**Supplementary Materials**

**Inclusion and Exclusion Criteria**

Eligibility criteria included the following, with “close relatives” defined as first- or second-degree blood relatives:

1. the subject or close relative (deleterious mutation in a first-degree relative confers a 50% prior probabiliy of an untested subject being a mutation carrier, while deleterious mutation in a second-degree relative confers a 25% prior probability of an untested subject being a mutation carrier ) had a known, deleterious *BRCA1* or *BRCA2* mutation; or
2. at least two ovarian or breast cancers (including DCIS) had been diagnosed among the subject or close relatives within the same lineage; or
3. the subject was of Ashkenazi Jewish ethnicity, with one first-degree or two-second degree relatives with ovarian or breast cancer; or
4. the subject was of Ashkenazi Jewish ethnicity and had a personal history of breast cancer; or
5. the probability of carrying a *BRCA1* or *BRCA2* mutation given family pedigree of breast and ovarian cancers as calculated by BRCAPRO [1] exceeded 20%.

When a diagnosis of breast cancer was required to meet any of these criteria, at least one breast cancer must have been pre-menopausal or, if menopausal status was unknown at time of diagnosis, then age at diagnosis was required to be ≤ 50 years.

It has since become apparent that some of these women are now known to ***not*** be at increased risk for ovarian cancer – *e.g.,* women with a site-specific breast cancer family history whose families lack a deleterious *BRCA1/2* mutation ([2] [3]), but they are nonetheless included in our analysis in line with the principle of “intention to treat.”

The subject was excluded from study participation if she:

1. had a personal history of ovarian cancer, including low malignant potential cancers (LMP), or primary papillary serous carcinoma of the peritoneum; or
2. had a close relative with a deleterious *BRCA1/2* mutation and the subject had tested *negative* for the same mutation; or
3. was less than 30 years of age; or
4. was currently pregnant or anticipating pregnancy during the study; or
5. was participating in other ovarian cancer early detection trials; or
6. had a current active malignancy (other than non-melanoma skin cancer); or
7. had been treated for metastatic malignancy within the prior five years (excluding hormonal therapies); or
8. had undergone intra-peritoneal surgery within the prior 3 months (laparoscopy or laparotomy); or
9. had a history of any medical conditions that would place the subject at risk related to phlebotomy, including but not limited to hemophilia or other bleeding disorders, chronic infectious disease, emphysema or serious anemia.

***Women who had clinical symptoms suggestive of ovarian cancer were also excluded***.

**ROCA Methods**

ROCA builds on statistical models that incorporate a doubling time for each tumor and an individual CA125 baseline for each woman. [2] The baseline is the expected value of CA125 (after natural log transformation) prior to the change-point. The doubling time *D* is related to the slope *s* of the linear increase of log(CA125) after the change-point by *D =* log(2)*/s*. To calculate the probability of a change-point given one or more CA125 values we utilized statistical modeling of serial CA125 data from previous screening studies. [3] [4] [5] This modeling estimated the distribution of rate of CA125 increase following a change-point in women diagnosed with ovarian cancer while on study, the distribution of the baseline CA125 level, and the natural variation in CA125 over time in women who were *not* diagnosed with ovarian cancer during each study. The age-specific ovarian cancer incidence rates for all females were obtained from US SEER tumor registries. For each woman, the initial odds based on the incidence rate for her age was updated with the odds ratio that her serial CA125 values represented a change-point *versus* a flat profile.

The ROCA calculation for the odds ratio measures the distance from each woman’s serial log(CA125) data to (1) a series of change-point profiles derived from women diagnosed with ovarian cancer in previous screening trials, and to (2) a series of flat profiles derived from women who did *not* develop ovarian cancer in the same trials [2]. This distance is the sum of squares between the log(CA125) value and the profile (change-point or flat) of expected values, normalized by the within woman variance of log(CA125) over time. The average over the distances gives a summary distance for each set of profiles. The ratio of these two distances provides an estimate of the odds factor attributable to CA125, which multiplies the initial odds of having ovarian cancer due to the woman’s age, to give the final odds of having ovarian cancer. The probability (or risk) of having ovarian cancer is then odds/(odds+1). Thus, ROCA built on these statistical models of serial CA125 values in historical cases and controls to calculate the probability that a CA125 increase in this current study was due to a disease-associated change-point rather than natural fluctuations. This probability of a change-point is a surrogate for the risk that the subject has undiagnosed ovarian cancer.

ROCA attains its power to detect ovarian cancer due to the much lower CA125 coefficient of variation (CV) between longitudinal CA125 results *within* individual women (median 12%, 95% population interval 6% - 18%, compared with the baseline CV *between* women of 36%. The example illustrated in Figure 1 of the main text has an even lower *within* woman CV of 10%. The choice of 35 U/mL as the single threshold with which to interpret CA125 for all women in current ovarian cancer screening strategies is adversely influenced by the higher CV *between* women of 36%, which results in much lower statistical power for the standard cut-point to identify undetected ovarian cancer.

**Sample Handling & CA125 Assay Instrumentation**

All peripheral blood samples for both studies were collected from venipunctures in 10 mL red top glass tubes, immediately spun down and the serum frozen at −80°C prior to batch shipping on dry ice, or individually shipped overnight wrapped in foam on a frozen ice pack in a Styrofoam container, to the two central labs.

The reportable range of the assay was 0.6 to 500 IU/mL, with a normal reference interval for females of less than 35 U/mL. Grossly hemolyzed or lipemic specimens were rejected without testing and a replacement blood draw requested. Assay CV on the E170 automated instrument was less than 4% on the basis of daily monitoring with quality control (QC) specimens (low QC mean = 32 U/mL, high QC mean = 93 U/mL).

**Specificity and Positive Predictive Value Standards**

Specificity and PPV standards were derived from general population screening trials. The standard CA125 test (>35 U/mL) has an annual specificity of 98%,[6] *i.e.,* 2% false-positives. We aimed to maintain the same 2% false-positive rate per ROCA test. With quarterly ROCA tests, in which each woman has her own baseline CA125 level, false-positive results between women are likely to be statistically independent. Thus, a 2% false-positive rate per quarterly ROCA test gives an 8% false-positive rate per year or an annual specificity of 92%, and therefore we set a lower bound of 90%. A PPV of 10% has been deemed the minimum acceptable level for screening programs in normal-risk women[7] and, by extension, we set the same value as the desired level for screening women at increased ovarian cancer risk.

**Supplementary Bibliography**

1. Berry, D.A., et al., *BRCAPRO validation, sensitivity of genetic testing of BRCA1/BRCA2, and prevalence of other breast cancer susceptibility genes.* J Clin Oncol, 2002. **20**(11): p. 2701-12.

2. Skates, S.J., D.K. Pauler, and I.J. Jacobs, *Screening Based on the Risk of Cancer Calculation From Bayesian Hierarchical Changepoint and Mixture Models of Longitudinal Markers.* Journal of the American Statistical Association, 2001. **96**(454): p. 429-439.

3. Jacobs, I., et al., *Multimodal approach to screening for ovarian cancer.* Lancet, 1988. **1**(8580): p. 268-71.

4. Einhorn, N., et al., *Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer.* Obstet Gynecol, 1992. **80**(1): p. 14-8.

5. Jacobs, I.J., et al., *Screening for ovarian cancer: a pilot randomised controlled trial.* Lancet, 1999. **353**(9160): p. 1207-10.

6. Skates, S.J., et al., *Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA 15-3, CA 72-4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions.* J Clin Oncol, 2004. **22**(20): p. 4059-66.

7. Jacobs, I. and R.C. Bast, Jr., *The CA 125 tumour-associated antigen: a review of the literature.* Hum Reprod, 1989. **4**(1): p. 1-12.

Supplementary Table 1

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **Year** | **# subjects with ROCA rec** | **# with normal rec** | **% with normal rec** | **# with intermediate rec** | **% with intermediate rec** | **# with elevated rec** | **% with elevated rec** |
| 2001 | 72 | 69 | 95.8% | 2 | 2.8% | 0 | 0.0% |
| 2002 | 1112 | 992 | 89.2% | 102 | 9.2% | 14 | 1.3% |
| 2003 | 1697 | 1525 | 89.9% | 153 | 9.0% | 19 | 1.1% |
| 2004 | 1992 | 1859 | 93.3% | 117 | 5.9% | 16 | 0.8% |
| 2005 | 2247 | 2116 | 94.2% | 115 | 5.1% | 16 | 0.7% |
| 2006 | 2169 | 2019 | 93.1% | 132 | 6.1% | 18 | 0.8% |
| 2007 | 1555 | 1488 | 95.7% | 59 | 3.8% | 7 | 0.5% |
| 2008 | 1271 | 1216 | 95.7% | 45 | 3.5% | 10 | 0.8% |
| 2009 | 1100 | 1052 | 95.6% | 38 | 3.5% | 10 | 0.9% |
| 2010 | 726 | 690 | 95.0% | 24 | 3.3% | 12 | 1.7% |
| 2011 | 310 | 289 | 93.2% | 14 | 4.5% | 7 | 2.3% |
| 2012 | 36 | 32 | 88.9% | 4 | 11.1% | 0 | 0.0% |
| 2013 | 22 | 17 | 77.3% | 4 | 18.2% | 1 | 4.5% |
| **Average** | **1101** | **1028** | **92.1%** | **62** | **6.6%** | **10** | **1.2%** |

Rec = recommendation

**LEGENDS**

**Supplemental Methods:** Provides detailed eligibility criteria, statistical models for building ROCA, sample handling and processing, and setting specificity and positive predictive value standards.

**Supplemental Table 1:** Frequency of ROCA recommendations at normal, intermediate, and elevated levels of risk by year on study.