

# UCSF

## UC San Francisco Previously Published Works

### Title

A comparison of the cyclic variation in serum levels of CA125 across the menstrual cycle using two commercial assays.

### Permalink

<https://escholarship.org/uc/item/0535z1pn>

### Journal

Biological research for nursing, 14(3)

### ISSN

1099-8004

### Authors

McLemore, Monica R  
Aouizerat, Bradley E  
Lee, Kathryn A  
[et al.](#)

### Publication Date


2012-07-01

### DOI

10.1177/1099800411412766

Peer reviewed

# A Comparison of the Cyclic Variation in Serum Levels of CA125 Across the Menstrual Cycle Using Two Commercial Assays

Biological Research for Nursing  
14(3) 250-256  
© The Author(s) 2012  
Reprints and permission:  
sagepub.com/journalsPermissions.nav  
DOI: 10.1177/1099800411412766  
<http://brn.sagepub.com>  


Monica R. McLemore, RN, MPH, PhD<sup>1</sup>, Bradley E. Aouizerat, PhD, MAS<sup>1</sup>,  
Kathryn A. Lee, RN, PhD, FAAN<sup>2</sup>, Lee-may Chen, MD<sup>3</sup>, Bruce Cooper, PhD<sup>4</sup>,  
Michael Tozzi, PharmD<sup>1</sup>, and Christine Miaskowski, RN, PhD, FAAN<sup>1</sup>

## Abstract

**Background:** Clinicians use CA125, a tumor-associated antigen, primarily to monitor epithelial ovarian cancer. However, CA125 lacks the sensitivity and specificity necessary for population-based screening in healthy women. The purpose of this study was to determine if serum concentrations of CA125 differed across the three phases of the menstrual cycle in healthy, premenopausal women using two commercially available assays. **Methods:** Healthy, Caucasian women between the ages of 18 and 39 were enrolled using strict criteria to exclude factors known to contribute to CA125 fluctuations. Menstrual cycle regularity was determined using calendars maintained by participants for 3 months. After cycle regularity was established, blood was drawn at three time points for CA125 determination using two commercial assays (i.e., Siemens and Panomics). **Results:** Regardless of the assay used, CA125 values were highest during menses. The CA125 values decreased 0.2 U/ml per day from menses to the end of the same cycle, which resulted in a net decrease of 5.8 U/ml across the cycle. **Conclusions:** The two commercial assays for CA125 determination demonstrated good concordance in terms of reference ranges regardless of epitope differences. While CA125 levels changed over the course of the menstrual cycle, these changes may not be clinically significant in healthy women. This study is the first to control for factors known to contribute to CA125 elevations; to quantify a decrease in CA125 levels across the menstrual cycle; and to confirm concordance in the relative decreases in serum CA125 levels across the menstrual cycle between two frequently used commercial assays.

## Keywords

CA125, menstrual cycle, ovarian cancer screening, premenopausal women

Clinicians use CA125, a tumor-associated antigen, to monitor epithelial ovarian cancer (Bast et al., 2005; Jacobs & Bast, 1989; Rubin & Sutton, 2004). However, CA125 alone lacks the sensitivity and specificity necessary for population-based screening in healthy women. Currently, researchers conducting longitudinal studies of healthy and high-risk women are evaluating the use of CA125 in combination with other modalities as a screening tool for ovarian cancer (Andersen et al., 2008; Buys et al., 2005; Duffy et al., 2005; Johnson et al., 2008; NCI, n.d.).

One barrier to the development of a sensitive and specific assay to screen for ovarian cancer is the low incidence of the disease worldwide (Crump, McIntosh, Urban, Anderson, & Karlan, 2000). In addition, researchers have attributed large interindividual variability in CA125 levels to differences in the commercial assays (Clement et al., 1995; Davelaar, van Kamp, Verstraeten, & Kenemans, 1998; Fiskén, Leonard, & Roulston, 1989; Martin & Blockx, 1997), fluctuations in levels during the phases of the menstrual cycle (Bon, Kenemans, Verstraeten, van Kamp, & Hilgers, 1995; Kan, Yeh, Ng, & Lou, 1992), ethnicity (John, Whittemore, Harris, & Intyre, 1993; Pauler et al.,

2001), menopausal status (Johnson et al., 2008; Pauler et al., 2001), and other benign conditions (Bon et al., 1999; Kafali, Artunc, & Erdem, 2007; Meden & Fattahi-Meibodi, 1998). These factors make it difficult to interpret any one individual's CA125 levels. Another major challenge in the interpretation of CA125 levels is whether variability in the epitopes used in the

<sup>1</sup> School of Nursing, Department of Physiological Nursing, University of California, San Francisco, CA, USA

<sup>2</sup> School of Nursing, Department of Family Health Care Nursing, University of California, San Francisco, CA, USA

<sup>3</sup> Department of Obstetrics, Gynecology, and Reproductive Sciences, University of California, San Francisco, CA, USA

<sup>4</sup> Dean's Office, School of Nursing, University of California, San Francisco, CA, USA

## Corresponding Author:

Monica R. McLemore, School of Nursing, Department of Physiological Nursing, University of California, 520 Frederick Street, #31, San Francisco, CA 94117, USA

Email: [mclmoremr@alumni.ucsf.edu](mailto:mclmoremr@alumni.ucsf.edu)

serum assays themselves are responsible for some of the reported differences in serum CA125 levels across the phases of the menstrual cycle (Clement et al., 1995; Davelaar et al., 1998; Fiskén et al., 1989; Martin & Blockx, 1997).

In previous studies exploring fluctuations in CA125 levels in healthy women, investigators included convenience samples of women without controlling for factors known to contribute to CA125 fluctuations (Bon et al., 1999; Crump et al., 2000; Kafali et al., 2007; Kan et al., 1992; Lehtovirta, Apter, & Stenman, 1990; Meden & Fattahi-Meibodi, 1998; Pauler et al., 2001). In the present study, we used strict enrollment criteria to control for these factors in a sample of healthy premenopausal women. To our knowledge, this study is the first to control for the known factors associated with CA125 fluctuations.

Additionally, the fluctuation of CA125 levels in healthy women is inconsistent with the assumptions that underlie traditional linear regression analysis. Researchers in 16 previous studies that evaluated CA125 fluctuations using multiple assays used linear statistical procedures that require normal Gaussian distributions (Bon et al., 1995, 1999; Bonfrer, Baan, Jansen, Lentfer, & Kenemans, 1994; Bonfrer et al., 1997; Clement et al., 1995; Davelaar et al., 1998, 2003; Fiskén et al., 1989; Kafali et al., 2007; Kenemans, van Kamp, Oehr, & Verstraeten, 1993; Kenemans, Verstraeten, van Kamp, & Mensdorff-Pouilly, 1995; Lehtovirta et al., 1990; Martin & Blockx, 1997; van Kamp, Verstraeten, & Kenemans, 1993; Yan, Ju, Liang, & Zhang, 1999; Yedema, Thomas, Segers, Doesburg, & Kenemans, 1992). The present study is the first to use more conservative statistical procedures that account for wide interindividual differences in CA125 levels.

Given the methodologic limitations in previous studies, the purpose of this study was to determine in a sample of healthy, premenopausal women if the absolute serum concentrations of CA125 differ across the three phases of the menstrual cycle using two commercially available assays for CA125 determination (i.e., Panomics CA125 enzyme-linked immunosorbent assay [ELISA] assay and Siemens CA125 ELISA assay).

## Method

### Sample Selection

Between June 2007 and February 2009, we approached 226 women about this study and enrolled 79 (35% response rate). The major reasons for refusal were scheduling conflicts ( $n = 74$ ), fear and/or refusal of blood draw ( $n = 8$ ), and a desire to become pregnant or begin hormonal birth control ( $n = 65$ ). For the 20 women who started but did not complete the study, reasons were that they did not establish a regular menstrual cycle ( $n = 11$ ), a first-degree relative developed cancer ( $n = 6$ ), or they became pregnant or started hormonal birth control ( $n = 3$ ). Thus, 59 women completed the entire study and 1 woman completed all but the final blood draw.

To be included in the study, women had to be able to read, write, and understand English; Caucasian and between the ages of 18 and 39; able to tolerate a venipuncture; and available for

three consecutive venipunctures. In addition, women had to have a regular menstrual cycle, defined as no less than 25 days and no more than 35 days with at least 3 days of bleeding for 3 consecutive months (Fehring, Schneider, & Raviele, 2006; Lee, Shaver, Giblin, & Woods, 1990). Participants also had to have a body mass index (BMI) between 18.5 and 29.9 kg/m<sup>2</sup>, which is consistent with the National Heart, Lung, and Blood Institute's guidelines for "normal." Researchers have reported an increased risk for ovarian cancer and increased serum levels of CA125 in samples of obese women (Rodriguez, Calle, Fakhrabadi-Shokooi, Jacobs, & Thun, 2002; Runnebaum & Stickeler, 2001). Women with diagnosed infertility (without hormonal treatment) who met all of the other inclusion criteria were eligible to participate.

We set our exclusion criteria because these factors could influence CA125 levels (Johnson et al., 2008; McLemore, Miaskowski, Aouizerat, Chen, & Dodd, 2009): having a personal history of ovarian, endometrial, lung, or colorectal cancer or a first-degree relative with such a history; being currently pregnant or less than 6 months postpartum; lactating currently; or having had an abortion or miscarriage within the last 3 months. We also excluded women with endometriosis, ovarian cysts, polycystic ovarian syndrome, or a history of pelvic inflammatory disease as well as any women participating in infertility treatment or ovum donation or who were taking hyperstimulatory medications or gonadotropins; women with any chronic illness that required routine nonsteroidal anti-inflammatory drug (NSAID) use (defined as daily, around-the-clock use for greater than 3 days prior to the onset of menses to the end of bleeding) and women who used systemic hormonal contraceptives; and women taking black cohosh or red clover supplements.

### Study Procedures

We recruited women within a 75-mile radius of the San Francisco Bay area using flyers and two Internet e-mail lists. Eligible participants provided written informed consent. The Institutional Review Boards at the University of California, San Francisco, and San Francisco General Hospital (SFGH) approved all procedures.

*Demographic and menstrual cycle data-collection procedures.* Demographic data included age, height, weight, age at first menses, pregnancy and contraceptive history, and current medications. We gave participants a study booklet with six calendars and monthly demographic sheets and instructed them to fill in the booklet for each month they were enrolled in the study.

We determined menstrual cycle regularity from calendars the participants completed. Participants marked an *X* on any day that they had bleeding and an *S* on any day that they had spotting. Participants maintained these calendars throughout the study. We excused participants from the study if we could not determine regularity within 6 months of enrollment.

*Blood sample procurement and processing.* We collected blood samples for ELISA of CA125, estradiol (E2), and progesterone (P) levels at three different phases of the menstrual cycle: menses (T1), follicular (T2; on Day 10  $\pm$  3 days of the cycle),

and luteal (T3; on Day 20  $\pm$  3 days of the cycle). We transported these samples to a Clinical Laboratory Improvement Amendment (CLIA)-approved facility within 12 hr of collection. We transported whole blood samples collected at the same time points to a research laboratory for additional CA125 analysis. We isolated plasma and placed aliquots in  $-80^{\circ}\text{C}$  storage within 48 hr of the blood draw.

### Measures

**CA125 determination.** We determined CA125 levels using two common commercial CA125 assays: Panomics CA125 EIA assay and the Siemens CA125 ELISA assay. We chose these assays based on their analytic precision and because they are two of the most common assays currently in clinical use. Of 35 possible assays, these two commercial assays had the smallest published coefficients of variation (3% and 7%, respectively) as well as the smallest intraassay and interassay variability. The Siemens CA125 ELISA assay (cat. no. 01678114) was completed in the CLIA-approved facility. The Panomics CA125 EIA assay (cat. no. BC-1013) was completed in a non-CLIA-approved research laboratory.

The Siemens CA125 assay was run in quadruplicate with any outliers removed. Both assays were performed according to the manufacturers' instructions. Samples measured using the Panomics EIA CA125 assay were assayed in triplicate. The reference values were  $<35$  U/ml and  $<21$  U/ml for the Siemens and Panomics assays, respectively. For the Siemens assay, the intraassay coefficient of variation (COV) was 3–7%. For the Panomics assay, the intraassay COV was 11.3–15.2% with a mean COV of 13.1%. Interassay COV ranged from 12.5 to 14.9% with a mean COV of 13.7%.

Two of the standard dilutions used in the Panomics assay with known CA125 values of 15 U/ml and 50 U/ml were run using the Siemens assay. Mean CA125 values reported using the Siemens assay were 13 U/ml and 52 U/ml. Standard dilutions from the Siemens assay were not available from the CLIA laboratory to run on the Panomics assay.

**E2 determination.** Serum E2 levels were determined using the Abbott Architect chemiluminescent immunoassay in the CLIA-approved facility. We drew E2 levels to confirm the participants' phase in the menstrual cycle. Serum E2 levels slowly increase from 100 pg/ml up to 400 pg/ml during the follicular phase and fall to less than 100 pg/ml in the first days of the luteal phase (Speroff & Fritz, 2005).

If E2 levels at T2 were not between 50 and 450 pg/ml, we instructed participants to maintain their calendar for an additional month. Similarly, if E2 levels at T3 were less than 10 pg/ml, we instructed participants to maintain their calendar for an additional month and repeat the lab work the next month. Two participants needed to maintain the calendar for an additional month due to low E2 at T2.

**P determination.** Serum P levels were determined using the Roche Modular E170 electro-chemiluminescent immunoassay

**Table 1.** Demographic Characteristics of Study Participants ( $N = 60$ )

Characteristic	Mean $\pm$ SD	Range
Age (years)	32.0 $\pm$ 4.5	21–39
Age at menarche (years)	12.6 $\pm$ 2.0	8–19
Height (inches)	64.7 $\pm$ 2.6	58.5–70.0
Weight (pounds)	139.0 $\pm$ 17.5	105–179
Body mass index (BMI) <sup>a</sup>	23.2 $\pm$ 2.4	17.5–29.2

<sup>a</sup> BMI calculated as  $\text{kg}/\text{m}^2$ .

in a CLIA-approved laboratory. We drew P levels to confirm the participants' phase in the menstrual cycle. The P levels are 0.3–1 ng/ml in the follicular phase, between 6 and 20 ng/ml during the luteal phase, and are less than 1 ng/ml during menses (Frasier, Jansen, Lobo, & Whitehead, 1998).

If P levels at T1 were greater than 1 ng/ml, we instructed participants to keep the calendar for an additional month and repeat the lab work the next month. If P levels at T2 or T3 were not between 1 and 28 ng/ml, we instructed participants to keep the calendar an additional month and repeat the lab work the next month. Two participants needed to maintain the calendar for an additional month due to elevated P values at T1. These participants were not the same individuals with abnormal E2 values.

### Statistical Analyses

We analyzed data using SPSS version 13.0 (IBM; <http://www-01.ibm.com/software/analytics/spss/downloads/>) and Stata version 9 (Stata; <http://www.stata.com>). We generated descriptive statistics on demographic characteristics and biomarkers. We determined menstrual cycle length for each participant and confirmed phase of cycle using E2 and P levels. We used random effects negative binomial regression to evaluate for changes in CA125 over the course of the menstrual cycle and paired *t*-tests to compare mean values of CA125 obtained from the two assays at each point in the menstrual cycle. We considered a *p*-value of  $<.05$  to be statistically significant.

## Results

### Demographic Characteristics

Table 1 shows demographic characteristics of the participants. Only 15 participants (25%) had ever been pregnant. Menstrual cycle length ranged from 25 to 35 days (mean = 28 days,  $SD = 1$  day) and bleeding during menses ranged from 3 to 9 days (mean = 5 days,  $SD = 1.5$  days). Over half of the women (56.7%) reported a medium flow, while 20% described their flow as light, 11.7% described their flow as heavy, and 11.7% stated that their flow varied from month to month. On average, 25% of the women reported spotting (requiring a panty liner) at some time during the month other than menses. Less than half (42%) of the women used NSAIDs for menstrual pain.

**Table 2.** Serum Values for CA125, Estradiol, and Progesterone During the Phases of the Menstrual Cycle

Variable/Time	N	Mean	SD	Minimum	Maximum	% above cutoff
<b>Estradiol (pg/ml)</b>						
T1	60	43.4	26.7	10	189	
T2	60	93.5	69.8	50	430	
T3	59	90.7	42.3	10	226	
<b>Progesterone (ng/ml)</b>						
T1	60	0.85	1.0	0.1	1.0	
T2	60	1.4	2.4	1.2	12.0	
T3	59	5.8	5.5	1.2	26.3	
<b>CA125 Siemens (U/ml)</b>						
T1	60	15.9	8.2	6	37	2
T2	60	12.2	5.4	5	34	0
T3	59	12.2	5.7	6	40	3
<b>CA125 Panomics (U/ml)</b>						
T1	60	27.2	13.5	8	99	19
T2	60	22.7	12.4	0	86	9
T3	59	22.7	12.9	5	67	11

Note. SD = standard deviation; T1 = menses (Day 1 of bleeding–Day 7); T2 = follicular (on Day 10 ± 3 days); T3 = luteal (on Day 20 ± 3 days); U = Units.

**Changes in E2 and P Across the Menstrual Cycle**

We have summarized E2 and P levels at each phase of the menstrual cycle in Table 2. We used random effects negative binomial regression to determine the change in E2 and P across the menstrual cycle. As shown in Table 3 and illustrated in Figure 1, E2 and P levels changed significantly across the menstrual cycle.

**Changes in CA125 Levels Across the Menstrual Cycle**

As shown in Table 2, mean values of CA125 using the Siemens assay ranged from 12.2 to 15.9 U/ml. The CA125 levels using the Panomics assay ranged from 22.7 to 27.2 U/ml. The percentage of participants above the reference values (i.e., CA125 values of >35 U/ml and >21 U/ml using the Siemens and Panomics assays, respectively) was highest (i.e., 19%) at T1 using the Panomics assay, which represented a third of the sample.

As shown in Table 3 and illustrated in Figure 1 for both assay systems, we found significant changes in CA125 levels across the menstrual cycle. When we transformed these data back to the original CA125 scale (U/ml), for every day of the menstrual cycle from menses to the start of the next cycle, CA125 decreased by 0.2 units using both assays. In other words, CA125 levels decreased 5.8 U/ml from the start of one menstrual cycle to the last day of that cycle.

**Differences in CA125 Between the Two assay Systems at Each Time Point in the Menstrual Cycle**

As shown in Figure 2, using paired *t*-tests, we found significant differences in CA125 levels between the two assay systems at

**Table 3.** Random Effects Negative Binomial Regression Models for Estradiol, Progesterone, and CA125 Across the Menstrual Cycle (in days)

Variable	Coef.	SE	Z	95% Confidence Interval		p Value
<b>E2</b>						
Days	0.016	0.004	3.85	0.008	0.024	.0001
Const.	0.936	0.128	7.34			
<b>P</b>						
Days	0.063	0.0129	4.86	0.038	0.088	.0001
Const.	-0.292	0.1943	-1.52			
<b>Siemens CA125</b>						
Days	-0.013	0.025	-5.19	-0.181	-0.0081	.0001
Const.	16.683	561.42	0.03			
<b>Panomics CA125</b>						
Days	-0.007	0.003	-2.13	-0.014	-0.0006	.03
Const.	2.556	0.200	12.73			

Note. Coef = coefficients (log scale); Const = constant; E2 = estradiol; P = progesterone; SE = standard error.

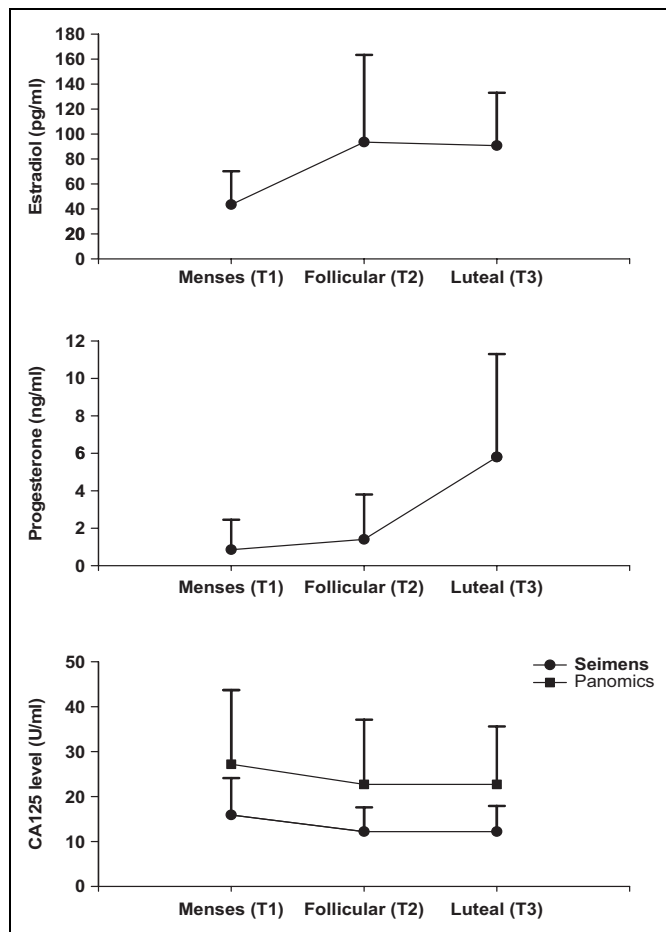
each phase of the menstrual cycle. At every time point, CA125 levels were lower (average of 12 U/ml) using the Siemens compared to the Panomics assay.

**Discussion**

Consistent with previous reports (Bon et al., 1999; Kafali et al., 2007; Kan et al., 1992; Lehtovirta et al., 1990; Meden & Fattahi-Meibodi, 1998), serum levels of CA125 in this sample of healthy women fluctuated across the menstrual cycle, with the highest levels found during menses. Changes in E2 and P values over time were also consistent with previous reports (Speroff & Fritz, 2005). The use of negative binomial regression to account for the unexplained between-subject differences in CA125 levels allowed for more precise estimates of the changes in CA125 levels over time.

Investigators have derived prior data on changes in CA125 across the menstrual cycle from studies of perimenopausal women or women with infertility problems (Bon et al., 1995, 1999; Crump et al., 2000; Kafali et al., 2007; Pauler et al., 2001), women who underwent tubal ligation or hysterectomy for benign disease (Bon et al., 1995, 1999; Kafali et al., 2007), or women at high risk for ovarian cancer (Johnson et al., 2008; Pauler et al., 2001), all conditions that are known contribute to CA125 variability. This study is the first to enroll a homogeneous cohort of healthy community-based women who met strict inclusion and exclusion criteria for study participation that controlled for these factors.

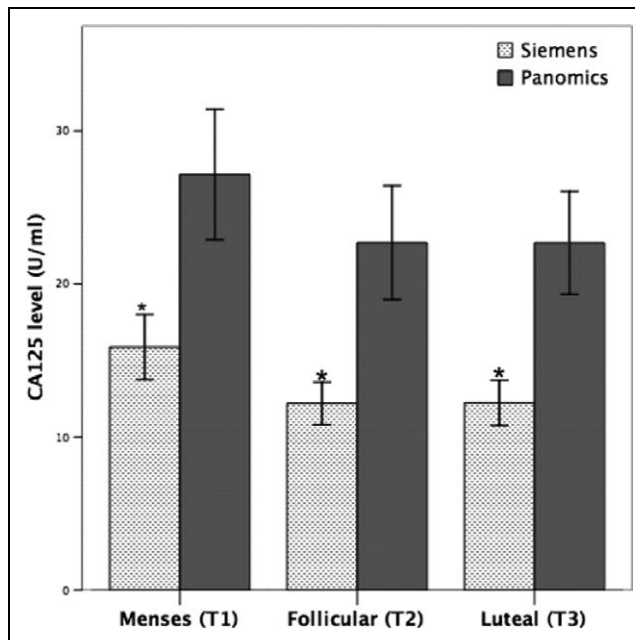
Mean CA125 values using both assays fluctuated in a narrow range under the reference values of <35 U/ml and <21 U/ml (for the Siemens and Panomics assays, respectively). However, the percentage of participants (19%) with CA125 values above the reference range of <21 U/ml at T1 using the Panomics assay is higher than the reported percentages of



**Figure 1.** Change over the phases of the menstrual cycle in serum levels of estrogen (pg/ml), progesterone (ng/ml), and CA125 (units/ml). All values were plotted as mean  $\pm$  standard deviation.

5.2–13.5% in previous studies (Kenemans et al., 1993; van Kamp et al., 1993). Three possible explanations for these findings exist. First, most participants in this study with values above 21 U/ml using the Panomics assay had CA125 values of 40 U/ml or less, with only one participant having a value of 99 U/ml, which skewed the group mean percentage. Second, the coefficients of variation for the Panomics assay were five-fold higher than for the Siemens assay, indicating wider intraassay variability. Finally, the Siemens assay was run in quadruplicate, and the reference lab reported mean values for three results. In contrast, the Panomics assay was run in triplicate and mean values for two results were reported when outliers were present.

The amount of change in CA125 over time (i.e., 0.2 U/ml per day) was identical using both assays, which suggests that despite the differences in reference values (<35 U/ml vs. <21 U/ml) the relative changes in CA125 are consistently measured regardless of assay system. This observation is consistent with the similar CA125 values obtained when the Panomics kit standard dilutions of 15 U/ml and 50 U/ml were run on the Siemens assay, with reported results of 13 U/ml and 52 U/ml, respectively. This observation is important because the assays used



**Figure 2.** Differences in mean CA125 values by assay system at each phase of the menstrual cycle. All values were plotted as means  $\pm$  standard deviations. \* $p < .0005$ .

in this study employ different epitopes (i.e., antigenic regions) recognized by antibodies (Nustad, Onsrud, Jansson, & Warren, 1998). The Siemens assay uses the OC125 antibody and Panomics uses the M11 antibody. However, without the reciprocal data (i.e., Siemens standards run on the Panomics kit), it is not possible to determine which kit is more accurate in determining serum CA125 levels.

Previous studies have reported varying relative differences in CA125 values using different assay systems with different epitope-antibody recognition sites (Bonfrer et al., 1994; Clement et al., 1995; Davelaar et al., 2003; Hornstein, Goodman, Thomas, Knapp, & Harlow, 1996; Kobayashi, Tamura, Satoh, & Terao, 1993; Lehtovirta et al., 1990; van Kamp et al., 1993; Yan et al., 1999). All of these previous studies used first-generation kits for CA125 that are no longer in use. In three of them (Davelaar et al., 2003; Kobayashi et al., 1993; Yan et al., 1999) investigators reported higher relative differences in CA125 than what we found. In five of these studies (Bonfrer et al., 1994; Clement et al., 1995; Hornstein et al., 1996; Lehtovirta et al., 1990; van Kamp et al., 1993) investigators reported relative differences of CA125 using multiple assay systems that were similar to the patterns we observed. However, the findings from the present study suggest that menstrual cycle and kit variability in CA125 levels persist even when the participants are carefully screened to include only women who are expected to show minimal fluctuations in CA125 levels.

Finally, controversy exists in the CA125 literature about appropriate reference ranges for healthy premenopausal women. Several studies have suggested that the upper limit for CA125 be increased to 65 U/ml in healthy premenopausal women, particularly if phase of the menstrual cycle is known (Bon

et al., 1995; Crump et al., 2000; Nguyen, Jacobson, & Patino-Paul, 1998; Pauler et al., 2001). All of these studies have included mixed samples of premenopausal and postmenopausal women, used single CA125 assays that were different than the ones used in our current study and are no longer in clinical use, and none controlled for factors known to contribute to fluctuations in CA125 levels.

The relative differences in CA125 levels observed in three of these studies (Bon et al., 1995; Crump et al., 2000; Nguyen et al., 1998) are similar to that found in our data. Crump and colleagues reported mean CA125 values of 24.7 U/ml, a median value of 16 U/ml, and a maximum value of 3600 U/ml. Nguyen and colleagues reported mean CA125 values of 19.3 U/ml ( $SD \pm 15.6$  U/ml) in premenopausal women and observed a statistically significant difference between CA125 values at menses ( $21.4 \pm 19.3$  U/ml) and T2, where mean values were  $14.0 \pm 9.1$  U/ml. Bon and colleagues reported mean CA125 values of 16 U/ml, with a median value of 13 U/ml and a maximum value of 113 U/ml. Despite the statistically significant decreases observed in CA125 from menses in all of these studies, including ours, we can only conclude that the menstrual cycle does not appear to be a strong modifier of CA125 levels in healthy women, given that these fluctuations generally occur within the normal reference ranges for each assay. Findings from this study need to be replicated in larger samples across several menstrual cycles to confirm the 25% decrease in CA125 levels at menses before any recommendations can be made about changes in “normal” reference ranges.

## Limitations

We need to acknowledge three limitations. First, we ran only two commercial assays, neither of which was used in previous studies. This difference does not allow for a direct comparison of relative CA125 results. However, we chose second-generation assays using similar antibodies with comparable COV values and analytical precision. In addition, the assays we examined herein are the two most common assays employed in research and clinically for the monitoring of CA125 in ovarian cancer patients. Second, while our sample size had sufficient power to detect changes in CA125 levels over time and differences between the two assays, investigators should run future samples in quadruplicate to ensure a similar number of data points and to minimize the impact of outliers. Third, these findings cannot be generalized to more heterogeneous samples because of this study's inclusion and exclusion criteria.

## Conclusion

The use of CA125 as a part of a comprehensive screening approach for ovarian cancer would be ideal if the sensitivity and specificity of single and serial measurements were improved. Knowledge of usual patterns of CA125 levels in healthy women is vital to understanding and interpreting these values. Additional research is warranted to determine the appropriate cutoff values for CA125 in healthy women based on multiple assay platforms.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The University of California, San Francisco, Alumni Association for the grant to support the purchase of reagents and lab supplies and the Alpha Eta chapter of Sigma Theta Tau for financial contribution to support the purchase of gift cards for the participants of the study.

## References

- Andersen, M. R., Goff, B. A., Lowe, K. A., Scholler, N., Bergan, L., Drescher, C. W., . . . Urban, N. (2008). Combining a symptoms index with CA125 to improve detection of ovarian cancer. *Cancer, 113*, 484-489.
- Bast, R. C. Jr., Badgwell, D., Lu, Z., Marquez, R., Rosen, D., Liu, J., & . . . Lu, K. (2005). New tumor markers: CA125 and beyond. *International Journal of Gynecologic Cancer, 3*, 274-281.
- Bon, G. G., Kenemans, P., Dekker, J. J., Hompes, P. G., Verstraeten, R. A., van Kamp, G. J., & Schoemaker, J. (1999). Fluctuations in CA 125 and CA 15-3 serum concentrations during spontaneous ovulatory cycles. *Human Reproduction, 14*, 566-570.
- Bon, G. G., Kenemans, P., Verstraeten, R. A., van Kamp, G. J., & Hilgers, J. (1995). Serum tumor marker immunoassays in gynecologic oncology: Establishment of reference values. *American Journal of Obstetrics and Gynecology, 174*, 107-114.
- Bonfrer, J. M. G., Baan, A. W., Jansen, E., Lentfer, D., & Kenemans, P. (1994). The technical evaluation of the three second generation CA125 assays. *European Journal of Clinical Chemistry and Clinical Biochemistry, 32*, 201-207.
- Bonfrer, J. M. G., Korse, C. M., Verstraeten, R. A., van Kamp, G. J., Hart, G. A., & Kenemans, P. (1997). Clinical evaluation of the Byk LIA-mat CA125 II assay: Discussion of a reference value. *Clinical Chemistry, 43*, 491-497.
- Buys, S. S., Partridge, E., Green, M. H., Prorok, P. C., Reding, D., & Riley, T. L. . . . PLCO Project Team. (2005). Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: Findings from the initial screen of a randomized trial. *American Journal of Obstetrics and Gynecology, 193*, 1630-1639.
- Clement, M., Bischof, P., Gruffat, C., Ricolleau, G., Auvray, E., Quillien, V., . . . Ferdeghini, M. (1995). Clinical validation of the new ELSA-CA 125 II assay: Report of a European multicentre evaluation. *International Journal of Cancer, 60*, 199-203.
- Crump, C., McIntosh, M. W., Urban, N., Anderson, G., & Karlan, B. Y. (2000). Ovarian cancer tumor marker behavior in asymptomatic healthy women: Implications for screening. *Cancer Epidemiology, Biomarkers, and Prevention, 9*, 1107-1111.
- Davelaar, E. M., Schutter, E. M., von Mensdorff-Pouilly, S., van Kamp, G. J., Verstraeten, R. A., & Kenemans, P. (2003). Clinical and technical evaluation of the ACS: OV serum assay and comparison with three other CA125-detecting assays. *Annals of Clinical Biochemistry, 40*, 663-673.

- Davelaar, E. M., van Kamp, G. J., Verstraeten, R. A., & Kenemans, P. (1998). Comparison of seven immunoassays for the quantification of CA 125 antigen in serum. *Clinical Chemistry*, 44, 1417-1422.
- Duffy, M. J., Bonfrer, J. M., Kulpa, J., Rustin, G. J., Soletormos, G., Torre, G. C., ... Zwirner, M. (2005). CA125 in ovarian cancer: European group on tumor markers guidelines for clinical use. *International Journal of Gynecologic Cancer*, 15, 679-691.
- Fehring, R. J., Schneider, M., & Raviele, K. (2006). Variability in the phases of the menstrual cycle. *Journal of Obstetrics, Gynecology, and Neonatal Nursing*, 35, 376-384.
- Fisken, J., Leonard, R. C., & Roulston, J. E. (1989). Immunoassay of CA125 in ovarian cancer: A comparison of three assays for use in diagnosis and monitoring. *Disease Markers*, 7, 61-67.
- Frasier, I. S., Jansen, R. P. S., Lobo, R. A., & Whitehead, M. I. (1998). *Estrogens and progesterones in clinical practice* (3rd ed.). Philadelphia, PA: Churchill Livingstone/Harcourt Brace.
- Hornstein, M. D., Goodman, H. M., Thomas, P. P., Knapp, R. C., & Harlow, B. L. (1996). Use of a second-generation CA125 assay in gynecologic patients. *Gynecologic and Obstetric Investigation*, 42, 196-200.
- Jacobs, I., & Bast, R. C. (1989). The CA125 tumour-associated antigen: A review of the literature. *Human Reproduction*, 4, 1-12.
- John, E. M., Whittemore, A. S., Harris, R., & Intyre, J. (1993). Characteristics relating to ovarian cancer risk: Collaborative analysis of seven US case-control studies: Epithelial ovarian cancer in black women. *Journal of the National Cancer Institute*, 85, 142-146.
- Johnson, C. C., Kessel, B., Riley, T. L., Ragard, L. R., Williams, C. R., & Xu, J-L, ... PLCO Project Team. (2008). The epidemiology of CA125 in women without evidence of ovarian cancer in the prostate, lung, colorectal and ovarian cancer (PLCO) screening trial. *Gynecologic Oncology*, 110, 383-389.
- Kafali, H., Artunc, H., & Erdem, M. (2007). Evaluation of factors that may be responsible for cyclic changes of CA125 levels during menstrual cycle. *Archives of Gynecology and Obstetrics*, 275, 175-177.
- Kan, Y. Y., Yeh, S. H., Ng, H. T., & Lou, C. M. (1992). Effect of menstruation on serum CA125 levels. *Asia Oceania Journal of Obstetrics and Gynaecology*, 18, 339-343.
- Kenemans, P., van Kamp, G. J., Oehr, P., & Verstraeten, R. A. (1993). Heterologous double-determinant immunoradiometric assay CA125 II: Reliable second-generation immunoassay for determining CA125 in serum. *Clinical Chemistry*, 39, 2509-2513.
- Kenemans, P., Verstraeten, A. A., van Kamp, G., & Mensdorff-Pouilly, S. (1995). The second generation CA125 assays. *Annals of Medicine*, 27, 107-113.
- Kobayashi, H., Tamura, M., Satoh, T., & Terao, T. (1993). Clinical evaluation of new cancer-associated antigen CA125 II in epithelial ovarian cancers: Comparison with CA125. *Clinical Biochemistry*, 26, 213-219.
- Lee, K., Shaver, J. F., Giblin, E. C., & Woods, N. F. (1990). Sleep patterns related to menstrual cycle phase and premenstrual affective symptoms. *Sleep*, 13, 403-409.
- Lehtovirta, P., Apter, D., & Stenman, U-H. (1990). Serum CA125 levels during the menstrual cycle. *British Journal of Obstetrics and Gynecology*, 97, 930-933.
- Martin, M., & Blockx, P. (1997). Comparison of one first and three second generation methods for the determination of CA 125. *Anticancer Research*, 17, 3171-3175.
- McLemore, M. R., Miaskowski, C., Aouizerat, B. E., Chen, L. -M., & Dodd, M. J. (2009). Epidemiological and genetic risk factors associated with ovarian cancer. *Cancer Nursing*, 32, 281-288.
- Meden, H., & Fattahi-Meibodi, A. (1998). CA 125 in benign gynecological conditions. *International Journal of Biological Markers*, 13, 231-237.
- National Cancer Institute (NCI). (n.d.). *Ovarian cancer research results from the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial: Fact sheet*. Retrieved August 12, 2008, from <http://previewqa.cancer.gov/cancertopics/factsheet/detection/plco-ovarian>
- Nguyen, H. N., Jacobson, A., & Patino-Paul, R. (1998). New reference levels for CA125 in pre-and postmenopausal women. *Primary Care Update for OB/GYNs*, 5, 157.
- Nustad, K., Onsrud, M., Jansson, B., & Warren, D. (1998). CA125-epitopes and molecular size. *International Journal of Biological Markers*, 13, 196-199.
- Pauler, D. K., Menon, U., McIntosh, M., Symecko, H. L., Skates, S. J., & Jacobs, I. J. (2001). Factors influencing serum CA125II levels in healthy postmenopausal women. *Cancer Epidemiology, Biomarkers, and Prevention*, 10, 489-493.
- Rodriguez, C., Calle, E. E., Fakhrabadi-Shokoohi, D., Jacobs, E. J., & Thun, M. J. (2002). Body mass index, height, and the risk of ovarian cancer mortality in a prospective cohort of postmenopausal women. *Cancer Epidemiology, Biomarkers, and Prevention*, 11, 822-828.
- Rubin, S. C., & Sutton, G. P. (2004). *Ovarian cancer* (2nd ed.). Philadelphia, PA: Lippincott, Williams & Wilkins.
- Runnebaum, I. B., & Stickeler, E. (2001). Epidemiological and molecular aspects of ovarian cancer risk. *Journal of Cancer Research and Clinical Oncology*, 127, 73-79.
- Speroff, L., & Fritz, M. A. (2005). *Clinical gynecologic endocrinology and infertility* (7th ed.). Philadelphia, PA: Lippincott, Williams & Wilkins.
- van Kamp, G. J., Verstraeten, A. A., & Kenemans, P. (1993). Discordant serum CA125 values in commercial immunoassays. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 49, 99-103.
- Yan, G., Ju, H., Liang, Z., & Zhang, T. (1999). Technical and clinical comparison of two fully automated methods for the immunoassay of CA125 in serum. *Journal of Immunological Methods*, 225, 1-8.
- Yedema, K. A., Thomas, C. M. G., Segers, M. F. G., Doesburg, W. H., & Kenemans, P. (1992). Comparison of five immunoassay procedures for the ovarian carcinoma-associated antigenic determinant CA125 in serum. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 47, 245-251.