Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

Cancer Epidemiology, Biomarkers & Prevention

Use of CA125 and HE4 Serum Markers to Predict Ovarian Cancer in Elevated-Risk Women

Beth Y. Karlan, Jason Thorpe, Kate Watabayashi, et al.

Cancer Epidemiol Biomarkers Prev 2014;23:1383-1393. Published OnlineFirst May 1, 2014.

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Use of CA125 and HE4 Serum Markers to Predict Ovarian Cancer in Elevated-Risk Women

Beth Y. Karlan[1], Jason Thorpe[4], Kate Watabayashi[4], Charles W. Drescher[4,5], Melanie Palomares[2], Mary B. Daly[6], Pam Paley[5], Paula Hillard[3], M. Robyn Andersen[4], Garnet Anderson[4], Ronny Drapkin[7], and Nicole Urban[4]

Abstract

Background: Serum markers are used before pelvic imaging to improve specificity and positive predictive value (PPV) of ovarian cancer multimodal screening strategies.

Methods: We conducted a randomized controlled pilot trial to estimate surgical PPV of a "2 of 3 tests positive" screening rule, and to compare use of HE4 as a first-line (Arm 1) versus a second-line (Arm 2) screen, in women at high and elevated risk for epithelial ovarian cancer (EOC) at five study sites. Semiannual screening was offered to 208 women ages 25 to 80 years with deleterious *BRCA* germline mutations and to 834 women ages 35 to 80 years with pedigrees suggesting inherited susceptibility. Annual screening was offered to 130 women ages 45 to 80 years (Risk Group 3) with epidemiologic and serum marker risk factors. Rising marker levels were identified using the parametric empirical Bayes algorithm.

Results: Both strategies yielded surgical PPV above 25%. Protocol-indicated surgery was performed in 6 women, identifying two ovarian malignancies and yielding a surgical PPV in both arms combined of 33% (95% confidence interval: 4%–78%), 25% in Arm 1 and 50% in Arm 2. Surgical consultation was recommended for 37 women (26 in Arm 1 and 11 in Arm 2). On the basis of 12 women with at least 2 of 3 tests positive (CA125, HE4, or imaging), an intent-to-treat analysis yielded PPV of 14% in Arm 1 and 20% in Arm 2.

Conclusions: Positive screens were more frequent when HE4 was included in the primary screen.

Impact: HE4 may be useful as a confirmatory screen when rising CA125 is used alone as a primary screen.

Cancer Epidemiol Biomarkers Prev; 23(7); 1383–93. ©2014 AACR.

Introduction

Epithelial ovarian cancer (EOC), including serous ovarian, fallopian tube, and primary peritoneal carcinomas, is the most lethal gynecologic malignancy. Early detection could potentially reduce EOC mortality, but because efficacy has not been demonstrated, national guidelines (1) do not recommend EOC screening in the general population. For women with or at high risk for a germline *BRCA* mutation, semiannual screening with transvaginal ultrasound (TVU) and CA125 testing is recommended starting at age 35 years, or 5 to 10 years earlier than the earliest age of EOC diagnosis in the family. The UK familial study of EOC screening reported that frequent screening and prompt surgical intervention are needed to detect cancer at an early stage (2).

Only 0.8% of women in the general population are diagnosed with EOC by age 70; 39% and 22% of *BRCA1* and *BRCA2* mutation carriers, respectively, will experience EOC by age 70 (3), but hereditary EOC accounts for only 10% to 15% of cases. Epidemiologic risk factors (4, 5) and risk-associated circulating biomarkers (6) could be useful to expand the conventional hereditary high-risk population and identify a cohort of elevated risk women who may benefit from screening. CA125 is a predictive marker for EOC that becomes increasingly powerful with proximity to diagnosis (7, 8) and may signal the presence of precursor lesions such as adnexal dysplasia (9).

Interpreting CA125 trends using a longitudinal algorithm to select women for imaging potentially improves screening performance (8, 10). This strategy is being tested in the three-arm UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS; ref. 11). Results of the prevalence screen suggest that a multimodal approach using rising CA125 annually to select women for imaging is more sensitive as well as more specific than using TVU annually in all women, yielding positive predictive value (PPV) of 35% (11). Data from a single-arm U.S. trial further support the potential efficacy of CA125 change over time as a means to triage women to TVU and surgery,

Authors' Affiliations: [1]Cedars-Sinai Medical Center, Los Angeles; [2]City of Hope Comprehensive Cancer Center, Duarte; [3]Stanford University, Palo Alto, California; [4]Fred Hutchinson Cancer Research Center; [5]Pacific Gynecology Specialists, Seattle, Washington; [6]Fox Chase Cancer Center, Philadelphia, Pennsylvania; and [7]Dana Farber Cancer Center, Boston, Massachusetts

Corresponding Author: Beth Y. Karlan, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048. Phone: 310-423-3302; Fax: 310-423-9753; E-mail: Beth.Karlan@cshs.org

doi: 10.1158/1055-9965.EPI-13-1361

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

demonstrating a PPV for early invasive EOC of 40% [95% confidence interval (CI), 12.2%–73.8%] and a specificity of 99.9% (95% CI, 99.7%–100%; ref. 12).

The human epididymis 4 gene *WFDC2* (HE4; refs. 13–15) could potentially contribute to a screening strategy. Serum HE4 is more specific than CA125 in discriminating women with malignant tumors from those with benign tumors (15, 16) and was recently approved by the FDA for this use in combination with CA125 (17). CA125 in combination with HE4 has not previously been prospectively examined in the screening setting. We performed a phase I randomized controlled trial (RCT) using HE4 as either a primary (first-line) or a confirmatory (second-line) test to select women for TVU and clinical follow-up. If 2 of the 3 tests (CA125, HE4, or imaging) are positive at the confirmatory screen, or if the confirmatory CA125 is sufficiently high, consultation with a gynecologic oncologist is recommended.

The parametric empirical Bayes (PEB) longitudinal algorithm (18, 19) is used to tailor marker thresholds to the individual woman based on her age and marker history. It provides a positive result at lower marker levels by accounting for marker history within each woman (18) without any sacrifice in specificity (19). Cut-off levels assigned by the PEB will be lower for most women than a single threshold rule with comparable specificity (19), yielding longer lead times for screen-detected cancers, as demonstrated using serial CA125 data from the PLCO trial (10). The PEB approach can be easily generalized to a panel that includes novel markers such as HE4. Requiring two tests such as CA125 and imaging (20), or CA125 and HE4 (21) both to be positive before recommending clinical follow-up can improve specificity dramatically.

Materials and Methods

We conducted a RCT to estimate surgical PPV of the "2 of 3 tests positive" rule, and to compare use of HE4 as a first-line versus a second-line screen, in multimodal screening of women at high and elevated risk for EOC.

Study populations

Participants were recruited at five sites: Cedars-Sinai Medical Center, City of Hope, Stanford University, Swedish Cancer Institute/Fred Hutchinson Cancer Research Center, and Fox Chase Cancer Center. Sites recruited eligible women through their high-risk EOC screening programs (Cedars-Sinai, Fox Chase, Swedish), specimen donation programs (Cedars-Sinai, Fred Hutchinson), or local oncology and gynecology practices (Cedars-Sinai, City of Hope, Stanford, Swedish). The Fred Hutchinson Cancer Research Center served as the national Coordinating Center.

Eligibility

Eligible participants were elevated-risk women aged 25 to 80 years, classified as belonging to the highest risk group for which they qualified. Risk Group 1 included women 25 to 80 years old carrying a deleterious *BRCA1* or *BRCA2* germline mutation. Risk Group 2 included women 35 to 80 years old from a high-risk family, including women meeting NCCN V4.2013 Breast and Ovarian Cancer Genetic Assessment Guidelines for referral to a genetics professional, and women with a deleterious mutation in HNPCC or *TP53* genes or a first- or second-degree relative positive for HNPCC. Risk Group 3 included women between 45 and 80 years old with epidemiologic risk factors (4, 5, 22, 23) or circulating proteins (6, 7) conferring EOC risk. The presence of either at least 3 of 6 risk factors [<1 year of oral contraceptive use, nulliparity, no breastfeeding, no tubal ligation, Ashkenazi Jewish, >1 year of menopausal hormone therapy (HT)], or a CA125, HE4, MMP7, or Mesothelin value exceeding the 95% population threshold, qualified a woman for Risk Group 3. Women were excluded if they had a personal history of EOC, no ovaries, abdominal surgery within the last 3 months, a current pregnancy, a medical condition precluding phlebotomy, untreated malignancy (other than non-melanoma skin cancer), or receipt of adjuvant chemotherapy or radiotherapy for cancer (tamoxifen, aromatase inhibitors, and/or GnRH agonist were allowed) within 3 months. Eligible women were enrolled if they provided informed consent, signed a medical records release form, and identified a care provider who agreed to receive screening results.

Randomization

At enrollment, participants were randomly allocated to screening that did or did not include HE4 in the primary screen by a computer algorithm generated by a statistician uninvolved in ascertainment of outcomes. Participants were randomized in blocks of 6 (3 to each arm) within study site and risk group.

Intervention

Two staged multimodal screening protocols were evaluated, as shown in Fig. 1, differing only in the design of the primary screen which included both CA125 and HE4 in Arm 1 and only CA125 in Arm 2. In both arms, if the primary screen was positive, a confirmatory blood test that included both CA125 and HE4 was used to select women for follow-up imaging and potentially for clinical follow-up. Screening was risk-based so that women in Risk Groups 1 and 2 were screened semiannually with early recall at 3 months. Women in Risk Group 3 were screened annually with early recall at 6 months. The staged approach selects women for increasingly costly tests and procedures so that PPV improves with each stage of the protocol. CA125 and HE4 were interpreted using the previously developed PEB longitudinal algorithm (above a threshold corresponding to 90%, 95%, or 99% specificity) to take advantage of rising trends in an individual woman's marker level as a signal of disease. The PEB determines the expected value of a marker for each individual woman based on her reference population and marker history. Age below or above 50 was used to define reference populations for the PEB, rather than

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

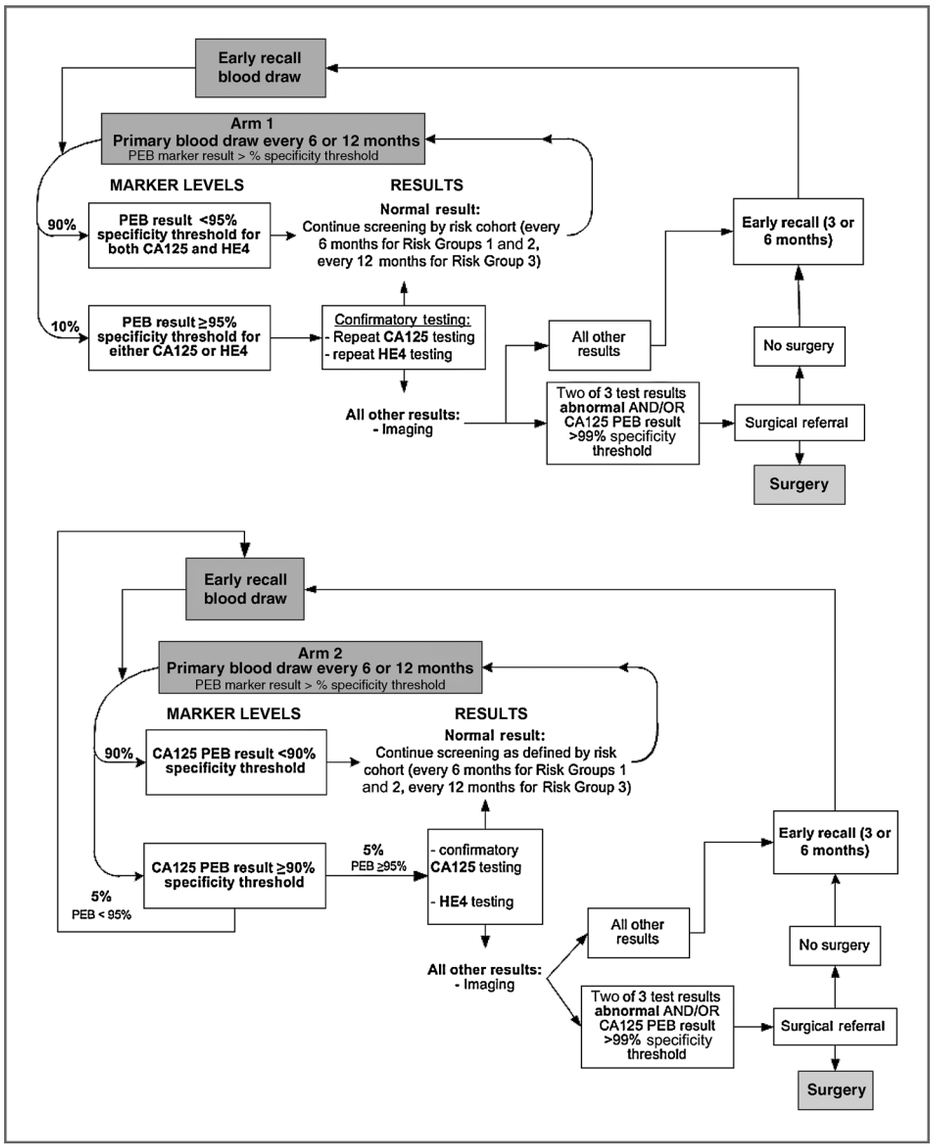

Figure 1. Overview of multimodal screening protocols by the study arm.

pre- and postmenopause (24), which can be difficult to define. The PEB rule is designed to account for personal characteristics of women that affect levels of the markers, such as ethnicity for CA125 (25) and age and smoking for HE4 (24) which were tracked in this study, by looking at the deviation from expected level based on marker level history. Positivity thresholds for CA125 and HE4 were set at 95% specificity regardless of arm, so that 5% of participants would be positive on each marker at each screen. Because both markers were used only in Arm 1, 10% of women in Arm 1 and 5% of women in Arm 2 were expected to return for a confirmatory test. In Arm 2 only, women with CA125 above a 90% specificity threshold had early recall.

If either CA125 or HE4 was positive at the confirmatory screen, the woman was asked to return for imaging. Surgical consult was recommended if 2 of the 3 tests were positive or, to avoid missing any cancers, if confirmatory CA125 exceeded the PEB 99% specificity positivity threshold. The design assured that surgical consultation was recommended for relatively few women. Clinical follow-up was coordinated by the study oncologist(s).

Laboratory analyses

Both CA125 and HE4 were measured at FHCRC on the Abbott Architect automated platform in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory using FDA-approved kits. Coefficients of variation (CV) for CA125 and HE4 are 4% and 6% respectively.

Imaging analyses

At each site, protocol-indicated TVU tests were performed by certified ultrasonographers, interpreted by a radiologist, and reported on standardized forms designed

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

to capture data required by a morphology-based index (26). Data were abstracted, entered into the research database by local study personnel, and reviewed centrally by the Coordinating Center research nurse. Women were considered postmenopausal if they had not had a menstrual period for at least one year. Screens in which the ovaries could not be visualized were considered negative. A scan was considered positive per study criteria if papillary nodules were present or if the volume of either ovary exceeded 20 cc in premenopausal and 10 cc in postmenopausal participants. Doppler studies were not performed regularly because their value has not been demonstrated consistently, the exam is operator dependent and poorly reproducible (27). However, the form, generally including morphology index information and sometimes including blood flow to the ovaries, was provided to referring physicians along with any accompanying report.

Outcome ascertainment

The primary outcome was surgical PPV defined as malignant lesions identified among protocol-indicated surgical procedures performed; intermediate outcomes included surgical consultations recommended and performed. Pathology reports were obtained for all women reporting surgical removal of ovaries and were reviewed centrally. Lesions of interest included serous tubal intraepithelial carcinoma (STIC) lesions as well as invasive epithelial malignancy of the ovary, fallopian tube, or peritoneum. Benign tumors, tumors of low malignant potential (LMP), and other types of malignancy were documented but were not the goal of screening.

Compliance with screening

Attendance at screens by participants was tracked and a missed screen was defined as a failure to attend a screen before the next one was due. When 12 months elapsed without a screening examination, participants were considered to have dropped out.

Statistical analyses

Results were analyzed as of October 31, 2013; the trial is ongoing. Surgical PPV was calculated as the number of malignancies identified divided by the number of protocol-indicated surgeries performed; an intent-to-treat analysis was also performed on the basis of recommendation for surgical consult in women with 2 of 3 tests positive. Participant characteristics were compared between study arms using Student *t* test for continuous variables, the χ^2 test for categorical variables, and Poisson regression for counts of relatives with breast and ovarian cancer. Fisher exact test was used to test for differences in the odds of having a positive primary or confirmatory screen between study arms, and for differences in the PPV based on the assigned treatment (intent-to-treat) between study arms. Statistical analyses were performed using the R statistical software (Version 2.14.0; The R Foundation for Statistical Computing). All statistical tests were two-tailed.

Results

Between February 1, 2010 and October 31, 2013, a total of 1,179 women were enrolled, of whom 1,172 had at least one screen and were included in analyses, 582 in Arm 1 and 590 in Arm 2, as shown in Fig. 2. Participants included 208, 834, and 130 women enrolled in Risk Groups 1, 2, and 3 respectively.

The rate of missed primary screens in Arms 1 and 2 was 1.1% and 1.0%, respectively; it was 0.8%, 1.4%, and 0% in Risk Group 1, 2, and 3 participants, respectively. Only 2.7% of study participants dropped out by being over 12 months late for a screening examination.

Clinical characteristics of women are reported in Table 1. There were no statistically significant differences between the study arms. Participants were predominantly between the ages of 45 and 65 (61%) and Caucasian (89%); about 19% were of Ashkenazi-Jewish descent. About 30% had a personal history of breast cancer and 33% had a family history of EOC. About 41% had undergone genetic testing, of whom 44% had a deleterious mutation. Approximately 85% had ever-used oral contraceptive pills and 26% had ever-used hormone replacement therapy (HRT). About 12% had prior tubal ligation, about 38% had prior hysterectomy, and 39% were nulliparous. While not statistically significant, three times as many women qualified for Risk Group 3 on the basis of elevated HE4 in Arm 1 than in Arm 2.

Outcomes for the "2 of 3 tests positive" strategy (both arms combined) are summarized in Table 2. A total of 37 women received a recommendation for surgical consult; 28 had the consult, 6 had bilateral salpingo-oophorectomy (BSO), and 2 were diagnosed with EOC, yielding overall surgical PPV of 33% (95% CI, 4%–78%). Twelve women received surgical referral based on the criterion "at least 2 of 3 tests positive"; of these, all had the consult, 4 had BSO, and 2 had EOC yielding surgical PPV of 50% and intent-to-treat PPV of 17%. Both CA125 and HE4 were positive on the 4 BSO cases and the 2 EOC were diagnosed in women who were positive on all three tests. One of the 2 participants who had BSO but no EOC was diagnosed with lung cancer based on her preoperative chest X-ray. Of the 25 women who were positive on CA125 alone, 16 had the consult, 2 had BSO, and none had EOC; two of these 25 were later found to have another malignancy (recurrent breast cancer and metastatic pancreatic cancer). Of the remaining 9, 4, and 5 women were in Risk Groups 1 and 2, respectively. Of these, three in Risk Group 1 were found to be pregnant and screening was suspended. Remaining women chose to attend an early recall examination; in all but one participant, CA125 returned to normal 3 months later.

PPV by study arm is reported in Table 3 by level of screen; reason for surgical recommendation is reported in terms of combinations of tests that were positive. On the basis of surgeries performed for all 37 women for whom surgical consult was recommended, PPV was 33% for both arms combined, 25% in Arm 1, and 50% in Arm 2. Because

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

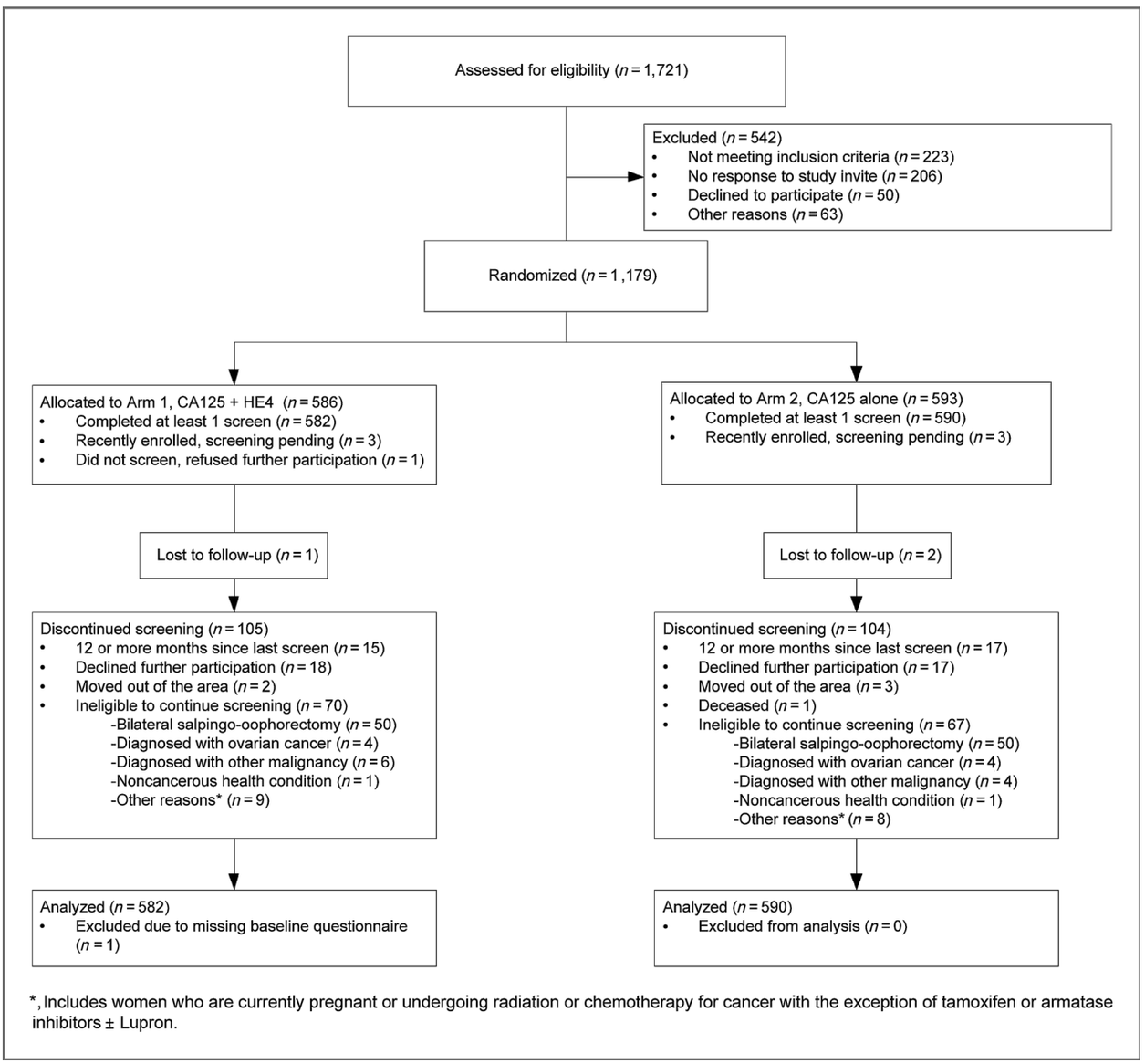

Figure 2. Participation in the randomized controlled trial by study phase and study arm.

surgery was seldom performed, we included an intent-to-treat analysis estimating PPV based on the 12 women with "at least 2 of 3 tests positive", yielding PPV estimates of 14% in Arm 1 and 20% in Arm 2, respectively. Among the same 12 women, based on surgeries actually performed, PPV was 33% in Arm 1 and 100% in Arm 2.

As reported in Table 3, three cancers were diagnosed in women with positive primary screens: twice as many cancers were identified in thrice as many positive screens in Arm 1 (2 cancers out of 233 positive screens) relative to Arm 2 (1 cancer out of 69 positive screens); primary screen PPV was 0.9% and 1.5% in Arms 1 and 2, respectively. The difference in PPV between arms was not statistically significant but there were significantly more positive primary screens in Arm 1 than in Arm 2: in each arm, 2,269 primary screens were performed, of which 233 and 69 were positive in Arms 1 and 2, respectively [OR = 3.5; refs. 2.6, 4.7; $P < 0.0001$, Fisher exact test).

At the confirmatory screen, one cancer was identified in each arm in twice as many confirmatory screens in Arm 1 (1/79 positive confirmatory screens) than in Arm 2 (1/33 positive confirmatory screens); confirmatory screen PPV was 1.3% and 3.0% in Arms 1 and 2, respectively, not statistically significantly different. However, there were more positive confirmatory screens in Arm 1 than in Arm 2 leading to 29 and 12 recommendations for surgical consult (OR = 2.4; refs. 1.2, 5.2; $P = 0.01$, Fisher exact test) in Arms 1 and 2, respectively.

Positive confirmatory marker screens resulted in imaging of 79 women in Arm 1 and 31 women in Arm 2, leading

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

Table 1. Summary of participant characteristics at baseline by study arm

Variable	Value	Arm 1: CA125 + HE4	Arm 2: CA125 alone	*P*
Study arm	Sample size	582 (49.7%)	590 (50.3%)	
Age at enrollment	Mean (SD)	52 (12)	52 (11)	0.80
Age at enrollment (categorical)	35 ≤ age ≤ 44	146 (25.1%)	151 (25.6%)	0.97
	45 ≤ age ≤ 54	185 (31.8%)	189 (32.0%)	
	55 ≤ age ≤ 64	171 (29.4%)	175 (29.7%)	
	65≤ age ≤ 85	80 (13.7%)	75 (12.7%)	
Self-reported race	White Caucasian	520 (89.3%)	527 (89.3%)	1.00
	Non-White	51 (8.8%)	62 (10.5%)	0.32
Ethnicity	Ashkenazi Jewish	119 (20.4%)	105 (17.8%)	0.27
	Hispanic	29 (5.1%)	25 (4.3%)	0.58
Risk group	RG1: BRCA mutation carrier	106 (18.2%)	102 (17.3%)	0.98
	RG2: Significant pedigree	411 (70.6%)	423 (71.7%)	
	RG3: Elevated markers	24 (4.1%)	24 (4.1%)	
	RG3: Epidemiologic risk algorithm	41 (7.0%)	41 (6.9%)	
Personal history of breast cancer	Yes	167 (28.9%)	180 (30.7%)	0.52
Number of first- or second-degree relatives with ovarian cancer	1	183 (31.4%)	209 (35.4%)	0.41
	2	39 (6.7%)	40 (6.8%)	
	3+	7 (1.2%)	10 (1.7%)	
Prior genetic test	Tested	229 (39.3%)	250 (42.6%)	0.28
Genetic test results among participants with a prior genetic test	Negative	103 (45.4%)	121 (51.1%)	0.56
	Variant(s) of unknown significance	10 (4.4%)	8 (3.4%)	
	Confirmed predisposition	103 (45.4%)	101 (42.6%)	
	Inconclusive	11 (4.8%)	7 (3.0%)	
Use of hormonal contraceptives	Ever used	487 (84.8%)	493 (84.6%)	0.94
	>1 year use	466 (80.1%)	472 (80.0%)	1.00
Use of HRT (E alone, or E+P)	Ever used	153 (26.3%)	152 (25.8%)	0.84
	One or more years	111 (19.1%)	105 (17.8%)	0.60
Prior tubal ligation	Yes	75 (13.0%)	64 (10.9%)	0.28
Nulliparity	Yes	220 (37.8%)	232 (39.3%)	0.63
Prior hysterectomy	Yes	51 (8.9%)	51 (8.8%)	1.00
Number of first- or second-degree relatives with breast cancer	1	220 (37.8%)	215 (36.4%)	0.77
	2	105 (18.0%)	120 (20.3%)	
	3+	85 (14.6%)	81 (13.7%)	
First- or second-degree relatives with BRCA1, BRCA2, or HNPCC	Yes	93 (16.0%)	96 (16.3%)	0.95
	No	269 (46.2%)	267 (45.3%)	
	Unknown	220 (37.8%)	227 (38.5%)	
Elevated markers among elevated marker risk group	HE4	3 (12.5%)	9 (37.5%)	0.09
	CA125	11 (45.8%)	7 (29.2%)	0.37
	MMP7	7 (29.2%)	8 (33.3%)	1.00
	Mesothelin	5 (20.8%)	5 (20.8%)	1.00
Smoking (past or present)	Ever smoker	190 (32.6%)	179 (30.3%)	0.41
Smoking among ever smokers	Current smoker	19 (10.0%)	19 (10.6%)	0.87
	Former smoker	171 (90.0%)	160 (89.4%)	

to 4 and 3 positive imaging exams in Arms 1 and 2, respectively. Surgical consultation was recommended (following 29 and 12 screens in Arms 1 and 2, respectively) if 2 of the 3 confirmatory tests were positive, or if CA125 exceeded the 99% PEB threshold for positivity. However, decisions regarding surgery depended on joint decision making by the woman, her primary care physician, and a surgeon if one was consulted. As shown in Table 2, 28 participants consulted with a surgeon but most did not undergo surgery, instead returning to screening with early recall. Only 6 women had BSO following a recommendation for surgical consult, 4 in Arm 1 and 2 in Arm 2, yielding a diagnosis of EOC in one woman in each arm. No EOC has been identified in the remaining women for whom surgical consult was recommended; all but 7 continued screening.

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

Table 2. Participants with a protocol-derived recommendation for surgical consultation by confirmatory test result and follow-up including surgical consult, surgical procedure, and diagnosis of EOC or other malignancy

Participant's test results	Recommendation for surgical consultation	Consultation completed	BSO performed	EOC lesion identified	Other cancer diagnosed
All 3 tests positive	2	2	2	2	0
CA125 and HE4 positive, TVS negative	5	5	2	0	1
CA125 and TVS positive, HE4 negative	4	4	0	0	0
HE4 and TVS positive, CA125 negative	1	1	0	0	0
CA125 positive, HE4 and TVS negative	25	16	2	0	2
Total	37[a]	28	6	2	3[b]

[a]Thirty-seven women accounted for a total of 41 recommendations.

[b]Recurrent breast cancer, metastatic pancreatic cancer (both were CA125 positive, HE4 and TVS negative), and suspected lung cancer (CA125 and HE4 positive, TVS negative). Of these 3 women, only the participant with suspected lung cancer had BSO during the trial.

Described in Table 4 are all eight EOC lesions identified in 108 BSOs performed in this high-risk study cohort, including the three signaled by screening. Six BSOs were protocol-indicated, as described above. Of the remaining 102, 62 were RRSOs performed in mutation carriers, 19 were in women with significant pedigrees whose only surgical indication was prevention, and 21 occurred in women with neither a documented mutation nor a significant pedigree for reasons other than or in addition to prophylaxis.

Two EOC lesions (#1 and #2) were classified as screen-detected (true-positive screens) because they were diagnosed following recommendation for surgical consultation: one stage IIC serous EOC diagnosed following the

Table 3. PPV by arm, level of screen, and reason for recommendation for gynecologic oncology consult

Study arm	CA125 + HE4 at primary screen	CA125 alone at primary screen	Both arms combined
Cancers diagnosed	4	4	8
Primary screens	2,269	2,269	4,538
Positive [% (95% CI)]	233 [10.3% (9.0%–11.6%)]	69 [3.0% (2.4%–3.8%)]	302 [6.7% (5.9%–7.4%)]
Cancers diagnosed following positive screen [PPV (95% CI)]	2 [0.9% (0.1%–3.1%)]	1 [1.4% (0.0%–7.8%)]	3 [1.0% (0.2%–2.9%)]
Confirmatory screens	217	64	281
Positive [% (95% CI)]	79 [36.4% (30.0%–43.2%)]	33 [51.6% (38.7%–64.2%)]	112 [39.9% (34.1%–45.8%)]
Cancers diagnosed following positive screen [PPV (95% CI)]	1 [1.3% (0.0%–6.9%)]	1 [3.0% (0.1%–15.8%)]	2 [1.8% (0.2%–6.3%)]
Ultrasound assessments	79	31	110
Positive [% (95% CI)]	4 [5.1% (1.4%–12.5%)]	3 [9.7% (2.0%–25.8%)]	7 [6.4% (2.6%–12.7%)]
Cancers diagnosed following positive screen [PPV (95% CI)]	1 [25.0% (0.6%–80.6%)]	1 [33.3% (0.8%–90.6%)]	2 [28.6% (3.7%–71.0%)]
Referrals for consultation with gynecologic oncologist	29	12	41
Cancers diagnosed [PPV (95% CI)]	1 [3.4% (0.1%–17.8%)]	1 [8.3% (0.2%–38.5%)]	2 [4.9% (0.6%–16.5%)]
Surgical procedures following a referral for consult	4	2	6
Cancers diagnosed [PPV (95% CI)]	1 [25.0% (0.6%–80.6%)]	1 [50.0% (1.3%–98.7%)]	2 [33.3% (4.3%–77.7%)]
Referrals for consultation with gynecologic oncologist (with 2 of 3 modalities positive)	7	5	12
Cancers diagnosed [PPV (95% CI)]	1 [14.3% (0.4%–57.9%)]	1 [20.0% (0.5%–71.6%)]	2 [16.7% (2.1%–48.4%)]
Surgical procedures following a referral for consult (with 2 of 3 modalities positive)	3	1	4
Cancers diagnosed [PPV (95% CI)]	1 [33.3% (0.8%–90.6%)]	1 [100.0% (2.5%–100.0%)]	2 [50.0% (6.8%–93.2%)]

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

Table 4. BSO and surgical pathologic findings

	Arm 1			Arm 2			Total		
	Cancer	No cancer	Total	Cancer	No cancer	Total	Cancer	No cancer	Total
BSOs performed:	4	50	54	4	50	54	8	100	108
Surgical indication:									
Protocol-indicated	1	3[a]	4	1	1[b]	2	2	4	6
RRSO (BRCA mutation)	1	29	30	3	29	32	4	58	62
BSO (significant pedigree)	0	9	9	0	10	10	0	19	19
BSO (other)	2[c]	9[d]	11	0	10[e]	10	2	19	21

Lesion detail:										
Patient	**Age**	**Mutation status**	**Cancer**	**Stage**	**Histology**	**Arm**	**CA125**	**HE4**	**PEB results**	**Surgical indication**
1	66	No testing reported	EOC	3c[f]	Serous	2	256.1	59.9	CA125 > 99%, HE4 > 95%	Protocol
2	61	No testing reported	EOC	2c	Serous	1	104.9	63.1	CA125 > 99%, HE4 > 95%	Protocol
3	55	BRCA2 mutation	FTC	1a	Serous	1	37.8	25.2	CA125>99%	Prophylactic
4	57	BRCA2 mutation	FTC	1a	Serous	2	7.3	34.0	Normal	Prophylactic
5	47	BRCA1 mutation	FTC	1a	Serous	2	3.9	30.4	Normal	Prophylactic
6	69	Not tested	FTC	0	Serous	1	10.7	35.3	Normal	Prophylactic
7	36	BRCA1 mutation	FTC	0	Serous	2	10.7	27.9	Normal	Prophylactic
Other lesions found:										
8	47	Negative	LMP	1c	Mucinous	1	8	33.3	Normal	Symptomatic

[a]One participant had BSO at the same time as an indicated laparotomy to correct a complication caused by prior gastric bypass surgery.
[b]Suspected lung cancer based on imaging at time of surgery.
[c]Participants presented with symptoms at time of surgery.
[d]Two participants were scheduled for BSO as part of treatment for breast cancer.
[e]Two participants diagnosed with endometrial cancer.
[f]Participant had a STIC lesion present in her right fallopian tube.

third screen in Arm 1, and one stage IIIC serous EOC diagnosed at the prevalence screen in Arm 2. Both had low-volume disease and were optimally cytoreduced with minimally invasive surgeries, but the one diagnosed at the prevalence screen recurred two years later. In both cases, CA125, HE4, and imaging were all positive at the confirmatory screen.

A mutation carrier diagnosed with stage 1A invasive fallopian tube cancer (#3) at RRSO tested positive for CA125 at a primary screen but a confirmatory screen was not performed. This lesion was counted as a screen-detected cancer (true positive) for the primary screen only, and ignored for estimation of the primary outcome surgical PPV and also the intent-to-treat PPV, as reflected in Table 3.

Two lesions (#4 and #5) were classified as missed invasive cancers (false negative screens), both in mutation carriers diagnosed with stage 1A invasive fallopian tube cancer found at previously planned RRSO following a normal primary screen.

Of the three remaining EOC lesions, two (#6 and #7) were noninvasive STIC lesions, one in a mutation carrier

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

and one in an untested women with a significant pedigree. Both were found at a previously planned RRSO following a normal primary screen.

Only one woman (#8) was symptomatic; she had a LMP mucinous tumor that was not considered significant due to the generally indolent clinical course of these lesions.

Discussion

We performed a pilot RCT to learn how best to use HE4 in a staged multimodal screening protocol, to inform the design of future screening protocols. To assess safety, we chose trial size to yield primary outcome estimation (for both arms combined) of a 95% CI for surgical PPV; we had 80% power to rule out inclusion of PPV of 10% or lower. We observed fewer surgeries and malignancies than expected, and observed an overall surgical PPV of 33%, with 95% CI of 4%–78%. The trial was not large enough to test the difference in PPV between arms, and no statistically significant difference was seen. Both screening protocols were found to be feasible and acceptable to women and physicians; neither resulted in excessive imaging, surgical consults, or surgical procedures.

Positive screens were more frequent when HE4 was included in the primary screen. Consistent with retrospective analysis of Prostate, Lung, Colorectal, and Ovary Cancer Screening Trial (PLCO) data (21), these data suggest that HE4 may be useful as a confirmatory test in a multimodal strategy using CA125 alone in the primary screen. Women were more likely to complete a recommended surgical consultation and a surgical procedure when rising CA125 was confirmed by rising HE4. Of 7 women who had positive confirmatory test results on both CA125 and HE4, 4 had surgery and 2 had ovarian malignancy, but women seldom had surgery if CA125 alone was positive.

Two EOC cases were preceded by positivity of all three tests. Both had low volume stage 3 disease that was optimally treated; one identified at the prevalence screen recurred with a progression free interval of 24 months. No advanced or symptomatic EOC cases were missed by screening, but no early-stage cancers were identified by screening. As shown in Table 4, five stage 0 and 1a tubal lesions were identified at RRSO; only one was preceded by elevation in CA125. These results are consistent with the literature suggesting that performance of the primary screen is critical and that better serum markers may be needed to reduce EOC mortality cost-effectively. Using a previously developed microsimulation model (28), we have recently reported that use of rising CA125 to select average-risk postmenopausal women for imaging as in the UKCTOCS is likely to reduce mortality by about 13% for a cost per year of life saved of about $89,000 (29). Selection of women at elevated risk for screening combined with use of HE4 in a confirmatory screen would improve cost-effectiveness and might improve efficacy somewhat, but better markers will likely be needed to achieve mortality reduction of 30%.

Emerging evidence suggests that salpingectomy offers a complementary means to reduce EOC mortality (30). In women with a mutation in *BRCA1* or *BRCA2*, occult malignancy of serous histology is frequently found in the fimbrial end of the fallopian tube at the time of RRSO (31–34). About half of such lesions are invasive, and long-term follow-up confirms their aggressiveness. Of 15 women with invasive lesions, 7 recurred and 3 died in 88 months of follow-up; of 17 women with noninvasive lesions, 1 recurred and none died in 80 months of follow-up (35). Serous EOC may originate in native serous epithelium of the fallopian tube in most mutation carriers and in many sporadic cases, suggesting that bilateral salpingectomy with ovarian retention (BSOR) may reduce cancer risk. We have previously noted different CA125 levels between women with tubal dysplasia and normal fallopian tubes at RRSO (36). Only one woman in this study elected BSOR; no significant lesions were identified. Her CA125 levels were 35, 88, 58, and 57 U/mL before BSOR, and 7, 6, 6, 9, and 8 U/mL following BSOR, suggesting the need for more research. Markers may contribute to a reliable risk classification tool to inform a woman's decision-making regarding elective BSO or BSOR at the time of hysterectomy (37, 38). The addition of BSOR to hysterectomy in women who do not carry *BRCA1/2* mutations was recently reported to show no negative effects on ovarian function or perioperative complications (39); efficacy of this approach remains to be demonstrated (40). Results from a recent online survey conducted by a national patient advocacy group showed that one-third of BRCA mutation carriers indicated definite interest in prophylactic salpingectomy with delayed oophorectomy as a risk-reducing surgery (41).

Limitations

This report represents a snapshot in time of an evolving EOC screening trial. Age-specific elevation thresholds for CA125 and HE4 were not used in the original design, but it was learned from trial data that due to a strong effect of age on HE4 in healthy participants, thresholds for HE4 should be defined for women of specific ages. We estimated age-based positivity thresholds from healthy participants, reporting positivity thresholds yielding 90%, 95%, 98%, and 99% specificity for age-defined populations of healthy women for HE4 and CA125 (24). We considered STIC lesions to be outcomes of interest, but did not include other putative precursor lesions such as small benign paratubal cysts (42) reported on the right ovary of a woman with rising HE4 who had BSO off-protocol. Costly abdominal and pelvic imaging such as CT or MRI was not performed in women with positive tests; performance of those technologies in the context of screening this high-risk population might be of interest. Marker levels were not measured post-BSO; CA125 and HE4 both fall with EOC surgery and rise with recurrence (43).

CA125 level may not elevate in patients with stage 1 ovarian cancer (44), suggesting the need for HE4 (or another promising biomarker) in the primary screen to

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

catch early disease. However, results suggest that a higher specificity should be required if HE4 is used in the primary screen, or that HE4 should be reserved for use in the second-line screen, to reduce call-backs for confirmatory screens. Both protocols were safe with PPV comparing favorably to results reported by efficacy trials to date. Data from this pilot trial may be useful for designing future risk assessment and prevention studies and for estimating the costs of conducting a full-scale efficacy screening trial should such a study be undertaken in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: B.Y. Karlan, J.D. Thorpe, C.W. Drescher, G.L. Anderson, N. Urban

Development of methodology: B.Y. Karlan, J.D. Thorpe, M.R. Palomares, N. Urban

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): B.Y. Karlan, J.D. Thorpe, K. Watabayashi, C.W. Drescher, M.R. Palomares, M. Daly, P.J. Paley, P.J. Hillard, R. Drapkin, N. Urban

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): B.Y. Karlan, J.D. Thorpe, C.W. Drescher, M.R. Palomares, P.J. Paley, G.L. Anderson, R. Drapkin, N. Urban

Writing, review, and/or revision of the manuscript: B.Y. Karlan, J.D. Thorpe, K. Watabayashi, C.W. Drescher, M.R. Palomares, M. Daly, P.J. Paley, P.J. Hillard, M.R. Andersen, G.L. Anderson, R. Drapkin, N. Urban

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.Y. Karlan, J.D. Thorpe, K. Watabayashi, M. Daly

Study supervision: B.Y. Karlan, C.W. Drescher, M. Daly, P.J. Hillard, N. Urban

Acknowledgments

The authors acknowledge helpful comments provided during the review of this article from Beth Schodin of Abbott Diagnostics. A grant of no-charge study materials from Abbott Laboratories is gratefully acknowledged. The authors also thank Kathy O'Briant, Hannah Purkey, and Christine Poulin for administrative and technical assistance. Trial registration: Clinicaltrials.gov Identifier, NCT01121640.

Grant Support

This work was supported by grants NCI P50 CA083636 (to N. Urban), NCI U01 CA152637 (to N. Urban), and NIH/NCATS Grant# UL1TR000124 and SIOP-06-258-01-COUN (to B.Y. Karlan).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received December 20, 2013; revised April 7, 2014; accepted April 22, 2014; published OnlineFirst May 1, 2014.

References

1. Genetic/familial high risk assessment: breast and ovarian 2006; 2006. v.1.2006. Available from: http://www.nccn.org/professionals/physician_gls/PDF/genetics_screening.pdf.
2. Rosenthal AN, Fraser L, Manchanda R, Badman P, Philpott S, Mozersky J, et al. Results of annual screening in phase I of the United kingdom familial ovarian cancer screening study highlight the need for strict adherence to screening schedule. J Clin Oncol 2013;31:49–57.
3. Chen S, Iversen ES, Friebel T, Finkelstein D, Weber BL, Eisen A, et al. Characterization of BRCA1 and BRCA2 mutations in a large United States sample. J Clin Oncol 2006;24:863–71.
4. Vitonis AF, Titus-Ernstoff L, Cramer DW. Assessing ovarian cancer risk when considering elective oophorectomy at the time of hysterectomy. Obstet Gynecol 2011;117:1042–50.
5. Morch LS, Lokkegaard E, Andreasen AH, Kjaer SK, Lidegaard O. Hormone therapy and different ovarian cancers: a national cohort study. Am J Epidemiol 2012;175:1234–42.
6. Urban N, Drescher C. Current and future developments in screening for ovarian cancer. Womens Health 2006;2:733–42.
7. Bjorge T, Lie A, Hovig E, Gislefoss R, Hansen S, Jellum E, et al. BRCA1 mutations in ovarian cancer and borderline tumours in Norway: a nested case-control study. Br J Cancer 2004;91:1829–34.
8. Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, Davies AP, et al. Screening for ovarian cancer: a pilot randomized controlled trial. Lancet 1999;353:1207–10.
9. Hermsen BBJ, von Mensdorff-Pouilly S, Berkhof J, van Diest PJ, Gille JJP, Menko FH, et al. Serum CA-125 in relation to adnexal dysplasia and cancer in women at hereditary high risk of ovarian cancer. J Clin Oncol 2007;25:1383–9.
10. 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 Oncol 2013;31:387–92.
11. 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 Oncol 2005;23:7919–26.
12. Lu KH, Skates S, Hernandez MA, Bedi D, Bevers T, Leeds L, et al. A 2-stage ovarian cancer screening strategy using the Risk of Ovarian Cancer Algorithm (ROCA) identifies early-stage incident cancers and demonstrates high positive predictive value. Cancer 2013;119:3454–61.
13. Schummer M, Ng W, Bumgarner R, Nelson P, Schummer B, Bednarski D, et al. Comparative hybridization of an array of 21,500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. Gene 1999;238:375–85.
14. Hough CD, Sherman-Baust CA, Pizer ES, Montz FJ, Im DD, Rosenshein NB, et al. Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer. Cancer Res 2000;60:6281–7.
15. Hellström I, Raycraft J, Hayden-Ledbetter M, Ledbetter J, Schummer M, McIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003;63:3695–700.
16. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol 2008;108:402–8.
17. Moore RG, Miller MC, Disilvestro P, Landrum LM, Gajewski W, Ball JJ, et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. Obstet Gynecol 2011;118:280–8.
18. McIntosh M, Urban N. A parametric empirical Bayes method for cancer screening using longitudinal observations of a biomarker. Biostatistics 2003;4:27–40.
19. McIntosh M, Urban N, Karlan B. Generating longitudinal screening algorithms using novel biomarkers for disease. Cancer Epidemiol Biomarkers Prev 2002;11:159–66.
20. Menon U, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol 2009;10:327–40.
21. Urban N, Thorpe JD, Bergan LA, Forrest RM, Kampani AV, Scholler N, et al. Potential role of HE4 in multimodal screening for epithelial ovarian cancer. J Natl Cancer Inst 2011;103:1630–4.
22. Koskela-Niska V, Lyytinen H, Riska A, Pukkala E, Ylikorkala O. Ovarian cancer risk in postmenopausal women using estradiol-progestin therapy - a nationwide study. Climacteric. 2013;16:48–53.

Published OnlineFirst May 1, 2014; DOI: 10.1158/1055-9965.EPI-13-1361

23. Koskela-Niska V, Pukkala E, Lyytinen H, Ylikorkala O, Dyba T. Effect of various forms of postmenopausal hormone therapy on the risk of ovarian cancer–a population-based case control study from Finland. Int J Cancer 2013;133:1680–8.
24. Urban N, Thorpe JD, Karlan BY, McIntosh MW, Palomares MR, Daly MB, et al. Interpretation of single and serial measures of HE4 and CA125 in asymptomatic women at high risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev 2012;21:2087–94.
25. Pauler D, Menon U, McIntosh M, Symecko H, Skates S, Jacobs I. Factors influencing aerum CA125II levels in healthy postmenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:489–93.
26. DePriest PD, Shenson D, Fried A, Hunter JE, Andrews SJ, Gallion HH, et al. A morphology index based on sonographic findings in ovarian cancer. Gynecol Oncol 1993;51:7–11.
27. Lutz AM, Willmann JK, Drescher CW, Ray P, Cochran FV, Urban N, et al. Early diagnosis of ovarian carcinoma: is a solution in sight? Radiology 2011;259:329–45.
28. Urban N, Drescher C, Etzioni R, Colby C. Use of a stochastic simulation model to identify an efficient protocol for ovarian cancer screening. Control Clin Trials 1997;18:251–70.
29. Drescher CW, Hawley S, Thorpe JD, Marticke S, McIntosh MW, Gambhir SS, et al. Impact of screening test performance and cost on mortality reduction and cost-effectiveness of multimodal ovarian cancer screening. Cancer Prev Res 2012;5:1015–24.
30. SGO Clinical Practice Statement: Salpingectomy for Ovarian Cancer Prevention; 2013 Nov [2013 Apr 1]. Available from: https://www.sgo.org/clinical-practice/guidelines/sgo-clinical-practice-statement-salpingectomy-for-ovarian-cancer-prevention/.
31. Crum CP, Drapkin R, Miron A, Ince TA, Muto M, Kindelberger DW, et al. The distal fallopian tube: a new model for pelvic serous carcinogenesis. Curr Opin Obstet Gynecol 2007;19:3–9.
32. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. Am J Surg Pathol 2007; 31:161–9.
33. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, et al. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. J Pathol 2007;211:26–35.
34. Medeiros F, Muto MG, Lee Y, Elvin JA, Callahan MJ, Feltmate C, et al. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. Am J Surg Pathol 2006;30: 230–6.
35. Powell CB, Swisher EM, Cass I, McLennan J, Norquist B, Garcia RL, et al. Long term follow up of BRCA1 and BRCA2 mutation carriers with unsuspected neoplasia identified at risk reducing salpingo-oophorectomy. Gynecol Oncol 2013;129:364–71.
36. Karlan BY, McIntosh M. The quest for ovarian cancer's Holy Grail: can CA-125 still be the chalice of early detection? J Clin Oncol 2007;25: 1303–4.
37. Tone AA, Salvador S, Finlayson SJ, Tinker AV, Kwon JS, Lee CH, et al. The role of the fallopian tube in ovarian cancer. Clin Adv Hematol Oncol 2012;10:296–306.
38. Anderson GL, McIntosh MW, Wu L, Barnett M, Goodman G, Thorpe JD, et al. Assessing lead time of selected ovarian cancer biomarkers: a nested case–control study. J Natl Cancer Inst 2010;102:26–38.
39. Morelli M, Venturella R, Mocciaro R, Di Cello A, Rania E, Lico D, et al. Prophylactic salpingectomy in premenopausal low-risk women for ovarian cancer: primum non nocere. Gynecol Oncol. 2013;129:448–51.
40. Gilks CB, Miller D. Opportunistic salpingectomy for women at low risk for development of ovarian carcinoma: the time has come. Gynecol Oncol 2013;129:443–4.
41. Holman LL, Friedman S, Daniels MS, Sun CC, Lu KH. Acceptability of prophylactic salpingectomy with delayed oophorectomy as risk-reducing surgery among BRCA mutation carriers. Gynecol Oncol 2014;133:283–6.
42. Dubeau L. The cell of origin of ovarian epithelial tumours. Lancet Oncol 2008;9:1191–7.
43. Schummer M, Drescher C, Forrest R, Gough S, Thorpe J, Hellstrom I, et al. Evaluation of ovarian cancer remission markers HE4, MMP7 and Mesothelin by comparison to the established marker CA125. Gynecol Oncol 2012;125:65–9.
44. Paramasivam S, Tripcony L, Crandon A, Quinn M, Hammond I, Marsden D, et al. Prognostic importance of preoperative CA-125 in International Federation of Gynecology and Obstetrics stage I epithelial ovarian cancer: an Australian multicenter study. J Clin Oncol 2005; 23:5938–42.