Research Article

Cancer Prevention Research

Large Prospective Study of Ovarian Cancer Screening in High-Risk Women: CA125 Cut-Point Defined by Menopausal Status

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Abstract

Previous screening trials for early detection of ovarian cancer in postmenopausal women have used the standard CA125 cut-point of 35 U/mL, the 98th percentile in this population yielding a 2% false positive rate, whereas the same cut-point in trials of premenopausal women results in substantially higher false positive rates. We investigated demographic and clinical factors predicting CA125 distributions, including 98th percentiles, in a large population of high-risk women participating in two ovarian cancer screening studies with common eligibility criteria and screening protocols. Baseline CA125 values and clinical and demographic data from 3,692 women participating in screening studies conducted by the National Cancer Institute–sponsored Cancer Genetics Network and Gynecologic Oncology Group were combined for this preplanned analysis. Because of the large effect of menopausal status on CA125 levels, statistical analyses were conducted separately in pre- and postmenopausal subjects to determine the impact of other baseline factors on predicted CA125 cut-points on the basis of 98th percentile. The primary clinical factor affecting CA125 cut-points was menopausal status, with premenopausal women having a significantly higher cut-point of 50 U/mL, while in postmenopausal subjects the standard cut-point of 35 U/mL was recapitulated. In premenopausal women, current oral contraceptive (OC) users had a cut-point of 40 U/mL. To achieve a 2% false positive rate in ovarian cancer screening trials and in high-risk women choosing to be screened, the cut-point for initial CA125 testing should be personalized primarily for menopausal status (50 for premenopausal women, 40 for premenopausal on OC, and 35 for postmenopausal women). *Cancer Prev Res; 4(9); 1401–8. ©2011 AACR.*

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doi: 10.1158/1940-6207.CAPR-10-0402

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Introduction

CA125 is currently used primarily to monitor ovarian cancer patients during chemotherapy and as a biomarker for early detection of recurrence. In these settings, the cut-point of 35 U/mL for a positive test has often been the standard, as recommended by Bast and colleagues (1). This cut-point was determined by studying 800 healthy blood center donors and finding that 98% of CA125 values were below 35 U/mL; in contrast, 80% of measurements from ovarian cancer cases were above 35 U/mL. Given this possible discrimination between patients with ovarian cancer and controls, CA125 has also been used as a first-line test in multiple ovarian cancer screening trials. For general population screening, the target population has most often been postmenopausal women, because this is the group in which most ovarian cancers occur. The initial use of CA125 as an ovarian cancer screening test was modeled on the clinical test for detection of cancer recurrence, namely a fixed cut-point usually at the reference level of 35 U/mL. Of 3 large ovarian cancer screening trials in postmenopausal women (2–4) and 1 in premenopausal women (5), 3 used cut-points of 35 U/mL and 1 used 30 U/mL (3), the latter choice because of concern about poor sensitivity of CA125 for detecting early-stage disease. However, although this cut-point may be appropriate in general, there are subgroups of patients in which the 98th percentile differs significantly from 35 U/mL, wherein a different cut-point may be appropriate. Limited information is available, though, about the characteristics of these subgroups or the appropriate cut-points for particular subgroups.

Furthermore, determination of these cut-points has become particularly relevant with the discovery of *BRCA1* and *BRCA2* because we can now identify a subgroup of women with a markedly increased annual incidence of ovarian cancer, approaching as high as 1% per year. Several screening trials are currently being conducted in women at high risk of, or known to carry, a deleterious mutation in *BRCA1* or *BRCA2* (5–8). In these trials, a significant proportion of women were premenopausal because of the younger-than-usual age-at-diagnosis for *BRCA*-related ovarian cancer. CA125 is known to have a high false positive rate amongst premenopausal women using the 35 U/mL cut-points. This is particularly relevant in a high-risk population, given the anxiety related to false positive results. As reported in one trial (8), there was significant anxiety reported because of false positive CA125 results.

Defining cut-points on the basis of factors predicting the CA125 distribution is a first step toward personalizing the screening test for each woman entering a screening trial. This report identifies factors that influence predicted CA125 blood levels through analyses of baseline data from 2 large prospective trials in high-risk women and provides CA125 cut-points for specific subgroups of women. These cut-points can be used for high-risk women choosing screening rather than risk-reducing surgery to manage their ovarian cancer risk and in future ovarian cancer screening trials in high-risk women.

Materials and Methods

The Cancer Genetics Network, a National Institute of Cancer (NCI)-funded network for research on inherited malignancies, initiated an ovarian cancer screening pilot strategy for women at increased genetic risk in 2001 (9). Eligible women were at high risk of ovarian and breast cancer because of either 2 or more breast and ovarian cancers, or a *BRCA1* or *BRCA2* mutation, in first- and/or second-degree blood relatives, with breast cancer in the subject counting toward the total. The screening strategy implemented ROCA (risk of ovarian cancer algorithm) on the basis of longitudinal CA125 values (10) from every 3 month testing with referral to transvaginal sonography (TVS) for an intermediate risk of having ovarian cancer, TVS, and review by a gynecologic oncologist for an elevated risk. Two other NCI programs, the ovarian Specialized Programs of Research Excellence (SPORE) and the Early Detection Research Network (EDRN), as well as 5 independent clinical sites subsequently joined the trial and contributed subjects. There were 2,352 high-risk women enrolled in the study at 25 U.S. sites. The aim of the study was to provide the first assessment of the operating characteristics of ROCA in high-risk women and to evaluate the acceptability of a screening protocol requiring every 3 month blood tests. At study entry, participants filled out a baseline questionnaire with demographic, menstrual and reproductive history, personal history of cancer and cancer treatment, use of tobacco, alcohol, caffeine, talc, and exposure to x-rays, medications (including hormones), abdominal symptoms, and family cancer history. Two years after the CGN study began, the Gynecologic Oncology Group (GOG) initiated a 2-arm, nonrandomized natural history study of high-risk women choosing between risk-reducing salpingo-oophorectomy (RRSO) and screening (6). The GOG study eligibility criteria and screening algorithm were the same as for the CGN study (with the exception that patients having had prior bilateral oophorectomy were ineligible for the GOG study), enabling data to be compared across the 2 studies. Incidentally, the 2 cohorts provided an estimate of the proportion of high-risk women (defined identically in the 2 studies) who had undergone clinical testing for deleterious germline mutations in *BRCA1*/*BRCA2*, that is, 27% (986/3,692), prior to study entry. Thus, the majority of women entered these screening trials without knowing their *BRCA* mutation status. With 1,442 women enrolled in the GOG screening arm, baseline data from 3,794 women were available for statistical modeling, providing a rich source for subgroup analysis of CA125 distributions and determinations of subgroup-specific cut-points (98th percentiles). Factors which a priori were expected to contribute to prediction of CA125 levels include menopausal status (11), number of ovaries, race (12), presence of uterus (13), and age.

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Of the 3,794 women recruited to the combined CGN/GOG study, subjects who were ineligible after enrollment and those missing either a baseline CA125 measurement or the baseline questionnaire were excluded. In addition, subjects who were diagnosed with ovarian cancer after enrollment were excluded. This process provided analyzable baseline data from 3,692 women; 2,251 women enrolled in the CGN study and 1,441 women enrolled in the GOG study. All peripheral blood samples for both studies were collected from venepunctures in 10 mL red top glass tubes (no additives or clotting enhancers), 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 central lab. All serum CA125 values were measured at the Reproductive Endocrine Unit Reference Laboratory at Massachusetts General Hospital on a Roche Elecsys immunoanalyzer, initially an Elecsys-2010 and then an E170 in 2006. 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 and not tested 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). Preliminary univariate analyses were conducted to determine the association between each baseline variable and log-transformed CA125 values. Standard statistical methods based on normal distributions (e.g., linear regression, ANOVA) are more appropriate for the log-CA125 scale because the distribution of log-CA125 values far more closely resembles a symmetric bell-shaped curve than the very skewed distribution of the nontransformed CA125 values. Statistical analyses of log-CA125 can be readily interpreted on the original CA125 scale by noting that to first order, percentage changes on the original CA125 scale (e.g., 10% change) are estimated by the factor effects (e.g., 0.1 change in log-CA125), median CA125s are estimated by exponentiated means from the log-CA125 scale, and the estimate of coefficient of variation (CV; e.g., 20% CV) is the SD on the log-CA125 scale (e.g., SD of 0.2).

It is well known that the CA125 distribution is strongly affected by menopausal status (11). Following confirmation of a very significant univariate difference in CA125 levels between pre- and postmenopausal women in this cohort, subjects were grouped according to menopause status, and all subsequent analyses were conducted separately within pre- and postmenopausal groups. A subject was designated as postmenopausal if: (i) more than a year elapsed since their last menstrual period (LMP) at time of baseline blood draw, or (ii) if the LMP date was not reported, the subject indicated their period had stopped for at least a duration of 3 regular cycles, or (iii) if there were no data to determine either of the above criteria, age exceeded 50. Missing values were replaced with "no" responses for categorical factors and with the median values for continuous variables. The potential bias introduced by this method for handling missing values was toward the null hypothesis of no effect, which resulted in a decreased likelihood that a given variable will be significantly associated with baseline CA125. This effect will be small because the percentage of missing values was very low, ranging from less than 1% for age to 7% for number of ovaries in postmenopausal women. Age defined menopausal status in only 2.6% of subjects, for all other subjects menopausal status was directly known. The preliminary univariate analyses compared log-CA125 across groups by using ANOVA for categorical variables and computed Pearson correlations for continuous variables. The SD of log-CA125 values was consistently close to 0.5 across different subgroups, equivalent to a CA125 CV of 50% between women within a subgroup, which supported the constant variance assumption of ANOVA. Variables that were significant ($P < 0.1$, by either ANOVA or Pearson ρ) in the preliminary univariate analyses were included in the initial multiple linear regression model. The factor with the least nonsignificant effect was removed, the model refitted, and the process iterated, until only variables with a significant impact on log-CA125 remained. Two-factor interactions for final main effects were fully examined but even though 2 were statistically significant in premenopausal women, they were not included in the final model because of the very small within subpopulation sample sizes (Black women who smoked, $n = 6$ and Asian women with irregular periods, $n = 9$). The final model estimated coefficients for the predictors of the CA125 distribution. To provide estimates on the CA125 scale, the median CA125 for each subgroup was estimated from exp(mean log-CA125) and the 98th percentile from exp(2.05 SDs + mean log-CA125) (mean specific to the subgroup, SD estimate pooled across subgroups). A quadratic term for age was included to account for potential nonlinearity in the effect of age. For comparison, a standard reference group of baseline factors was defined for pre- and postmenopausal subjects. Generally, the standard profile reflected a woman with "no" responses for each factor (i.e., "unexposed" to the factors that affect baseline CA125) and whose values for continuous variables such as age are set at fixed values. For premenopausal women, the reference group consisted of 50-year-old women from the CGN study cohort who were white, had regular menstrual periods, and were not current users of oral contraceptives (OC) or cigarettes. The reference group in postmenopausal subjects corresponded to 50-year-old women from the CGN study cohort who were white, had 2 intact ovaries, had never used fertility drugs, were not current smokers, and who experienced menopause at age 45. Median and cut-point CA125 estimates for a factor reflect the estimated change in CA125 for a subgroup defined by changing the factor compared with the standard profile. Changes were considered statistically significant if $P < 0.05$. However, although some changes could be statistically significant because of the relatively large sample size, these differences could be of sufficiently small magnitude that they would be unlikely to be of major clinical

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significance. Changes were considered of minor clinical importance if the change was less than 10%.

Results

The univariate 98th percentile CA125 cut-point among premenopausal women in this study was 49 U/mL. Among postmenopausal women, the CA125 cut-point was 34 U/mL, which recapitulated the standard cut-point for CA125 of 35 U/mL. Table 1 presents the factors, cut-points, median, sample sizes, and the P value for the difference within factor levels, stratified by menopausal status. Only factors with a univariate statistical significance ($P \leq 0.1$) for CA125 in either pre- or postmenopausal subjects or both are displayed in Table 1. The effect estimates in Table 1 reflect no adjustment for the impact of other factors.

Premenopausal women in the reference group had a 98th percentile cut-point of 52 U/mL for CA125 in the multivariate analysis, significantly higher than 36 U/mL in postmenopausal women in the reference group ($P <$ 0.0001). Because of the large impact of menopausal status, the effects of other factors were examined within each menopausal group. Table 2 summarizes the factors that were significant in the multivariate linear models in pre- and postmenopausal subjects, adjusting for the presence of multiple factors simultaneously. As in the univariate analyses, the value of 36 U/mL recapitulated the standard clinical cut-point of 35 U/mL for postmenopausal women, as expected. Several factors (e.g., hormone replacement therapy, *BRCA1* and *BRCA2* mutation status, family history of ovarian cancer, and hysterectomy) were significant in the univariate analysis but no longer significant in the multivariate analysis. This is likely because of confounding between these variables and other factors that had a stronger correlation with CA125 values. Analyses of risk categories (low, intermediate, and mutation carrier) given family history may refine this conclusion but require collection of detailed pedigrees not available in this study.

Results of secondary factors in premenopausal women

On the basis of the univariate results, current use of OCs was associated with a large and significant reduction in CA125, with the 98th percentile cut-point of 39 U/mL among current users. Other factors associated with a statistically significant (although smaller) reduction in CA125 among premenopausal women in the univariate analyses included former or current smoking (cut-points: 43 U/mL and 48 U/mL, respectively), irregular periods (cut-point: 44 U/mL), Ashkenazi Jewish heritage (cut-point: 47 U/mL), and presence of a (self-reported) deleterious *BRCA1* or *BRCA2* mutation (cut-point: 46 U/mL). Premenopausal women with a family history of ovarian cancer had a clinically minor but statistically significant reduction in CA125 (cut-point: 48 U/mL) compared with women who reported no ovarian cancer family history (cut-point: 50 U/mL). CA125 among premenopausal women of Asian descent was significantly lower (cut-point: 38 U/mL) compared with women of other ancestry. Finally, the univariate analyses suggested that women in the GOG cohort had a lower CA125 cut-point (47 U/mL) compared with women in the CGN cohort (51 U/mL).

The effects of each factor listed in the middle column within menopausal groups (pre-, post-) of Table 2 comprise the important estimates from the multivariate analysis because these estimates enable the joint effect of any combination of factors to be estimated. Current use of OCs in premenopausal women had the greatest impact on predicted CA125 levels, with levels 26% lower than those who were not currently using OCs ($P < 0.0001$). Premenopausal women with Asian background had CA125 levels that were 24% lower than non-Asians ($P = 0.009$). CA125 levels were 15% lower in premenopausal current smokers than in nonsmokers ($P = 0.0003$). Having irregular periods corresponded to an 11% decline in CA125 values compared with those reporting regular cycles ($P = 0.01$). Premenopausal women in the GOG cohort had CA125 levels that were, on average, 7% lower compared with women in the CGN cohort ($P = 0.002$).

Considering a premenopausal OC user of non-Asian descent who is a current smoker with irregular cycles provides an example of the additive effect of multiple factors on predicted CA125 levels. The individual effects of these factors were reductions of 26%, 15%, and 11%, respectively (Table 2). For a premenopausal woman with all these factors, the reduction is the sum of these values, namely 52%, reducing the CA125 cut-point from 52 to 25 U/mL. This shows the large change in cut-point because of the cumulative effect of multiple secondary factors.

Results of secondary factors in postmenopausal women

Among postmenopausal women, the univariate results suggest that several factors are associated with a significant reduction in CA125, compared with the overall 98th percentile cut-point of 34 U/mL. These include prior use of fertility treatment (cut-point: 28 U/mL), black race (cut-point: 27 U/mL), and prior removal of both ovaries (cut-point: 30 U/mL). Hysterectomy was associated with a very subtle clinical effect, but statistically significant reduction of about 2 U/mL in the CA125 cut-point in postmenopausal women. As was found in premenopausal women, postmenopausal women in the GOG cohort have a significantly lower CA125 cut-point (31 U/mL) compared with postmenopausal members of the CGN cohort (34 U/mL).

On the basis of results from the multivariate model as summarized by the right hand columns of Table 2, the estimated CA125 cut-point among postmenopausal women in the reference group (i.e., women with "no" responses for all significant categorical factors, with current age and age at menopause equal to 50 and 45, respectively) was 36 U/mL. Compared with this profile, postmenopausal black women had a 22% reduction in predicted CA125 levels ($P = 0.0008$) and having had prior oophorectomy or prior use of fertility drugs reduced predicted CA125 levels

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Table 1. Study population: univariate analysis of CA125 median and cut-point (98th percentile) by menopausal status and other defining subgroup factors (pre-SD = 0.54, post-SD = 0.47)

	Premenopausal subjects			Postmenopausal subjects		
Factor	**98th percentile CA125 (median)**	***n* (%)**	***P***	**98th percentile CA125 (median)**	***n* (%)**	***P***
OCs			<0.0001			0.2
Never	**50** (16.8)	400 (20%)		**32** (12.3)	482 (29%)	
Former	**51** (17.1)	1,291 (63%)		**33** (12.7)	1,174 (71%)	
Current	**39** (13.1)	345 (17%)		**NA (NA)**	0 (0%)	
No. of intact ovaries			0.04			0.0005
0	NA (NA)	0 (0%)		**30** (11.4)	282 (17%)	
1	**57** (19.0)	50 (2%)		**35** (13.1)	80 (5%)	
2	**49** (16.2)	1,986 (98%)		**34** (12.8)	1,294 (78%)	
Irregular menstrual periods			0.003			0.5
Yes	**44** (14.6)	181 (9%)		**32** (12.3)	168 (10%)	
No	**49** (16.5)	1,855 (91%)		**33** (12.6)	1,488 (90%)	
Black			0.2			0.004
Yes	**45** (14.9)	68 (3%)		**27** (10.4)	52 (3%)	
No	**49** (16.4)	1,968 (97%)		**33** (12.6)	1,604 (97%)	
Asian			0.005			0.9
Yes	**38** (12.7)	35 (2%)		**33** (12.7)	10 (1%)	
No	**49** (16.4)	2,001 (98%)		**33** (12.5)	1,646 (99%)	
Ashkenazi Jewish			0.05			0.2
Yes	**47** (15.6)	400 (20%)		**32** (12.2)	333 (20%)	
No	**49** (16.5)	1,636 (80%)		**33** (12.6)	1,323 (80%)	
Hysterectomy			0.4			0.02
Yes	**45** (14.9)	25 (1%)		**32** (12.0)	531 (32%)	
No	**49** (16.3)	2,011 (99%)		**34** (12.8)	1,125 (68%)	
Fertility treatment			0.9			0.02
Yes	**49** (16.4)	86 (4%)		**28** (10.8)	51 (3%)	
No	**49** (16.3)	1,950 (96%)		**33** (12.6)	1,605 (97%)	
Smoking history			0.05			0.3
Never	**50** (16.7)	1,304 (64%)		**34** (12.7)	950 (57%)	
Former	**43** (14.4)	170 (8%)		**30** (11.5)	131 (8%)	
Current	**48** (16.0)	562 (28%)		**33** (12.4)	575 (35%)	
Family Hx of ovarian cancer			0.04			0.2
Yes	**48** (15.9)	1,047 (51%)		**33** (12.3)	730 (44%)	
No	**50** (16.7)	989 (49%)		**34** (12.7)	926 (56%)	
BRCA1/2 mutation			0.04			0.3
Yes	**46** (15.3)	361 (63%)		**30** (12.1)	156 (38%)	
No	**50** (16.8)	210 (37%)		**31** (12.7)	259 (62%)	
Breast cancer			0.2			0.06
Yes	**50** (16.7)	604 (30%)		**34** (12.8)	954 (58%)	
No	**48** (16.2)	1,432 (70%)		**32** (12.2)	702 (42%)	
Family Hx of breast cancer			0.1			0.9
Yes	**49** (16.5)	1,653 (81%)		**33** (12.5)	1403 (85%)	
No	**47** (15.7)	383 (19%)		**33** (12.6)	253 (15%)	
HRT			0.9			0.1
Never	**49** (16.3)	1,464 (72%)		**33** (12.6)	733 (44%)	
Former	**49** (16.2)	456 (22%)		**34** (12.7)	652 (39%)	
Current	**49** (16.4)	116 (6%)		**31** (11.8)	271 (16%)	
Population			0.001			0.0004
CGN	**51** (16.9)	1,127 (55%)		**34** (12.9)	1,124 (68%)	
GOG	**47** (15.6)	909 (45%)		**31** (11.8)	532 (32%)	

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Table 2. Multivariate linear model–CA125 cut-point (98th percentile) for each factor, percent change, and median CA125 value

	Premenopausal subjects			Postmenopausal subjects		
Factor	**98th percentile**	**Effect (*P*)**	**Median CA125**	**98th percentile**	**Effect (*P*)**	**Median CA125**
Baseline	**52**	–	17.8	**36**	–	13.9
Black	**47**	−11% (0.090)	16	**29**	−22% (0.0008)	11.1
Asian	**41**	−24% (0.0090)	14.1		NS[a]	
Irregular periods	**47**	−11% (0.0100)	16.1		NA[b]	
Current OC use	**40**	−26% (<0.0001)	13.8		NA	
No. of intact ovaries						
0		NS		**30**	−18% (<0.0001)	11.6
1				**37**	2% (0.7000)	14.1
Fertility treatment		NS		**30**	−17% (0.0100)	11.7
Current smoking	**45**	−15% (0.0003)	15.4	**33**	−8% (0.0500)	12.7
Menopause age						
45|age = 50		NA		**35**	0.4% per year	13.6
50|age = 55				**36**	given	13.7
55|age = 60				**36**	age = 50 (0.01)	14
Age						
<35 (30)	**49**	Nonlinear (0.03)	16.9	NA	Nonlinear (0.01)	NA
35–45 (40)	**54**		18.5	NA		NA
45–55 (50)	**52**		17.8	**36**		13.9
55–65 (60)	NA		NA	**36**		13.7
> 65 (70)	NA		NA	**37**		14.2
GOG	**48**	−7% (0.002)	16.6	**31**	−13% (<0.0001)	12.1

[a]Not statistically significant ($P > 0.05$).
[b]Not applicable given menopause subgroup.

by 18% and 17%, respectively ($P < 0.0001$, $P = 0.01$); removal of one ovary had no impact. The association with current smoking was not as pronounced in postmenopausal women; the 8% reduction just reached statistical significance. Age at menopause and current age had statistically significant but clinically marginal effects. Factors identified by our study and predictive of CA125 levels among normal risk postmenopausal women in a U.K. study (12) were Black race, Asian race, hysterectomy, regular smoking, age at menopause, and current age. Women from the GOG study group had 13% lower CA125 levels ($P < 0.0001$). Considering a current smoker with previous use of fertility drugs provides an example of the additive effect of multiple factors in postmenopausal women. The individual effects of these 2 factors were reductions of 8% and 17%, respectively. For a postmenopausal woman with both these factors, the reduction was the sum of these 2 values, namely 25%, reducing the cut-point from 36 to 27 U/mL.

Discussion

The significant differences in median CA125 levels and 98th percentile CA125 cut-points between the pre- and postmenopausal reference groups and specific subgroups shown in our study indicate that the choice of 35 U/mL as a global cut-point in women undergoing ovarian cancer screening needs to be reconsidered. The strongest primary clinical effect was due to menopausal status, with premenopausal women in the reference group having a significantly higher cut-point of 52 U/mL compared with the standard 35 U/mL for the postmenopausal reference group and 40 U/mL for premenopausal women on OC.

Although it is well known that premenopausal women have higher levels of CA125 than postmenopausal women, the analysis reported herein provides the best available quantification of this aspect of CA125 interpretation. A reference level of about 50 U/mL is equivalent in premenopausal women to a reference level of 35 U/mL in postmenopausal women in that 2% of premenopausal women without ovarian cancer will have levels exceeding 50 U/mL, resulting in similar false positive rates in premenopausal women as in postmenopausal women. In one study with approximately equal numbers of pre- and postmenopausal women, premenopausal women had significantly higher anxiety than postmenopausal women, and 2 premenopausal women withdrew from screening because of anxiety from false positive CA125 results (8). Whether this higher

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threshold for postscreening workups should be used for premenopausal women requires balancing multiple clinical considerations, such as the lower rate of false positive CA125 results which may reduce ovarian cancer risk–related anxiety (8), the lower sensitivity expected for ovarian cancer cases because of a higher cut-point and the larger number of years of life lost by a premenopausal woman who experiences early death because of ovarian cancer than for the postmenopausal woman. In this context, it is rational (albeit not yet proven worthwhile) to provide equivalent CA125 cut-points for premenopausal women rather than the standard used for postmenopausal women. At present, pre- and postmenopausal high-risk women are both managed by using the 35 U/mL cut-point. Our data suggest that, at the very least, the use of the standard CA125 cut-point in premenopausal women is likely to cause a high false-positive screening rate, as a consequence of ignoring factors that predict higher baseline CA125 levels.

Relative to menopausal status, factors of secondary clinical importance defined multiple subgroups in which clinically and statistically significant differences in the CA125 cut-point were observed. Applying subgroup-specific CA125 cut-points would individualize the screening test for the initial blood draw to each woman as defined by her baseline factors and better define the CA125 level above which a screening intervention would be indicated. The cut-point for any subgroup defined by a combination of factors was estimated from the reference group cut-point modified by the cumulative percentage change for these factors, as illustrated above for a current smoker with previous use of fertility drugs. An individualized cut-point could improve the interpretation of the first CA125 test in ROCA-based studies when no longitudinal values are available. For subsequent CA125 tests, ROCA relies on comparison with the baseline CA125 for each woman, which is less precisely defined when there are only few previous CA125 values. Further analyses along the lines presented herein should improve prediction of the CA125 baseline of woman by accounting for menopausal status, race, and other factors identified in this study and thereby improve ROCA when the baseline CA125 of a woman is still being established.

The multivariate linear model identified race (Black, Asian), irregular periods, OC use, bilateral oophorectomy, and history of fertility treatment as important factors worthy of consideration when interpreting a CA125 measurement, particularly in the context of an ovarian cancer screening trial. External support of most factors identified by our study that predict the CA125 distribution in high-risk postmenopausal women derives from a U.K. study (12) of normal risk postmenopausal women identifying the same or similarly defined factors. Caution needs to be exercised for subgroups with significant effects but small sample sizes, for example, premenopausal Asian race, $n = 35$. Two other factors related to age, namely age at menopause and current age had statistically significant effects but had only minor clinical impact.

It is important to remember though that efficacy of screening in a high-risk population has not yet been shown. Results from this large U.S.-based study as well as a similar study in the United Kingdom will provide estimates for the sensitivity of personalized CA125 cut-points and of ROCA for early-stage disease in high-risk women. While additional markers may increase sensitivity compared with CA125 alone, such markers have yet to be identified. Two reports (16, 17) of 30 candidates measured in preclinical serum samples and matched control samples from the large NCI Prostate, Lung, Colorectal, and Ovarian (PLCO) screening trial indicated CA125 was superior to all other potential markers, and no marker added significantly to CA125 sensitivity. Until these trials report their final results, there are currently no convincing data to suggest that any screening strategy is associated with a significant reduction in ovarian cancer mortality or shift to early-stage disease, including the study reported herein, and that RRSO is currently considered the most effective risk-reducing strategy (14, 15). Should a woman choose screening despite the lack of evidence showing benefit relative to these final endpoints, the interpretation of ad hoc CA125 tests may still be guided by this discussion of CA125 98th percentile cut-points.

The significantly lower threshold in women in the GOG study cohort compared with those in the CGN cohort (for GOG vs. CGN: 48 vs. 52 U/mL in premenopausal women and 31 vs. 36 U/mL in the postmenopausal women) was an unanticipated finding in this analysis. Both studies used the same eligibility criteria, and procedures for handling, processing, and shipping blood samples were standardized. CA125 values were measured in the same laboratory. One possible explanation for this effect could be unrecognized differences between populations accrued through an oncology clinical trials oncology group (GOG) versus those accrued by epidemiologic registries, from which a large segment of the CGN population was recruited. Because of this difference in CA125 cut-points between the CGN and GOG cohorts, the multivariate models described in Table 2 were fitted separately in the 2 cohorts. The variables in the model had a comparable effect (i.e., same direction of the change in CA125 and to a comparable extent) in the separate analyses, supporting the estimates of effects listed in Table 2 on predicted CA125 levels from the analysis of the combined data from these 2 cohorts. A second possible contributing factor is the GOG trial started 2 years later resulting in a differential because of the upgrade of the immunoanalyzer. The consistency between the separate analyses may indicate that CA125 effects are more reliably estimated than percentiles.

Clearly, as these large ovarian cancer screening studies in high-risk women have indicated, there are many women who may choose not to undergo RRSO at the current time. If ovarian cancer screening is considered, our analysis may provide a more refined, individualized approach to interpreting a CA125 test rather than

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simply applying one threshold of 35 U/mL. In our study, premenopausal women have a significantly higher cutpoint of 50 U/mL compared with the standard 35 U/mL for postmenopausal women and 40 U/mL for premenopausal women on OC. This study lays the groundwork for personalizing the CA125 test in a prospective screening trial in high-risk women. This is a step toward the ultimate goal that integrates epidemiologic, genetic, and longitudinal biomarker results into a subject-specific screening algorithm.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

The ROCA study was supported mostly by research grants/contracts from NCI (CA078284, CA078134, CA078164, CA078156, CA078148, CA078146, CA078174, CA078157, CA078142, HHSN2612007440000C, CA086381, and CA152990) to sites in the Cancer Genetics Network, the Ovarian SPORE program, and the Early Detection Research Network; Fujirebio Diagnostics Inc., supported the study for one year after NCI funding ended. P. Mai and M.H. Greene were supported by the Intramural Research Program, NCI/NIH. The Gynecologic Oncology Group's participation (GOG0199) was supported by its Cancer Prevention and Control Committee. Programming assistance from Ying Zhou MS is gratefully acknowledged. In addition to the clinical site principal investigators who enrolled more than 50 patients in the CGN study and are listed as authors, the following clinical sites and PI's contributed patients to the study and are gratefully acknowledged: Holly Gallion MD (Magee Women's Hospital 46), Powel Brown MD (Baylor College of Medicine 41), Alex Miller MD (University of Texas Health Sciences Center San Antonio 38), Paula Ryan MD PhD (Massachusetts General Hospital 21), Judy Garber MD PhD (Dana Farber Cancer Institute 20), Henry Lynch MD (Creighton University 19), James Evans MD PhD (University of North Carolina 15), Henry Lynch MD (EDRN High Risk Registry 12), Lee-May Chen MD (University of California San Francisco 10), Olufunmilayo Olopade MD (University of Chicago 3), Thomas Caputo MD (Cornell University 3). The CGN study was conducted under the auspices of the Cancer Genetics Network with the support of the CGN PI's which is gratefully acknowledged: Deborah Bowen PhD (Fred Hutchinson Cancer Center), Claudine Isaacs MD (Georgetown University), Constance Griffin MD (Johns Hopkins University), Geraldine Mineau PhD (Huntsman Cancer Institute), Joellen Schildkraut PhD (Duke University), Louise Strong MD (MD Anderson Cancer Center), Susan Domchek MD (University of Pennsylvannia), Gail Tomlinson MD PhD (University of Texas Southwestern Medical Center), Dennis Ahnen MD (University of Colorado), Hoda Anton-Culver PhD (University of California Irvine), Sharon Plon MD PhD (Baylor College of Medicine), James Evans MD PhD (University of North Carolina), William Wood MD (Emory University), Alex Miller MD (University of Texas Health Sciences Ctr. at San Antonio).

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Received December 28, 2010; revised March 19, 2011; accepted May 13, 2011; published online September 5, 2011.

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