes, 91% (579/636) of tumours secreted CA-125. Clinically-diagnosed mucinous and endometrioid tumours had similar median PCDP durations to HGSC at 14.0 and 15.7 months, respectively (Table 1). Clear cell tumours had the shortest median PCDP duration at 10.1 months (Table 1). Low-grade serous tumours had the longest median duration at 33.6 months. Low-grade serous, clear cell, and mucinous cancers had median doubling times of 6.0, 5.7, and 4.7 months, respectively (Table 1). Endometrioid tumours had a median doubling time of 3.3 months, comparable to HGSC (Table 1).

Discussion

This report provides comprehensive evidence, based on a prospective clinical trial spanning two decades, of the OC CA-125 preclinical detectable phase (PCDP) and *in-vivo* tumour doubling time. Of women with HGSC, 93% (504/541) secrete CA-125. The median *in-vivo* tumour doubling time is 2.9 (IQR 2.3–3.7) months, with 95% of HGSCs doubling in 1.5 to 7.6 months. There is a median window of 15.2 (IQR 13.1–16.9, 95% IPR 9.6–21.8) months prior to clinical diagnosis during which HGSCs are detectable using serial CA-125. During this window, HGSC is in early stage for 11.9 (IQR 10.5–13.1, 95% IPR 7.5–16.5) months. The PCDP is biomarker specific. Hence, these findings are relevant only to ovarian cancers where serum CA-125 levels are significantly elevated over baseline, which comprised 93% of HGSC and 79% of non-HGSC in our dataset.

The findings have far-reaching implications for both early detection and routine clinical care of women with HGSC. For screening, this suggests that there is possibly only one and maybe two opportunities for detection prior to clinical diagnosis using annual CA-125 screening. These results support the shorter 3-6-monthly screening intervals adopted for screening high-risk women. Additionally, the doubling time of three months implies that

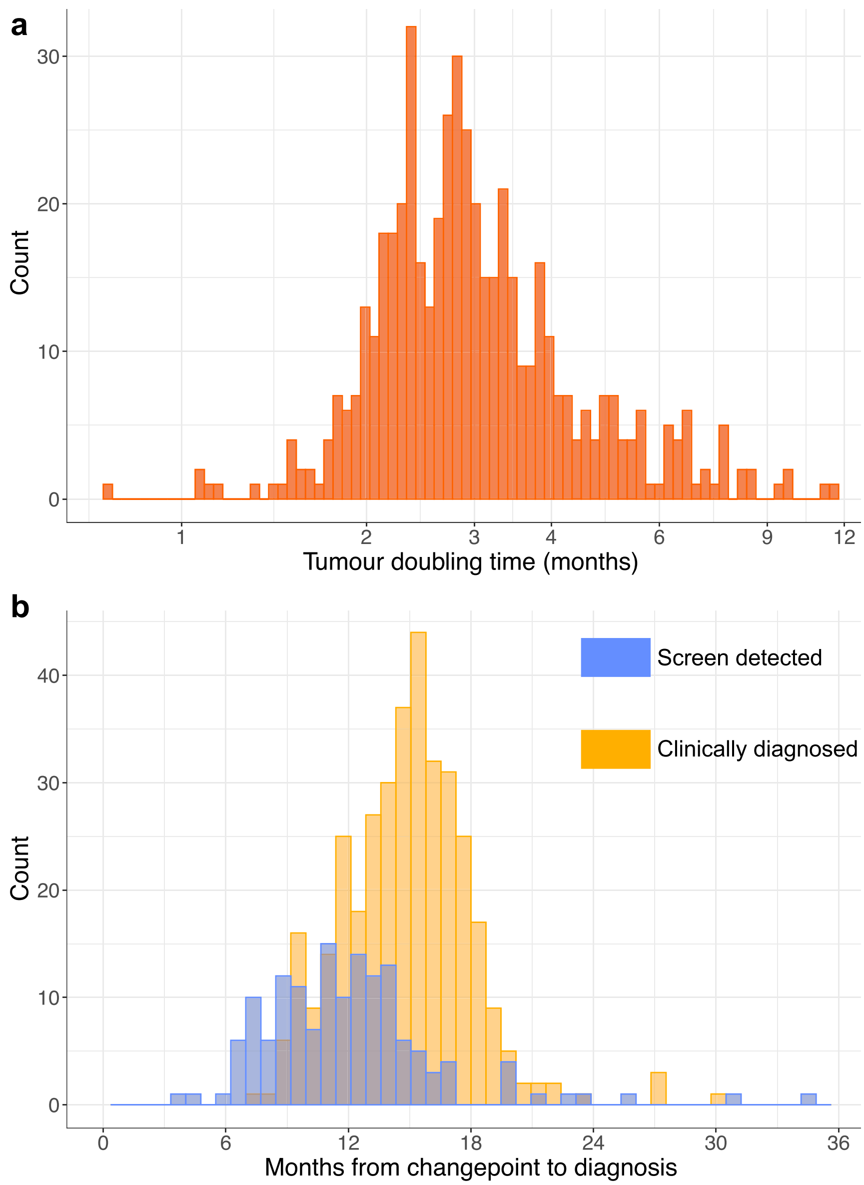

Fig. 3: **High-grade serous cancer tumour doubling time and preclinical detectable phase duration.** a) Histogram of tumour doubling times for 504 high grade serous tumours secreting CA-125. b) Histogram comparing preclinical detectable phase durations for 143 CA-125 secreting cases detected by screening and 335 CA-125 secreting cases that were clinically diagnosed.

workups following a positive screening test should be expeditiously scheduled to maximise the opportunity for detection in early stage. For routine care, the short doubling time provides urgent impetus to reduce diagnostic intervals from clinical presentation to primary physician to diagnosis and treatment, with implications for diagnostic pathways, treatment guidelines, and waiting-time targets.[15]

Overall 91% (579/636) of invasive epithelial OCs and 93% (504/541) of HGSCs secreted CA-125. This is in line with the literature where 80–90% of OCs are reported to have a CA-125 > 35 U/ml at diagnosis.[16] The median PCDP duration for clinically-diagnosed HGSC was 15.2 (IQR 13.1–16.9, 95% IPR 9.6–21.8) months (Table 1). The Shizuoka Cohort Study on Ovarian Cancer Screening (SCSOCS),[17] using a single CA-125 measurement 2 months to 9.4 years prior to diagnosis from 168 serous OCs, reported a mean interval from slightly elevated CA-125 (≥35–65 U/ml) to clinical diagnosis of 16.8 months (1.4 years). Longitudinal preclinical CA-125 measurements (as in UKCTOCS) enable estimation of the PCDP duration per case, which provides a

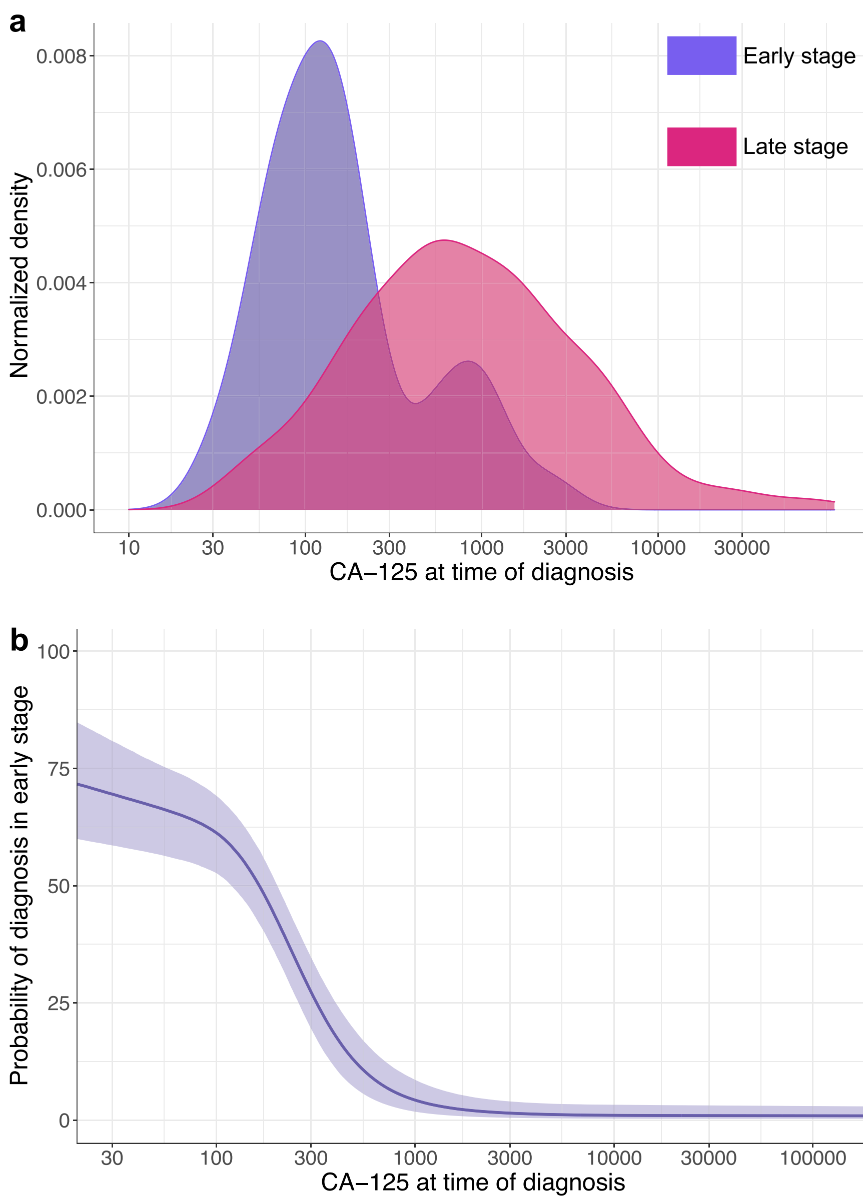

Fig. 4: **CA-125 at diagnosis in clinically-diagnosed, early and late-stage high-grade serous cancer and probability of early-stage disease.** a) Normalised density of CA-125 on the day of diagnosis for 38 early stage and 297 late-stage, clinically diagnosed cases. b) Curve showing probability of early-stage high grade serous cancer at 1000 CA-125 levels equally spaced on the log scale ranging from 10 U/ml to 100,000 U/ml. Shaded region denotes 95% interval.

distribution of PCDP durations across cases. Estimating such a distribution with single timepoint preclinical measurements per individual is not possible. Despite the limitations of a single timepoint measurement per case, significantly lower proportions (25%) of serous cases secreting CA-125 and combining high and low grade serous cancers in the analysis, the 16.8 month estimate using a CA-125 cut-off aligns with our 15.2 month median CA-125 PCDP estimate. Ishizawa et al. estimated the HGSOC sojourn time from PLCO data to be 21.6 months (1.8 years).[18] We consider this to be an overestimate, probably due to use of the continuous time Markov chain model which assumes an exponential distribution for overall sojourn time where the most common value (mode) is 0 years. When we modelled the PCDP of each case using individual data from UKCTOCS, we observed a near symmetric distribution with a mode of 15 months (Fig. 3b).

During this median PCDP window, HGSC is in early stage for 11.9 (IQR 10.5–13.1, 95% IPR 7.5–16.5) months. For this analysis, we estimated the CA-125 value on the date of diagnosis for all CA-125-secreting, clinically-diagnosed cases from the individual case's fitted change point model. This addresses the lag time between the CA-125 measurement during the pre-operative work up and the date of histopathological diagnosis through surgery/biopsy.

Based on modelling tumour growth rates *in silico* using preclinical TVS measurements, the median TVS preclinical detectable phase duration for HGSC was estimated to be 21.1 months (1.76 years).[19] However, almost 50% of the simulated HGSC growth curves never reached the minimum detection threshold.[19] In contrast, 93% of HGSC tumours in the present analysis secreted CA-125. Gambhir and colleagues modelled ovarian tumour growth and CA-125 shedding *in silico* and found that the minimally detectable tumour size by elevated serum CA-125 (>35 U/ml) ranged from 0.11 mm^3 to 3610 mm^3.[20] All of this suggests that while some HGSCs can be detected early by both TVS and increased CA-125 levels, many are only detectable by increasing CA-125 levels alone. This is reflected in the lower sensitivity observed for TVS screening, especially during incidence screening in the ultrasound arm (61.5%; 95% CI: 52.6–69.9) compared to the CA-125-based multimodal screening arm (86.2%; 95% CI: 80.8–90.6) in UKCTOCS.[21]

The precise relationship between the CA-125 PCDP and the preclinical interval from onset of serous tubal intraepithelial carcinoma (STIC) to clinically diagnosed HGSC remains unclear. Brown and Palmer estimated the interval from STIC to clinically diagnosed HGSC to be 5.1 years, with more than 4 years as STIC and stage I/II cancers, and approximately 1 year as stage III/IV cancers.[22] Recent studies based on genetic analyses have estimated the average interval from onset of STIC to ovarian carcinoma to be 6–7 years[23,24] with an estimate of 2 years from onset of ovarian carcinoma to metastasis in a subset of three cases of advanced stage HGSC.[23] CA-125 levels were normal in a cohort of ten cases with STICs.[25] Combined with our observation that 93% of HGSCs secreted CA-125, this suggests that CA-125 enters the circulation primarily after progression to invasive carcinoma, and our CA-125 PCDP estimates likely reflect the interval from invasive carcinoma to clinical diagnosis.

The median CA-125 PCDP for screen detected HGSC was 11.6 months. It is important to note that this is not the sojourn time which is akin to the PCDP of clinically diagnosed cases. In addition to the interval from clinical referral to surgery (median 1.8 months/8 weeks), the PCDP duration for screen-detected HGSC includes the interval for protocol driven repeat CA-125 and TVS undertaken to decrease false positives and ensure an overall specificity of 99.8%.[2] This interval from annual test to diagnosis was significantly longer in women at intermediate/elevated risk with annual CA-125 < 35 U/ml (6.9 months/30 weeks; IQR 4.1–9.9 months/18–43 weeks) compared to those with ≥35 U/ml (2.8 months/12 weeks; IQR 1.6–4.4 months/7–19 weeks).[26] The current interval from annual test to diagnosis is one of the limitations of the current MMS strategy. Future approaches need to minimise this interval, for example by incorporating more sensitive second line tests or identifying new biomarkers to complement CA-125.

Significant research has been conducted to identify serum biomarkers that complement CA-125.[27] The most studied is HE4,[27] but recently, other biomarkers have been reported to improve both sensitivity and specificity.[28,29] LINE-1 ORF1p has shown promise as a biomarker exclusively produced and secreted by cancers, and it is detectable in 90% of OCs and 90% of fallopian tube precursor lesions.[28] Analyses of tumour DNA in blood and other accessible fluids (e.g. uterine lavage or cervical swabs) are another method for early detection.[29,30] The PCDP for these new biomarkers will be dependent on their lead time over CA-125.

HGSC tumours had a short *in-vivo* tumour doubling time (median 2.9 months) based on CA-125 levels. Previous analyses of HGSC have identified four molecular subtypes differing in prognosis and *MUC16* gene expression.[13,14,31] It is likely that these subtypes have varying tumour doubling times, which could explain the wide range we observed. However, the precise relationship between molecular subtype and preclinical serum CA-125 dynamics remains unknown. We do not have information on such molecular subtypes in UKCTOCS. We are not aware of human preclinical invasive HGSC studies correlating serum CA-125 with tumour size. There are however multiple studies on CA-125 related tumour cell kinetics in metastatic and recurrent phase and doubling of CA-125 is a well-established OC progression criterion.[32] In the International Cancer Benchmarking Partnership (ICBP) study, the median interval from first clinical presentation to diagnosis in the best performing of nine high-income jurisdictions was 2.2 months (66 days), with 25% of patients having an interval of over 4.4 months (133 days).[33] This interval is subject to significant health system variations which are magnified in ovarian cancer where the symptoms are non-specific with many efforts across the globe to reduce it. The key driver is the adverse impact that any delays can have on quality of life of the patient and their family. We provide another reason to reduce this time in ovarian cancer by providing data on tumour progression. Our observed median tumour doubling time suggests that in one in four of patients cancers will double in volume while awaiting treatment. It underscores the urgency to reduce the interval from symptomatic presentation to treatment. A similar urgency applies to minimising the time taken for second line tests and diagnostic workup in OC screening approaches.

Screening frequency is a crucial parameter in early detection strategies. In both the United Kingdom Familial Ovarian Cancer Screening Study (UKFOCSS) and the Cancer Genetics Network/Gynecologic Oncology Group trials, MMS at 3–4 months intervals year resulted in a significant downstaging of HGSC.[6,7] In our analysis, 99.7% (296/297) of late-stage, clinically-diagnosed HGSCs had a window greater than 6 months for diagnosis in early stage and 46% (137/297) had a window greater than 12 months. This suggests that general population screening every 6 months would result in 99.7% of women having at least one screening opportunity for early-stage HGSC detection compared to 46% with annual testing. However, selection of screening frequency in any screening program needs to consider cost effectiveness and acceptability.

In contrast to HGSC, 79% (75/95) of non-HGSC tumours secreted CA-125. Previous work has suggested that non-HGSCs show less dynamic CA-125 secretion compared to HGSC.[34] Our results indicate that CA-125 is secreted by the majority of non-HGSC tumours, although the urgency for early detection of such tumours is much lower due to typical presentation at an early stage.[34,35] Low grade serous tumours had approximately double the PCDP duration of HGSC (Table 1), in keeping with previous observations that they are indolent and slower growing.[35] Endometrioid (15.3 months) and mucinous (14.0 months) tumours had median CA-125 PCDPs comparable to HGSC (15.2 months). However, a majority of non-HGSCs were diagnosed in early stage (Table 1), as in other studies.[36,37] A key contributory factor is likely their slower median doubling times compared to HGSC (Table 1). More work is required to determine variation in doubling time of non-HGSCs as overall numbers per histotype in our analysis ranged from 19 to 33 cases, despite inclusion of all analysable non-HGSCs diagnosed in over 150,000 women followed for a median of 16.3 years.

Strengths of UKCTOCS have been detailed previously[2] and include number of participants, multicentre design, and high adherence to trial protocol. Follow-up using national registries and independent outcomes review ensured minimal ascertainment bias. The number of cases and the availability of longitudinal preclinical samples enabled us to capture a wide distribution of CA-125 PCDP durations and tumour doubling times. Furthermore, most preclinical OC reports to date have studied germline *BRCA1* mutation carriers, which represent only 10% of diagnosed HGSC.[38] The exclusion of high risk women from UKCTOCS enabled us to estimate these parameters for the 90% of HGSCs that are sporadic.

A key limitation was that the majority of cases were White and all were postmenopausal. Many factors influence CA-125 levels, including ethnicity, menopausal status, benign ovarian disease, and obesity.[34,39] In particular, CA-125 levels have been shown to be lower in African and Asian women compared to Caucasian women.[39] However, through comparing each case to her baseline CA-125 level, individualised interpretation of serial CA-125 measurements accounted for factors like ethnicity and obesity, ensuring minimal contribution of such epidemiological factors to CA-125 increases. Thus, increases in CA-125 levels during the PCDP were driven by invasive OC growth. Before these findings are applied to premenopausal women, it would be important to perform similar analyses in cohorts from screening trials that included premenopausal women.

Most women underwent annual screening. More frequent screening would have provided greater granularity of CA-125 levels providing more accurate estimates of the PCDP and doubling time. However, the inclusion of CA-125 at time of diagnosis provides unbiased estimates of both these measures. In addition, in 10% of women, more frequent CA-125 levels (6 and 12 weekly) were available. While most CA-125 measurements were assayed centrally in the UKCTOCS laboratory, some CA-125 values at diagnosis were extracted from medical records. While there can be inter-lab variability in CA-125 measurements, external CA-125 values were from clinically accredited laboratories within the National Health Service where CA-125 assays were monitored by an External Quality Assessment Scheme. Another issue was that requirement of at least two CA-125 measurements per case resulted in 67% (636/925) of invasive epithelial OCs being analysable. However, analysed cases were representative of all invasive epithelial OCs in UKCTOCS based on the near equality of distributions for histotype and stage (Supplemental Data pp5-6). Finally, while a majority of the cases are likely to be sporadic OC as women with high-risk family history were excluded and referred to the UKFOCSS,[7] formal genetic testing was not undertaken. Studies have since revealed that 50% of individuals with pathogenic variants in the *BRCA* genes are not identified using family history.[40] External validation using other datasets such as from the PLCO trial, where up to six annual CA-125 levels are available per participant in the screened arm, would add validity to the findings.[8]

There are multiple innovative strategies such as population genetic testing for cancer susceptibility genes, bilateral salpingectomy and oophorectomy in high-risk women and increasing use of opportunistic salpingectomy in average-risk women that are likely to reduce HGSC incidence in the future. However, there will still be need for a screening program for the majority of average risk women. Currently, there is no general population screening program for OC. The results of this analysis, quantifying tumour behaviour before diagnosis, will enable researchers to develop and benchmark future screening approaches. It also provides urgent impetus for reduction of diagnostic workup and treatment intervals in routine clinical practice.

Contributors
SJS, JSB, UM, and IJJ conceptualised the study and SJS and JSB devised the underlying statistical models. JSB did the literature search. AR and UM extracted the dataset. JSB undertook the formal statistical analysis

and modelling and SJS verified this analysis. JSB and SJS prepared the tables and figures. JSB, SJS, and UM drafted the manuscript. All authors contributed to the interpretation of the data and revision of the manuscript. JSB, SJS, AR, and UM accessed and verified the underlying data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing statement

The UKCTOCS protocol is available on the trial website. The individual participant data that underlie the results reported in this Article, after deidentification, will be available beginning 12 months after publication. A data dictionary defining each field in the set will be made available. Researchers will need to state the aims of any analyses and provide a methodologically sound proposal. Proposals should be directed to u.menon@ucl.ac.uk. Data requestors will need to sign a data access agreement and in keeping with patient consent for secondary use, obtain ethical approval for any new analyses. Following all necessary approvals and mandatory training required for access to UKCTOCS data, the researchers will be given access to the data, which is housed within the UCL Data Safe Haven.

Declaration of interests

UM had stock ownership, awarded by University College London (UCL) until October, 2021, in Abcodia, which holds the licence for risk of ovarian cancer algorithm (ROCA). UM and MKBP have received grants and AG-M, AR, and MB have been funded by grants from the Medical Research Council (MRC), Cancer Research UK, the National Institute for Health Research (NIHR), and The Eve Appeal. UM and AG-M have also received grants from the Australian National Health and Medical Research Council (NHMRC), MRC Proximity to Discovery Industrial Connectivity Award, and Innovate UK Grant. UM and AG-M report research collaboration contracts with iLOF (intelligent Lab on Fiber), Micronoma, Imperial College London, Dana Farber Cancer Institute (DFCI), QIMR Berghofer Medical Research Institute, Mercy Bioanalytics, and University of Innsbruck. UM additionally reports research collaboration contracts with RNA Guardian and DFCI. UM holds patent number EP10178345.4 for Breast Cancer Diagnostics. UM received an honorarium for a lecture from the New York Obstetrical Society (USA), and was reimbursed for travel and accommodations by New York Obstetrical Society. UM has also been a member of Tina's Wish Scientific Advisory Board (USA) and Research Advisory Panel, Yorkshire Cancer Research (UK). She has been a member of International Alliance for Cancer Early Detection (ACED); data monitoring committee for the mixed COVID-19 vaccines study in India; Trial Steering Committee, NOVEL; Trial Steering Committee, PROTECTOR. AG-M is a member of ACED Gynaecological Cancer Working Group and is ACED codirector Research Domain Trials. MKBP was an Associate Member of the EME funding committee while the project was active. SJS co-developed ROCA in 1995, which was patented by Massachusetts General Hospital, MA, USA, and Queen Mary University of London, London, UK, and is owned by these universities (the patent has expired). Massachusetts General Hospital and Queen Mary University of London granted a licence for the ROCA to Abcodia in 2014. SJS reports stock options from SISCAPA Assay Technologies for participation on a board. SJS received grants and JSB has been funded from the US National Cancer Institute. SJS additionally reports funding from Concord (MA) Detect Ovarian Cancer Early Fund and Mercy Bioanalytics. SJS participated in clinical and scientific advisory boards for Mercy BioAnalytics, SISCAPA Assay Technologies, and Guardant Health (for which he was paid consulting fees). IJJ reports grants from Eve Appeal Charity, MRC, Cancer Research UK, and NIHR during the conduct of the study. IJJ codeveloped the ROCA in 1995. Massachusetts General Hospital and Queen Mary University of London granted a licence for the ROCA to Abcodia in 2014. IJJ was non-executive director, shareholder, and consultant to Abcodia and has rights to royalties from sales of the ROCA. IJJ founded (in 1985), was a trustee of (2012–14), and is now an Emeritus trustee (2015–present) of The Eve Appeal, one of the funding agencies for UKCTOCS. LF reports MRC funding for the psychosocial group of the UKCTOCS study 2001–13, paid to University of Sussex. AJM was a member of NIHR Health Technology Assessment and Efficacy and Mechanism Evaluation editorial board (2012–22). RM reports funding from The Eve Appeal, Rosetrees Charity, Barts Charity, Yorkshire Cancer Research, Ovacure, British Gynaecological Cancer Society, AstraZeneca, North East London Cancer Alliance, and GSK. RM reports consulting fees from Everything Genetics Limited. NS received honoraria from AstraZeneca–MSD and GSK for participation in advisory boards. All other authors declare no competing interests.

Acknowledgements

Researchers at MGH were supported by funds from the National Cancer Institute (NCI) Early Detection Research Network (U01 CA152990, U2C CA271871), Concord (MA) Detect Ovarian Cancer Early Fund, and NCI (U01 CA260758). Researchers at UCL were supported by the MRC Clinical Trials Unit at UCL core funding (MC_UU_00004/01).

We thank the volunteers without whom the trial would not have been possible and everyone involved in the conduct and oversight of UKCTOCS. UKCTOCS was funded by the MRC (G9901012 and G0801228), Cancer Research UK (C1479/A2884), and the UK Department of Health, with additional support from The Eve Appeal. The long-term follow-up for UKCTOCS was supported by the NIHR (Health Technology Assessment grant 16/46/01), Cancer Research UK, and The Eve Appeal.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105554.

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