Effects of Personal Characteristics on Serum CA125, Mesothelin, and HE4 Levels in Healthy Post-menopausal Women at High-Risk for Ovarian Cancer

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ABSTRACT

Objective: To evaluate if serum levels of candidate ovarian cancer biomarkers vary with individual characteristics of healthy women who are likely candidates for an ovarian cancer screening program. **Methods**: We analyzed serum CA125, mesothelin and HE4 levels in a sample of 155 healthy post-menopausal women at increased risk for developing ovarian cancer based on personal and family cancer history. Information on reproductive, family and medical histories, lifestyle factors and anthropometry was collected by self-report. Twenty-two factors were examined using univariate and multiple linear regression models for the three biomarker levels. **Results**: In the multivariate models, CA125 levels were significantly higher in women who had used talcum powder (P=0.02) and were lower in women who were parous (P=0.05). Mesothelin levels were significantly higher in older women (P=0.01) and lower in heavier women (P=0.03). HE4 levels were higher in older women (P=0.001) and in women who began menstruating at an older age (P=0.03). **Conclusions**: CA125, mesothelin and HE4 levels in healthy, post-menopausal women at increased risk for ovarian cancer are significantly associated with a few ovarian cancer risk factors. Since the effects of these personal characteristics on these serum markers are not large, their incorporation in screening algorithms may be unnecessary. This is true especially if a longitudinal algorithm is used because the marker level at the previous screen reflects personal characteristics such as age, BMI, and age of menarche. Understanding the influence of personal factors on levels of novel early detection markers in healthy, unaffected women may have clinical utility in interpreting biomarker levels.

INTRODUCTION

Considerable effort is underway to identify screening strategies that accurately diagnose ovarian cancer in its early stages when it is most treatable (1). Biomarkers that can be measured in blood products are of particular interest for their potential to provide a low-cost, noninvasive screening modality suitable for application to large populations. CA125 is the most commonly used currently available ovarian cancer biomarker (1, 2). It is a high-molecular weight glycoprotein that is elevated in the serum of 75% to 90% of patients with epithelial ovarian tumors (3). It is employed as a diagnostic tool for ovarian cancer (4), to assess response to ovarian cancer treatment (5) and to monitor for recurrence (6). As a diagnostic marker CA125 is associated with a high false-positive rate among women with benign gynecologic conditions (7) and its sensitivity in early stage disease is inadequate (1). When used to screen asymptomatic postmenopausal participants in the Prostate, Lung, Colorectal and Ovarian Cancer Screening trial, abnormal CA 125 levels were found in 1.4% of the women and the positive predictive value for CA125 was only 3.7% (after excluding tumors of low malignant potential) (8).

The identification of new cancer biomarkers to replace or complement CA125 is urgently needed and currently underway (6). Mesothelin (1, 9-11) and HE4 (12, 13) are two of the most promising novel ovarian cancer biomarkers and are of particular interest as candidate early detection markers. Their diagnostic performance has been well-studied and they are recognized for their ability to complement CA125. Briefly, mesothelin is an epithelial biomarker that predicts ovarian cancer with sensitivity levels of 38% and 27% at 95% and 98% specificity, respectively (14). The AUC for cases versus controls is >0.70 (14). In addition, mesothelin has been shown to improve the diagnostic

performance of CA125. Results from McIntosh et al. (15) demonstrate that sensitivity at 98% specificity increases from 79% for CA125 alone to 87% when CA125 is used in a composite marker with mesothelin. HE4 is commonly overexpressed in ovarian cancer tissue and elevated in the serum of patients with ovarian cancer (13, 16). The sensitivity of HE4 is 62% at 95% specificity and 55% at 98% specificity, and the AUC value for cases versus controls is >0.84 (14). These levels of sensitivity and specificity rival those of CA125, particularly in distinguishing patients with ovarian cancer from those with nonmalignant gynecologic conditions (12). Immunohistochemical analysis of HE4 has been reported to complement CA125, identifying 32% of cancers that do not express CA125 (10).

The application of biomarkers to ovarian cancer early detection, including their validation in serial preclinical samples such as those from the Prostate, Lung, Colorectal and Ovarian Cancer Screening trial or the Women's Health Initiative, requires the development of algorithms or decision rules that select women for additional testing and/or diagnostic surgery. Since the prevalence of ovarian cancer is low and definitive diagnosis requires expensive and potentially morbidity-causing surgery, high specificity for a screening program is required. Recent research has focused on evaluating change over time in marker levels and strategies for combining markers into panels (17-19). These approaches generally aim to improve lead-time and sensitivity while maintaining specificity. Importantly both screening thresholds and specificity depend largely on the behavior of biomarkers in healthy, unaffected women. Therefore it is plausible that overall screening performance can be improved by accounting for factors influencing the level of markers in healthy, unaffected women.

Furthermore, before clinical utility of the markers for early detection can be established through a prospective randomized controlled trial, they must be validated in preclinical samples obtained prior to diagnosis. Such samples were collected in the Prostate, Lung, Colorectal and Ovarian Cancer Screening trial and in the Women's Health Initiative, and are becoming available for validation purposes. If patient characteristics other than presence or absence of disease have a noteworthy influence on marker levels and these effects are not accounted for in selecting thresholds for positivity, the performance of the markers for disease classification may be impaired. The objective of this study was to identify and quantify the effects of personal characteristics, including lifestyle factors, on the levels of three promising candidate ovarian cancer early detection markers: CA125, mesothelin and HE4. We focused our research on postmenopausal women at increased ovarian cancer risk since these women are ideal candidates for testing novel ovarian cancer screening strategies.

MATERIALS AND METHODS

Study Population

The women included in this report are participants in the Seattle-based Ovarian Cancer Early Detection Study (OCEDS). This was an Institutional Review Board-approved study initiated in 2003 to evaluate screening using longitudinal CA125 levels and transvaginal sonography for the early detection of ovarian cancer in women at increased risk for the disease based on personal or family history of breast cancer, family history of ovarian cancer, or the presence of a BRCA 1 or 2 mutation among first or second degree relatives. Specific OCEDS eligibility criteria are outlined in Table 1. All

participants provided informed consent prior to enrollment. The screening protocol involved CA125 measurements at baseline and quarterly thereafter. Longitudinal CA125 values were interpreted using the parametric empirical Bayes method (17, 18), which evaluates within women changes over time in CA125 levels. Screening using transvaginal sonography was conducted at baseline and annually thereafter. Additional transvaginal sonography examinations were conducted at follow-up for women with elevated CA125 results.

OCEDS participants were considered eligible for this study if they did not report bilateral oophorectomy and if they were 50+ years of age or post-menopausal at the time of a blood collection for which serum biomarker levels were available. For women <50 years of age, menopausal status was defined by self-reported natural menopause, use of hormone replacement therapy (HRT), or reported hysterectomy. Of the 285 women enrolled in OCEDS at the time of this report, 155 were eligible for this study (Figure 1).

All women in the defined study population were followed for ovarian cancer incidence through data linkage to the Puget Sound Surveillance Epidemiology and End Results cancer registry for 2 years from the date of the blood sample that was used in our analyses.

Defining the Personal Characteristics

Data related to reproductive history, personal or family diagnoses of cancer, overall health issues (such as hospitalization or surgery), medical information directly related to ovarian and breast health, use of medications, and personal habits including

smoking and caffeine use were obtained from self-administered OCEDS questionnaires and were measured by self-report. The personal characteristics previously studied by Pauler et al. (20) for influencing CA125 levels in healthy, postmenopausal average risk women were examined, including age, personal history of breast cancer, age at menarche, race, parity, hysterectomy, and use of oral contraceptives, HRT, talcum powder, tobacco, and caffeine. We also evaluated self-reported height, weight, fertility drug use, pain medication use, and tubal ligation. Since the focus of this research is on high-risk women, we also evaluated the influence of having a BRCA1/BRCA2 mutation, a first-degree relative with ovarian cancer, a second-degree relative with ovarian cancer, a first-degree relative with breast cancer, or a second-degree relative with breast cancer.

Data on current age was calculated based on the date of the blood draw and the self-reported birth date. Body mass index (BMI; in kg/m^2) was calculated for subjects who provided data for both height and weight. Race was defined as white or non-white based on self-report of any race other than white or Caucasian. Being parous was defined as reporting having had at least one pregnancy that lasted beyond 6 months (all deliveries, both term and preterm). A caffeine user includes individuals reporting current consumption of one or more cups of coffee per day. Smokers include participants reporting current or previous smoking. In addition, the number of self-reported cigarettes smoked per day was assessed. A talcum powder user is defined as an individual reporting current or past application of talcum powder to the genital area one or more times per month. Individuals who report genetic testing that demonstrates BRCA1 or BRCA2 mutations are defined as mutation carriers or carriers of variants with undetermined significance. HRT use was defined as ever taking pills that contained

female hormones, such as estrogen or estrogen plus progesterone (other than birth control pills). Hormonal contraceptive use was defined as reporting ever having used birth control pills, patches, or implants. Fertility drug use was defined as reporting ever having ever taken drugs to stimulate ovulation. Pain medication use was defined as reporting ever having used aspirin, Tylenol, nonsteriodal anti-inflammatory drugs, or similar drugs. For family history of ovarian or breast cancer, a first-degree relative was defined as a parent, sibling, or child. Second-degree relatives include grandmothers, aunts, and nieces. Personal history of breast cancer was defined as having breast cancer prior to enrollment in OCEDS.

Measurement of CA125, HE4 and Mesothelin

All biomarkers were measured in serum by sandwich ELISA on a Luminex platform without multiplexing. Monoclonal antibodies (mAb) were used in the assays for CA125 and HE4 (21); a commercially available polyclonal antibody and a novel biobody (22) were used in the mesothelin assay (14).

CA125 and HE4 serum levels were assessed using bead based immunoassays performed as described by Scholler et al. (21). Briefly, complementary anti-CA125 mAbs X306 and X52 were purchased from Research Diagnostics, Inc (RDI). Complementary anti-HE4 mAbs 3D8 and 2H5 were kind gifts from Dr. Ingegerd Hellstrom (12). Anti-CA125 X52 mAb and anti-HE4 3D8 mAb were biotinylated using the EZ-Link sulfo-NHS-biotinylation kit (Pierce) according to the manufacturer's instructions and dialyzed against PBS (Fisher BioReagents) using a dialysis slide (Slide-a-Lyzer 7kDa molecular weight cutoff; Pierce). Biotinylated detection antibodies were incubated with

phycoerythrin-conjugated streptavidin (Bio-Rad Laboratories Inc., or Becton Dickinson Pharmingen). Bead-based assays were carried out in 96-well MultiScreen GV filter plates (Millipore Corporation) using a vacuum manifold (Millipore) to drain assay reagents. For conjugating anti-HE4 2H5 to beads, bead activation buffer was made with 0.1 mol/L sodium phosphate (NaH_2PO_4; pH 6.2; Sigma-Aldrich). 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce) and N-hydroxysulfosuccinimide (Pierce) were diluted to 38 mg/mL and to 109 mg/mL, respectively in activation buffer. The coupling buffer was made with 0.05 mol/L of 2-(N-Morpholino) ethanesulfonic acid (pH 5.0; Sigma-Aldrich). PBS supplemented with 1% bovine serum albumin was used for bead blocking and storage buffers. Anti-CA125 X306 was conjugated to beads using a Bio-Plex amine coupling kit (Bio-Rad). All incubations were carried out for 30 minutes (unless otherwise specified) in PBS supplemented with 1% bovine serum albumin (Sigma-Aldrich) for HE4 and mesothelin assays, and assay buffer (Bio-Rad) for CA125 assays. Washes were performed with PBS supplemented with 0.05% Tween 20 (Sigma-Aldrich) for HE4 and mesothelin assays and wash buffer (Bio-Rad) for CA125 assays. Plates were analyzed with the Bio-Plex Array reader (Bio-Rad).

For the CA125 assay, 5 μg/mL of anti-CA125 mAb X306 was coupled to carboxy-coated beads. Antibody-coated beads were incubated with 4-fold diluted patient sera and captured antigen was detected with 2 μg/mL of biotinylated anti-CA125 mAb X52 followed by SA-PE. This procedure has been found to yield values that are strongly correlated ($r > 0.90$) with the research standard CA125II RIA from Fujirebio Diagnostics, Inc. (FDI) (21). For the HE4 assay, anti-HE4 2H5 was coupled to beads at a

concentration of 10 μg/mL. Antibody-coated beads were incubated with 10-fold diluted sera. Captured antigens were detected with 2 μg/mL of biotinylated 3D8 followed by a 10 minute incubation with 1000-fold diluted phycoerythrin-conjugated streptavidin. The bead-based HE4 assay yields values that are strongly correlated (r =0.95) with a plate-based assay using the same mAbs (21).

The mesothelin bead-based assay employed a polyclonal antibody and a novel biobody and was conducted as previously described (14). The anti-mesothelin polyclonal antibody (pAb) was purchased from R&D Systems. The complementary anti-mesothelin antibody was an *in vivo* biotinylated single-chain Fragment variable secreted by yeast (biobody no. 7) (23). Biobodies were preincubated with PJ31S PhycoLink Streptavidin-R-Phycoerythrin (Prozyme). For the bead-based assays, carboxy-coated beads were conjugated to anti-mesothelin polyclonal antibody at a concentration of 50 μg/mL. To capture antigen, antibody-coated beads were incubated with 5-fold diluted sera. Biobody no. 7 at a concentration of 1 ug/mL was preincubated with 2,000-fold diluted PJ31S on ice and in the dark. Bead-captured antigens were detected with biobodies preincubated with PJ31S. This novel assay correlates well with a bead-based assay using anti-mesothelin mAbs (14).

Statistical Methods

Serum levels of CA125, mesothelin and HE4 were log-transformed and standardized to have mean 0 and SD 1. This transformation induces the same measurement scale for all three markers, allowing them to be easily compared to one another (24).

Univariate linear regression methods (25) were used to evaluate the association of each personal characteristic with CA125, HE4 and mesothelin levels. For analysis purposes, women who did not report a condition or use of a medication were assumed not to have the condition or use the medication. Factors that yielded a $P \leq 0.20$ in the univariate models were included in a multivariate linear regression model (25) for each marker and backwards elimination methods (26) were applied to yield the model reported. In this approach, all of the selected factors are included in the initial model and then the variable with the highest P value is eliminated from the model in a stepwise fashion until all of the remaining variables are statistically significant. The overall predictiveness of the model is summarized by an R^2 statistic, which can be interpreted as the fraction of variation in biomarker levels that is "explained" by the variables included in the model.

STATA statistical software package (version 9.0, Stata Corporation) was used for these analyses. All statistical tests were two-sided and considered to be statistically significant at $P < 0.05$.

RESULTS

Data for the personal characteristics and lifestyle factors included in our analyses were obtained from self-report. The following characteristics had 100% response rate: age at blood draw, age at menarche, hormone contraceptive use, family history of breast cancer, and family history of ovarian cancer. A <5% nonresponse rate was obtained for the following characteristics: height, weight, BMI, race, parity, talcum powder use, HRT use, fertility drug use, hysterectomy, tubal ligation, and personal history of breast cancer.

Report of caffeine consumption, smoking, use of pain mediation, and BRCA1/BRCA2 mutation was missing for 18%, 59%, 22%, and 83% of the women, respectively. All of the women who self-reported smoking included the total number of cigarettes smoked every day.

Table 2 summarizes the personal characteristics and lifestyle factors in this study population. Median age of the women was 55 years. In this high-risk population, 49.0% reported a personal history of breast cancer, 63.2% reported one or more first-degree relatives with breast cancer, 34.8% reported one or more first-degree relatives with ovarian cancer, and 5.2% reported having a BRCA1/BRCA2 mutation.

The univariate linear regression results for CA125, mesothelin and HE4 are reported in Table 3. Since the marker values were log-transformed and standardized, each factor's coefficient can be interpreted as the deviation from zero for a one unit change in the corresponding factor. For example, talcum powder use was associated with a 0.32 SD increase in the log-transformed CA125 levels (P=0.04). The log-transformed mesothelin levels increased 0.02 SD for every 1 year increase in age (P=0.05) and 0.04 SD for each additional cigarette smoked per day (P=0.03). Similarly, the log-transformed HE4 levels increased 0.06 SD for every 2 year increase in age (P<0.001), 0.14 SD for every year increase in age at menarche (P= 0.03), 0.40 SD among parous women (P=0.03), and 0.37 SD among women who consumed at least one cup of coffee per day (P=0.04); HE4 levels decreased 0.35 SD among women who reported one or more second-degree relatives with breast cancer (P=0.05).

Using these univariate analyses to guide variable selection for the multivariate modeling, the initial multivariate model for CA125 included parity, number of cigarettes

smoked per day, talcum powder use, HRT, use of pain medications, and reported first-degree relative(s) with ovarian cancer. The initial multivariate model for mesothelin included age at the time of blood draw, weight, body mass index, number of cigarettes smoked per day, known BRCA1/BRCA2 mutation, and first-degree relative(s) with breast cancer. For HE4 the model began with age at the time of blood draw, age at menarche, parity, coffee use, number of cigarettes smoked per day, talcum powder use, known BRCA1/BRCA2 mutation, HRT, hormonal contraception use, first-degree relative(s) with breast cancer, and second-degree relative(s) with breast cancer.

After backwards elimination of nonsignificant predictors, the final multivariate models suggest that these personal characteristics explain a fraction of biomarker variability (4% for CA125, 6% for mesothelin, and 22% for HE4) (Table 4). Talcum powder use was associated with a 0.39 SD increase (P=0.02) and parity was associated with a 0.28 SD decrease (P=0.05) in CA125 levels. Mesothelin levels increased 0.02 SD for every year increase in age at the time of blood draw (P=0.01) and decreased 0.04 SD for every unit change in BMI (kg/m^2; P=0.03). HE4 levels increased 0.06 SD for every year increase in age at the time of blood draw (P=0.001) and 0.13 SD for every year increase in menarche (P=0.03).

DISCUSSION

Early detection is a critical focus of ovarian cancer research because of its potential to reduce suffering and morbidity. One promising screening strategy involves using change over time in a panel of serum markers that includes CA125 to select women for additional testing with imaging. The application of biomarkers and biomarker panels

to ovarian cancer early detection requires the development of algorithms or decision rules that improve sensitivity and maintain high specificity. Since the behavior of markers in healthy, unaffected women largely defines screening thresholds and hence specificity, we undertook this study to evaluate personal factors that influence the levels of CA125, mesothelin and HE4 in healthy, high-risk postmenopausal women.

Our analysis demonstrates that serum levels of all three candidate ovarian cancer early detection markers are significantly associated with a few personal characteristics of women. In the final multivariate models, CA125 levels appear to track with some documented ovarian cancer risk factors: CA125 is higher in talcum powder users and is lower in parous women. Similarly, age was a significant predictor of higher mesothelin and HE4 levels. We also observed an inverse correlation between mesothelin and BMI. While statistically significant, the impact of these factors on marker levels is not large, suggesting that rarely will these variables need to be accounted for in decision rules that include these specific markers. The largest effects were noted for CA125 where talcum powder use was associated with a 0.39 SD increase ($P = 0.02$) and being parous was associated with a 0.28 SD decrease ($P = 0.05$). These factors explained only 4% of the variability in CA125 levels, suggesting there is limited value in adjusting screening thresholds for these factors. However, age at the time of the blood-draw and age at menarche explained 22% of the variability in HE4 levels. Although these factors are statistically significant, their relevance to screening algorithms and the decision for whether these covariates need to be adjusted for screening algorithms, may depend on the intended screening algorithm. Although cross-sectional rules might need to account for some of these effects, longitudinal algorithms, which are adjusted based on previous

marker values, do not, as long as the covariates are not time-varying. Variables that do not vary by time, or that vary according to predicable trends (such as age), will be adjusted for automatically and efficiently by the previous screening history measurement (17). The most comprehensive study of the influence of personal characteristics on CA125 levels among healthy women was provided by Pauler et al. (20). In a sample of 18,748 ovarian cancer-free postmenopausal women, the personal factors that significantly lowered CA125 levels included hysterectomy, smoking, and caffeine consumption. Conversely, CA125 levels significantly increased with age of menarche, age of menopause, and among women with a prior non-ovarian cancer diagnosis or a history of ovarian cysts. Race also significantly influenced CA125 levels in this population, with Caucasian women having the highest levels of CA125, followed by Asian women and then African women. In the current study of high-risk women, associations in the same direction were found but they were not statistically significant, perhaps because of the much smaller sample size (although important differences in study populations cannot be ruled out). We found talcum powder use and parity to be significant predictors of CA125 levels, neither of which were identified by Pauler et al. These results could reflect the high risk women in the current study or they could represent a chance finding. Confirmation in other studies would help to clarify the importance of these effects.

We believe this is the first report to describe factors that influence levels of the novel markers HE4 and mesothelin in healthy women. We found that age was a significant predictor of both HE4 and mesothelin levels. The results of our analysis also revealed a significant negative trend between BMI and mesothelin levels. Interestingly, a negative correlation between BMI and prostate-specific antigen has been reported in men

40 to 59 years of age (27). Given the minimal effort required to collect age information and calculate BMI, these personal attributes could easily be adjusted for when evaluating cancer biomarker levels.

Our sample size limits our ability to detect associations of less than a third of a standard deviation between the lifestyle factors and the biomarker levels. Additional limitations include imprecise information on menopausal status and reliance on self-reported information particularly for variables with high social desirability attributes (e.g., weight, BMI, smoking). Of the personal attributes we found to be significant predictors of the biomarker levels, BMI is most likely to have high misclassification rates. In fact, it has been shown that study participants will often overestimate their height and underestimate their weight (27), both of which could introduce bias. We are unable to separate the effects of estrogen from progestin on biomarker levels. The frequency of minority women, documented mutation carriers and fertility drug use was too low to provide reliable estimates of their effects.

Since we did not adjust for multiple comparisons, some of our findings are likely to be false positives. The Bonferroni method of adjustment (in which alpha is divided by the total number of comparisons) substantially limits the likelihood of a type I error, but may be too conservative in this setting since we evaluated the influence of 22 factors on three biomarkers (i.e.: resulting in a total of 66 comparisons). In this scenario, we would expect approximately three of our statistically significant results to be obtained by chance alone. However, despite this fact, our results suggest that some personal characteristics may influence the levels of the biomarkers. These factors may be appropriately accounted for by using longitudinal screening algorithms for ovarian cancer screening.

This analysis examined a high-risk postmenopausal population. Our objective was to determine whether individual characteristics influence levels of ovarian cancer biomarkers in women who are most likely to participate in an ovarian cancer screening program. We excluded 35 women who reported bilateral oophorectomy since women without ovaries are unlikely to participate in this type of cancer screening program. In a sensitivity analyses, we repeated these analyses including these women. The results did not change for CA125 or mesothelin. When women with bilateral oophorectomy were included in the analysis of HE4, age at menarche was no longer statistically significant.

The primary objective of this study was to evaluate the influence of personal characteristics, including lifestyle factors on levels of promising ovarian cancer early detection markers in a population of ostensibly healthy high-risk postmenopausal women likely to participate in a screening program. We identified a few personal factors that are significantly associated with levels of CA125, HE4 or mesothelin, but statistical significance does not imply clinical relevance or discriminatory power (28). Our findings provide important reassurance that screening algorithms based on these three markers need not include complicated adjustments for these variables, especially if a longitudinal algorithm such as the parametric empirical Bayes method (17) is employed because it inherently accounts for personal characteristics that may change systematically over time (such as age) as well as those that differentiate among women. Larger studies evaluating the influence of personal factors on the levels of novel early detection markers in healthy individuals will be needed as retrospective and prospective validation studies go forward.

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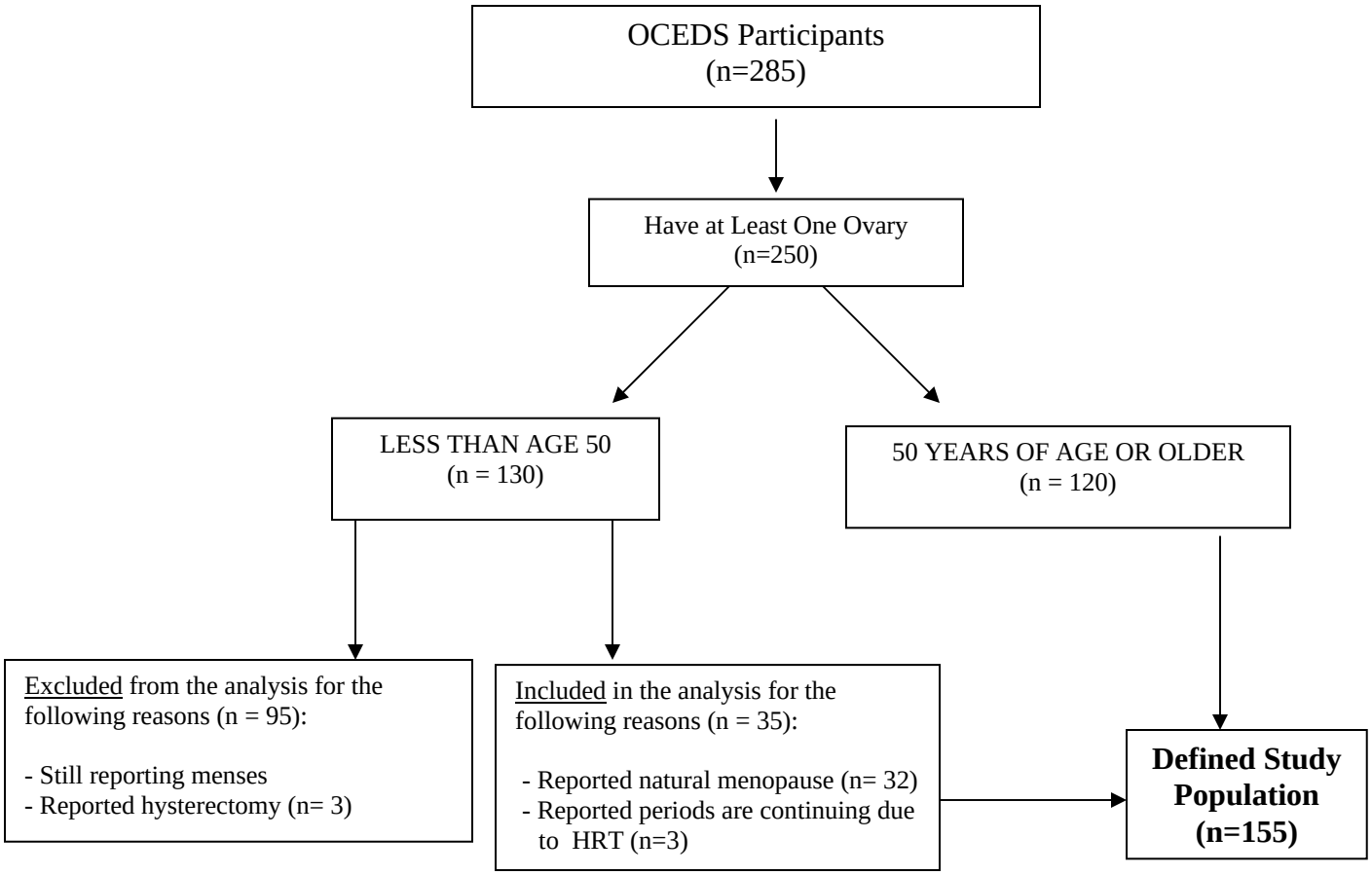

Figure 1. Eligibility schema of the decision rule used to define menopause status and derive the defined study population.

Table 1. OCEDS inclusion and exclusion criterion.

Inclusion Criteria

1. Women must meet one of the following criteria to be considered high-risk for ovarian cancer:
    - The family contains at least two ovarian or breast cancers among the subject or first and second degree relatives of the subject. This condition is satisfied by multiple primary cancers in the same person. Where breast cancer is required to meet this criterion, at least one breast cancer must be pre-menopausal (age at diagnosis less than 50 years if age at menopause is unknown).
    - The subject is of Ashkenazi Jewish ethnicity with one first degree or two second degree relatives with breast cancer, or the subject is of Ashkenazi ancestry and has had breast cancer herself. Where breast cancer is required to meet this criterion, at least one breast cancer must be pre-menopausal (age at diagnosis less than 50 years if age at menopause is unknown)..
    - The subject has tested positive for a BRCA I/II mutation
    - The subject has a first or second degree relative with a BRCA I/II mutation.

Exclusion Criteria

The subject is, has, or has had one of the following criteria:

1. Ovarian cancer or peritoneal carcinomatosis.
2. A first or second degree relative with a BRCA I/II mutation and she had tested negative for the same mutation herself.
3. Has no ovaries and has not tested positive for a BRCA I/II mutation.
4. Less than 30 years of age.
5. Currently pregnant or anticipating pregnancy during the study.
6. Participating in other ovarian cancer early detection trials.
7. Psychiatric or psychological or other conditions which prevent a fully informed consent.
8. Currently untreated malignancy (other than non-melanoma skin cancer).
9. Currently receiving adjuvant chemotherapy or radiation therapy for cancer (excluding tamoxifen). Patients who are being treated for local disease may enroll 3 months after completion of the last treatment (excluding tamoxifen).
10. Treatment (excluding tamoxifen) for prior metastatic malignancy within the past five years.
11. Intraperitoneal surgery within the last 3 months (laparoscopy or laparotomy).
12. A history of any medical conditions that would place the subject at risk as a result of the blood donation. Such conditions include but are not limited to: hemophilia or other bleeding disorders, chronic infectious disease, emphysema or serious anemia.

Table 2. Distribution of the personal characteristics among the study population.

Lifestyle Factors*	**Study Population (n=155)**
Personal Characteristics	
Age at blood draw -years, median (range)	55 (32-83)
Age at menarche - years, median (range)	12 (9-17)
Height - inches, median (range)[±]	65 (55-72)
Weight - lbs, median (range) [Π]	149 (105 – 282)
BMI - kg/m^2, median (range) [δ]	24.8 (17.4 – 43.9)
Race - non-white, n (%)	3 (1.9)
Parous - at least one birth, n (%)	102 (65.8)
Caffeine - currently consume one or more cups of coffee per day, n (%)	69 (44.5)
Smoker – current for former, n (%)	29 (18.7)
Number of cigarettes smoked per day, median (range)	10 (1 – 20)
Talcum powder - ever used one or more times per month, n (%)	39 (25.2)
BRCA1/BRCA2 Mutation Carrier, n (%)	8 (5.2)
Medication Use	
HRT - (estrogen, progesterone, or both), n (%)	60 (38.7)
Hormonal Contraceptives - ever use oral birth control, patches, implants, n (%)	121 (78.1)
Fertility Drugs - ever use, n (%)	12 (7.7)
Pain Medication - ever use Aspirin, Tylenol, Ibuprofen, etc., n (%)	114 (73.5)
Gynecologic Surgery	
Hysterectomy, n (%)	16 (10.3)
Tubal ligation, n (%)	34 (21.9)
Cancer History	
Personal history of breast cancer, n (%)	76 (49.0)
One or more 1° relatives with breast cancer, n (%)	98 (63.2)
One or more 2 ° relatives with breast cancer, n (%)	100 (64.5)
One or more 1° relatives with ovarian cancer, n (%)	54 (34.8)
One or more 2° relatives with ovarian cancer, n (%)	42 (27.1)

* Data on the lifestyle factors were obtained from self-report.
[±] Data for height is missing for 2 subjects.
[Π] Data for weight is missing for 3 subjects.
[δ] BMI was calculated from self-reported height and weight. The results exclude 4 subjects with missing BMI data.

Table 3. Univariate linear regression results for the association between each personal characteristic and CA125, mesothelin, and HE4.

Covariates	CA125		Mesothelin		HE4	
	coefficient*	p-value	coefficient*	p-value	coefficient*	p-value
Personal Characteristics						
Age at blood draw – years	-0.003	0.75	0.02	0.05	0.06	<0.001
Age at menarche – years	0.01	0.80	0.07	0.26	0.14	0.03
Height at baseline – inches	-0.003	0.90	-0.01	0.63	-0.02	0.57
Weight at baseline – lbs	-0.004	0.87	-0.004	0.10	-0.001	0.79
Body Mass Index - kg/m^2	-0.001	0.95	-0.03	0.08	-0.003	0.87
Race - non-white	-0.49	0.33	-0.22	0.73	-0.35	0.58
Parous - at least one birth	-0.20	0.18	0.07	0.70	0.40	0.03
Coffee drinker - currently consume ≥1 cups of coffee per day	-0.08	0.56	0.103	0.55	0.37	0.04
Number of cigarettes smoked per day	-0.03	0.09	0.04	0.03	0.03	0.10
Talc – ever used one or more times per month	0.32	0.04	0.19	0.55	0.37	0.06
BRCA1/BRCA2 Mutation Carrier	-0.15	0.64	-0.67	0.08	-0.74	0.06
Medication Use						
HRT - (estrogen, progesterone, or both)	0.23	0.11	0.16	0.37	0.30	0.10
Hormonal Contraceptives - ever use birth control, patches, implants	0.06	0.71	0.06	0.76	-0.32	0.13
Fertility Drugs - ever use	-0.31	0.40	0.40	0.36	-0.32	0.48
Pain Medication - ever use Aspirin, Tylenol, Ibuprofen, etc.	0.29	0.07	0.21	0.27	-0.03	0.88
Gynecologic Surgery						
Hysterectomy	0.02	0.94	0.26	0.35	-0.18	0.53
Tubal Ligation	0.20	0.23	0.08	0.70	0.17	0.41
Cancer History						
Personal history of breast cancer	-0.13	0.35	0.13	0.43	-0.08	0.64
One or more 1° relatives with breast cancer	-0.12	0.42	0.27	0.13	-0.33	0.07
One or more 2 ° relatives with breast cancer	0.14	0.35	-0.05	0.79	-0.35	0.05
One or more 1° relatives with ovarian cancer	-0.23	0.11	0.02	0.93	-0.03	0.87
One or more 2° relatives with ovarian cancer	-0.05	0.78	-0.14	0.47	0.004	0.98

* Biomarker values have been log transformed and standardized to mean=0 and SD=1; therefore, each factor's coefficient can be interpreted as the deviation from zero for a one unit change in the corresponding factor.

Table 4. Final multivariate linear regression models for CA125, mesothelin, and HE4.

Marker	Overall Model Fit		Variable	Coefficient*	p-value
	Adjusted R^2	F-statistic p-value			
CA125					
	0.04	0.02	Talc use	0.39	0.02
			Parous	-0.28	0.05
Mesothelin					
	0.06	0.008	Age at blood draw	0.02	0.01
			BMI	-0.04	0.03
HE4					
	0.22	0.001	Age at blood draw	0.06	0.001
			Age at menarche	0.13	0.03

* Biomarker values have been log transformed and standardized to mean=0 and SD=1; therefore, each factor's coefficient can be interpreted as the deviation from zero for a one unit change in the corresponding factor.