UCSF

UC San Francisco Previously Published Works

Title

Differences in the Molecular Species of CA125 Across the Phases of the Menstrual Cycle.

Permalink

<https://escholarship.org/uc/item/25f4q0fv>

Journal

Biological research for nursing, 18(1)

ISSN

1099-8004

Authors

McLemore, Monica R
Miaskowski, Christine
Lee, Kathryn
et al.

Publication Date

2016

DOI

10.1177/1099800414565879

Peer reviewed

Differences in the Molecular Species of CA125 Across the Phases of the Menstrual Cycle

Monica R. McLemore, RN, MPH, PhD¹,
Christine Miaskowski, RN, PhD, FAAN¹,
Kathryn Lee, RN, PhD, FAAN, CBSM¹,
Lee-may Chen, MD^{1,2}, and Bradley E. Aouizerat, MAS, PhD¹

Abstract

Background: CA125, a tumor-associated antigen, is primarily used to monitor epithelial ovarian cancer. There is evidence that different species of CA125 exist; however, it is not known if any of these species are present in healthy women during the menstrual cycle and if they are associated with serum concentrations of CA125. The purpose of this study was to determine if the molecular species of CA125 differ across the three phases of the menstrual cycle in healthy women. **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 Western blot analysis. **Results:** Western blot analysis yielded 17 distinct profiles (i.e., patterns of species) of CA125, with 80% of the sample exhibiting 5 common profiles. No differences in demographic characteristics and serum CA125 values were found among the various CA125 profiles. **Conclusions:** Different molecular species of CA125 exist in healthy women with regular menstrual cycles. These data provide evidence that CA125 is not a homogeneous molecular species. Future research should evaluate the molecular composition and the clinical importance of these species.

Keywords

CA125, molecular speciation, menstrual cycle

Molecular speciation is the classification of distinct groups of similar entities (King & Stansfield, 2002). Members of a distinct molecular species must have at least one common precursor (Tiollais, Galibert, & Boiron, 1971), exhibit differences in electrophoretic mobility from other species (Tiollais et al., 1971), show different responses to the same experimental conditions as compared to members of other species (Stewart et al., 1975), or contain a different number of residues or nucleotides from other species (Stewart et al., 1975; Tiollais et al., 1971). These original criteria continue to be valuable for current molecular speciation studies (Andersch-Bjorkman, Thomsson, Holmen-Larsson, Ekerhovd, & Hansson, 2007; Bouanène et al., 2012; Jankovic & Milutinovic, 2008; Jankovic & Tapuskovic, 2005; Nustad, Onsrud, Jansson, & Warren, 1998; Saldova et al., 2013).

Molecular speciation studies of prostate-specific antigen (PSA) have allowed clinicians and researchers to distinguish which species of PSA are associated with prostate cancer, benign conditions of the prostate, or normal metabolic changes associated with aging (Meany, Zhang, Sokoll, Zhang, & Chan, 2009). PSA values were rescaled when the different molecular species of PSA (i.e., PSA, free PSA, and percent free PSA) were identified in the serum of healthy men and survivors of

prostate cancer. The primary objective in rescaling PSA values was to decrease the number of unnecessary prostate biopsies and diagnostic scans, as well as to decrease anxiety in men with elevated PSA levels that were reflective of benign conditions (Gelman et al., 2001).

CA125 is a tumor-associated antigen used to monitor epithelial ovarian cancer (OC; Bast et al., 2005; Bast & Spriggs, 2011). However, the molecular species of CA125 are not well defined (Weiland, Martin, Oehler, & Hoffmann, 2012). CA125 is secreted by all of the tissues of the female reproductive tract as a large glycoprotein and is degraded by proteolysis during transport throughout the circulatory system (Bidart et al., 1999; Hollingsworth & Swanson, 2004; Jankovic & Milutinovic, 2008; Maeda et al., 2004; Nustad et al., 1998).

¹ University of California, San Francisco, CA, USA

² Helen Diller Family Comprehensive Cancer Center, San Francisco, CA, USA

Corresponding Author:

Monica R. McLemore, University of California, San Francisco, 2 Koret Way, N431H, San Francisco, CA 94143.

Email: Monica.mclemore@ucsf.edu

Tumor-derived CA125 is degraded in serum, ascites, and the supernatant of tumor cell lines, which results in subunits of varying sizes (Bouanène et al., 2012; Jacobs & Bast, 1989; Nustad et al., 1998). In laboratory studies, researchers have observed that the oligosaccharides of tumor-derived and pregnancy-associated CA125 are added to the peptide core of CA125 as posttranslational modifications, which results in differences in size and glycosylation profiles (Biskup et al., 2013; Chen et al., 2013; Saldova et al., 2013;). *MUC16*, the gene that encodes for the peptide core of CA125 (O'Brien et al., 2001; O'Brien, Beard, Underwood, & Shigemasa, 2002), has been observed to be alternatively spliced in OC tumor samples, ascites, and supernatant from tumor cell lines (Maeda et al., 2004; O'Brien et al., 2001, 2002; Yin & Lloyd, 2001).

Serum CA125 levels fluctuate during the menstrual cycle in healthy women, with the highest levels observed during menses (Bon, Kenemans, Verstraeten, van Kamp, & Hilgers, 1995; Bouanène et al., 2012; Kafali, Artunc, & Erdem, 2007; McLemore et al., 2012). We hypothesized that these fluctuations in CA125 levels might be due in part to different species being present at each phase of the menstrual cycle. Despite evidence that different species of CA125 exist in OC and pregnancy (Bidart et al., 1999; Hamilton et al., 2002; Jankovic & Tapuskovic, 2005; Maeda et al., 2004; Nustad et al., 1998), it is not known if these species are (1) present in healthy women, (2) present at specific time points in the menstrual cycle, or (3) associated with serum concentrations of CA125. Therefore, the purpose of the present study was to determine whether the molecular species of CA125 differ across the three phases of the menstrual cycle within and across healthy women with regular menstrual cycles and to evaluate for differences in demographics and menstrual cycle characteristics as well as serum levels of CA125 by CA125 profile.

Materials and Methods

Study Design and Participants

In a previous study examining changes in CA125 over time (McLemore et al., 2012), our power analysis showed that 60 participants were sufficient. We used data from these same 60 participants for this analysis. We enrolled healthy, Caucasian women between the ages of 18 and 39 using strict criteria to exclude factors known to contribute to fluctuations in serum CA125. Specifically, participants were excluded if they (1) had a previous personal history of ovarian, endometrial, lung, or colorectal cancer; (2) had a first-degree relative with a history of ovarian, endometrial, lung, or colorectal cancer; (3) were pregnant or had been postpartum for less than 6 months, were lactating, or had an abortion or miscarriage within the last 3 months; (4) had endometriosis, ovarian cysts, polycystic ovarian syndrome (PCOS), or a recent history of pelvic inflammatory disease (PID); (5) were participating in infertility treatment or ovum donation currently and were on hyperstimulatory medications or gonadotropins; (6) had severe premenstrual syndrome/premenstrual dysphoric disorder; (7) had chronic

illnesses requiring routine nonsteroidal anti-inflammatory medications (NSAIDs); (8) were using hormonal contraceptives; (9) were taking herbal supplements (i.e., black cohosh, red clover). The Institutional Review Boards at the University of California, San Francisco, and San Francisco General Hospital (SFGH) approved all procedures, and eligible participants provided written informed consent.

We determined menstrual cycle regularity using calendars that participants maintained for 3 months. After we established cycle regularity, we collected blood samples for Western blot analysis of CA125 at three different phases of the menstrual cycle: menses (T_1), follicular (T_2 ; on Day 10 of the cycle, ± 3 days), and luteal (T_3 ; on Day 20 of the cycle, ± 3 days) and transported them to the research laboratory. The same member of the research team (M.M.) collected all of the blood samples at locations convenient to participants (primarily at their homes or places of employment). Participants notified the researcher by phone at the start of menstruation and set up appointments for the T_2 and T_3 blood draws.

We obtained CA125 enzyme-linked immunosorbent assay (ELISA) values for all samples using a common commercially available assay (Seimens, catalog number 01678114). Plasma was isolated and aliquots were placed in -80°C storage within 48 hr of the blood draw. We obtained plasma samples from an OC patient with a CA125 ELISA value of 2,700 units per milliliter (U/ml) from the UCSF Cancer Center Ovarian Cancer Tumor Bank and used them as a positive control.

Measurements and Materials

We purchased the primary antibody (1° Ab), X75, from Novus Biologicals (Littleton, CO, cat. no. NB600-1468). The secondary Ab (2° Ab), conjugated to horseradish peroxidase for visualization, was a goat anti-mouse antibody purchased from Jackson ImmunoResearch Laboratories (West Grove, PA, cat. no. 115-035-062). We purchased Immobilon-P 45 μm polyvinylidene fluoride (PVDF) membranes from Millipore (Bedford, MA, cat. no. TM151-1), HiMark pre-stained high molecular weight protein standard (ladder) from Invitrogen (Carlsbad, CA, cat. no. L5699), and Super Signal West Femto electrochemiluminescence reagent from Thermo Scientific (Rockford, IL, cat. no. 34096).

Gel Electrophoresis and Immunoblotting. For each participant ($N = 60$) at each of the three time points, 0.5 μl of plasma was mixed with 2 μl of sample buffer and 5 μl of deionized water and heated for 30 min at 65°C . After 10 min of cooling under nonreducing conditions, sample mixtures were loaded onto the gel lanes in the following order: molecular weight standard, positive control sample, empty lane, Participant 1 sample at T_1 , empty lane, Participant 1 sample at T_2 , empty lane, Participant 1 sample at T_3 , empty lane until all lanes were filled.

We loaded 26-well, 3–8% gradient Tris-Acetate Criterion XT precast gels, XT-Tricine buffer, and 4X-Xt sample buffer for sample resolution (Bio-Rad, Hercules, CA, cat. no. 345-0131). Gels were subjected to electrophoresis at 100 volts until

the dye front exited the bottom of the gel (i.e., approximately 115 min). Gels were removed from cassettes and equilibrated in Tris-Glycine, 20% methanol (MeOH) transfer buffer for 10 min. Proteins were transferred to the PVDF membrane for 1.5 hr at 400 milliamps at 4°C. After labeling, the blots were immediately immersed in 100% MeOH and placed on filter paper to dry for a minimum of 30 min.

Blots were rehydrated prior to immunoblotting in 100% MeOH and rocked for 5 min in Tris-buffered magnesium chloride. Blots were blocked using 5% milk (generic powdered, non-fat) in 25 ml Tris-buffered saline (TBS) and 0.1% Tween. Blots were incubated with 1° Ab at 4°C overnight. The 1° Ab preparation was X75 at a 1:1850 dilution (i.e., 8.11 µl in 5% milk in 15 ml TBS and 0.1% Tween). After incubation, blots were washed quickly in TBS-0.1% Tween twice and then 3 times on a rocking apparatus for 5 min. Once washed, the blots were then incubated with the 2° Ab for 45 min at room temperature. The 2° Ab preparation was goat anti-mouse IgG at a 1:500,000 dilution in 5% milk in 25 ml TBS and 0.1% Tween. Blots were rinsed quickly in TBS and 0.1% Tween twice and then 3 times on a rocking apparatus for 15 min. Blots were immediately treated with electrochemiluminescent reagents per the manufacturer's instructions and were exposed for four different durations: 30 s, 1 min, 4 min, and 10 min.

Image Acquisition. The radiographic films of all autoradiogram exposures of the Western blots were scanned using an Epson Perfection 4990 flatbed photo scanner. The resolution for all scans was 300 dots per inch (DPI) and all files were imported into Fireworks 4™ software and converted into 24-bit, portable network graphic images (.png). Radiographic films were annotated using the ladder included on each blot, allowing the visible bands to be documented at a kilodalton (kDa) level resolution. All visible bands were included in the annotation, and the darkest (i.e., most abundant) band was chosen as the referent band for each film.

Profile Recognition and Counting. Scanned images were compared on screen using referent bands. Initial banding patterns were documented using the location of the referent band. An iterative process was used to determine distinct patterns, and a template schematic for each pattern was created (Figure 1). The primary investigator (M.M.) performed the initial profile assignment with final adjudication conducted by two investigators (M.M. and B.E.). This process was repeated until consensus was reached. Samples that represented the exemplars of each profile were selected and used as a referent to confirm assignment for each sample. Final adjudication of the profiles was conducted with the research team using the digital and printed images of each profile. The number of samples exhibiting a specific profile was counted.

Statistical Analyses

Data were analyzed using Stata Version 11. Descriptive statistics were generated on demographic characteristics. Frequencies for

each CA125 profile were calculated. Exploratory analysis of variance (ANOVA) was used to examine for differences in age, body mass index (BMI), menstrual cycle length, and prior pregnancy status by CA125 profile. Negative binomial regression models were used to examine for differences in mean CA125 levels at each time point by CA125 profile due to the skewed distribution of serum CA125 values and high dispersion. A *p* value of <.05 was considered statistically significant.

Results

Demographic Characteristics

As shown in Table 1, the mean age of the 60 participating women was 32 years (*SD* = 4.5 years). 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). The mean values of CA125 ranged from 12.2 U/ml to 15.9 U/ml.

Profiles of CA125 Species

As shown in Figure 1, Western blot analysis yielded 17 distinct CA125 profiles in healthy women with regular menstrual cycles, with 5 of these occurring in more than one participant. More than 80% of the participants exhibited one of these 5 profiles (i.e., A = 15 participants, B = 12 participants, C = 11 participants, D = 6 participants, and E = 4 participants). The 12 participants with rare profiles (Profile R) represent 20% of the overall sample (Figure 2).

Differences in Demographics and Serum CA125 Values by Profile

We conducted exploratory ANOVA, excluding Profiles E and R (*n* = 16 participants). We excluded Profile E due to the low number of participants (*n* = 4) and Profile R because each participant had a unique profiles (*n* = 12). Age at enrollment, age at menarche, BMI, menstrual cycle length, and ever-pregnant status did not differ significantly among Profiles A through D (Table 2). Additionally, within each profile, CA125 values did not differ significantly across menstrual phase (Table 3).

Discussion

In the present study, we used CA125 profiles, based on size in kDa, to identify molecular species. We identified 17 CA125 profiles using Western blot analysis in healthy women with regular menstrual cycles. Consistent with previous studies of molecular speciation of CA125, the species identified in our study exhibited reproducible differences in electrophoretic mobility. Also, consistent with previous studies that included Western blot analysis of CA125 (Andersch-Bjorkman et al., 2007; Jankovic & Tapuskovic, 2005; Kobayashi, Tamura, Satoh, & Terao, 1993; Nustad et al., 1998; O'Brien et al., 2001, 2002), our data show species ranging from 31 kDa to

Figure I. Profiles of the species recognized by OC125 antibody.

460 kDa. Most of the referent bands in our data appear at the level of 117 kDa or higher, indicating that CA125 may primarily be a higher molecular weight species in healthy women with regular menstrual cycles. Of note, only two participants had

changes in CA125 profiles across the menstrual cycle and they both had unique profiles. Within the five common profiles, CA125 appeared unchanged across the menstrual cycle. In other words, regardless of the phase of the menstrual cycle

Table 1. Demographic Characteristics of Study Participants.

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
Menstrual cycle bleeding (days)	5.0 $\pm$ 1.5	3–9
Menstrual cycle length (days)	28.0 $\pm$ 1.0	25–35
Height (in.)	64.7 $\pm$ 2.6	58.5–70.0
Weight (lb)	139.0 $\pm$ 17.5	105–179
BMI (kg/m ²)	23.2 $\pm$ 2.4	17.5–29.2
CA125 (units/ml)		
T ₁	15.9 $\pm$ 8.2	6–37
T ₂	12.2 $\pm$ 5.4	5–34
T ₃	12.2 $\pm$ 5.7	6–40

Note. *N* = 60. BMI = body mass index; T = time.

Figure 2. Frequency of CA125 profiles (*N* = 60). Each participant in Profile R had a unique pattern (*n* = 12).

(i.e., menses, follicular, and luteal), the profiles appeared identical. This finding suggests that the species of CA125 does not change over time associated with a single menstrual cycle. Our previous work examining the same cohort of women showed a statistically significant decrease of absolute serum concentration of CA125 from the menses to the follicular phases (McLemore et al., 2012). However, we did not detect a statistically significant difference in CA125 serum concentration by menstrual cycle phase in the current study. We speculate that there are two possible explanations for this finding. The first is that, while differences in the relative abundance of each species exist for each profile, the antibody detects all species and was not able to differentiate among the individual species. The second possibility is that the effects were more subtle than we were able to detect in the available sample size.

It is important to note two major differences between our data and other published studies that investigated the molecular species of CA125: the type of biospecimens we evaluated and the more stringent criteria we used to determine the sample. Investigators have used five tissue types for CA125 molecular

Table 2. Exploratory Analysis of Variance of Age, Body Mass Index (BMI), and Menstrual cycle Length by CA125 Profile.

Variable	Sum of squares	df	Mean square	F	Significance
Age at enrollment					
Between groups	166.48	5	33.29	1.78	.132
Within groups	1009.98	54	18.70		
Total	1176.46	59			
Age at menarche					
Between groups	9.42	5	1.84	.44	.821
Within groups	203.26	47	4.33		
Total	212.68	52			
BMI					
Between groups	16.66	5	3.33	.57	.726
Within groups	317.92	54	5.88		
Total	334.58	59			
Menstrual cycle length					
Month 1					
Between groups	4.50	5	0.90	.38	.861
Within groups	128.35	54	2.37		
Total	132.85	59			
Month 2					
Between groups	2.78	5	0.56	.34	.888
Within groups	89.15	54	1.65		
Total	91.93	59			
Month 3					
Between groups	8.19	5	1.64	.77	.576
Within groups	108.65	51	2.13		
Total	116.84	56			
Month 4					
Between groups	3.62	4	0.90	.48	.753
Within groups	13.30	7	1.90		
Total	16.92	11			

Table 3. Cross-Sectional Negative Binomial Regression Models of Serum CA125 Levels by CA125 Profile.

CA125 Profile	Observed coefficient	Bootstrap SE	z	p > (z)	95% Confident interval
T ₁					
Profile B	-.015	.196	-0.08	.937	[-.399, .369]
Profile C	.072	.198	0.36	.719	[-.317, .460]
Profile D	-.267	.193	-1.39	.165	[-.645, .109]
Profile E	-.336	.212	-1.58	.114	[-.753, .081]
T ₂					
Profile B	-.043	.147	-0.29	.769	[-.333, .246]
Profile C	-.036	.151	-0.24	.812	[-.332, .260]
Profile D	-.130	.219	-0.59	.552	[-.561, .300]
Profile E	-.161	.189	-0.85	.394	[-.532, .209]
T ₃					
Profile B	-.027	.122	-0.22	.823	[-.267, .212]
Profile C	-.267	.137	-1.95	.051	[-.535, .001]
Profile D	-.139	.184	-0.76	.450	[-.501, .222]
Profile E	-.274	.164	-1.67	.096	[-.596, .048]

Note. Wald χ^2 (df: 4): 6.36, *p* = .173, reference pattern: A.

speciation studies: ascites (Jacobs & Bast, 1989; Nustad et al., 2002; Whitehouse & Solomon, 2003), cell lines in culture available commercially for purchase (Nustad et al., 1998,

2002; Whitehouse & Solomon, 2003), serum or plasma from healthy women with or without OC but with no documentation of menstrual cycle (Bouanène et al., 2012; Jacobs & Bast, 1989; Nustad et al., 1998, 2002; Tuzun et al., 2009), tumor tissue (Bouanène et al., 2012; Jacobs & Bast, 1989; Nustad et al., 2002; Tuzun et al., 2009), and tissues of the female reproductive tract from healthy volunteers (i.e., cervical and/or endometrial samples, fetal and/or placental tissue; Andersch-Bjorkman et al., 2007; Jankovic & Tapuskovic, 2005; Nustad et al., 1998, 2002; Whitehouse & Solomon, 2003). CA125 obtained from these different tissue types has had wide variability in molecular weight using Western blotting methods, ranging from 750 to 15 kDa (Jacobs & Bast, 1989; Nustad et al., 1998, 2002; Whitehouse & Solomon, 2003).

In the present study, we used serum samples derived from human participants. Only three previous studies have used samples derived from healthy human participants (Andersch-Bjorkman et al., 2007; Bouanène et al., 2012; Jankovic & Tapuskovic, 2005). The most recent study (Bouanène et al., 2012) used serum from healthy women and patients with epithelial OC. The authors measured serum CA125 using enzyme immunoassay, but do not report the values. They do report abundant CA125 bands at 40 kDa; however, two participants had bands at >2,000 kDa. The next most recent study (Andersch-Bjorkman et al., 2007) used cervical mucus collected at different phases across the menstrual cycle, and the oldest study (Jankovic & Tapuskovic, 2005) obtained samples from first-trimester human placentas at between 6 and 12 weeks' gestation. While the authors did not report ELISA values for the cervical mucus samples, they did note distinct bands between 50 kDa and 300 kDa across all participants, and banding patterns changed within each woman across the menstrual cycle (Andersch-Bjorkman et al., 2007), where the menstrual cycle was confirmed using transvaginal ultrasound and midluteal-phase serum progesterone levels. The authors did report ELISA values for the placental samples, with an average value of 6,000 U/ml and a single broad band at 205 kDa (Jankovic & Tapuskovic, 2005). Our data include bands ranging from 41 kDa to 460 kDa. Despite the use of healthy human samples, each study used a different tissue type, which could explain the variation in the observed species.

Studies of the molecular species of CA125 have been difficult to interpret because of the heterogeneity of tissue types and selection criteria of those tissues included in previous studies. For example, researchers previously used ascites, which is abdominal fluid removed from OC patients during tumor reduction surgery, for molecular speciation studies (O'Brien et al., 2001, 2002). In their 2001 study, O'Brien and colleagues blotted the extracellular repeat region of CA125 using three antibodies (i.e., OC125, K95, and M11), which yielded similar bands of varying intensities all at 32 kDa. However, in the 1998 O'Brien study (O'Brien, Hanimoto, Konishi, & Gee, 1998), investigators blotted all of the isolated CA125 and observed bands at 200 kDa, between 60 and 55 kDa, and at 30 kDa. In both of these studies, the ascites was taken from patients with Stage 3 or 4 tumors, and the data were not stratified by stage

of tumor, which may contribute to the variation observed in the profiles of CA125.

Additionally, two studies examining the molecular species of CA125 used cell culture medium for CA125 analysis. The first study used media from the Henrietta Lacks (HeLa) cell line (the cells that differentiate into amnion are called the WISH cell line; Nustad et al., 1998). The researchers initially used gel filtration to separate CA125, which resulted in fractions of 750, 210, 55, and 15 kDa. This analysis showed banding patterns of CA125 above 200 kDa with no banding occurring under 200 kDa. The second study used cell culture media from the HOC-I cell line (Kobayashi et al., 1993). In the aggregate, these researchers found species with masses ranging from 30 kDa to 750 kDa. Given the multivalent nature of CA125 (Jankovic & Milutinovic, 2008; Jankovic & Tapuskovic, 2005; McLemore & Aouzierat, 2005; Nustad et al., 1998) and the documented variation of its measurement in serum (Andersch-Bjorkman et al., 2007; Bidart et al., 1999; Bon et al., 1995; Jankovic & Milutinovic, 2008; Wong et al., 2003), stringent selection criteria for the samples used for molecular speciation studies may provide crucial information about tissue-associated or tissue-specific CA125 species.

Limitations

Two limitations need to be acknowledged. First, we observed two of the patterns identified in this study (D and E) in 10 or fewer participant samples, which only allowed for exploratory statistical analyses. Second, it is unknown what the differences in the species represent and it remains to be determined whether these species have different compositions (e.g., residues, nucleotides, and amino acids) that result in different functions.

Conclusions

These data provide concrete evidence that CA125 is not a homogeneous molecular species in the serum of healthy women across the menstrual cycle. Future research needs to validate these observations and evaluate the clinical importance and molecular compositions of these species as well as the relationship between these species and the serum ELISA values of CA125.

Author Contribution

MML contributed to conception and design, acquisition, analysis, and interpretation; drafted and critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. CM contributed to conception and design, analysis, and interpretation; drafted and critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. KL contributed to design, analysis, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. L-mC contributed to the acquisition, analysis, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work

ensuring integrity and accuracy. BA contributed to conception and design, acquisition, analysis, and interpretation; drafted and critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

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

Funding

The author(s) 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

- Andersch-Bjorkman, Y., Thomsson, K. A., Holmen-Larsson, J. M., Ekerhovd, E., & Hansson, G. C. (2007). Large scale identification of proteins, mucins and their O-glycosylation in the endocervical mucus during the menstrual cycle. *Molecular & Cellular Proteomics*, 6, 708–716.
- Bast, R. C., 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.
- Bast, R. C., & Spriggs, D. R. (2011). More than a biomarker: CA125 may contribute to ovarian cancer pathogenesis. *Gynecologic Oncology*, 121, 429–430.
- Bidart, J. M., Thuillier, F., Augereau, C., Chalas, J., Daver, D., Jacob, N., . . . Voitot, H. (1999). Kinetics of serum tumor marker concentrations and usefulness in clinical monitoring. *Clinical Chemistry*, 45, 1695–1707.
- Biskup, K., Braicu, E. I., Sehouli, J., Fotopoulou, C., Tauber, R., Berger, M., & Blanchard, V. (2013). Serum glycome profiling: A biomarker for diagnosis of ovarian cancer. *Journal of Proteome Research*, 12, 4056–4063.
- 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.
- Bouanène, H., Saibi, W., Mokni, M., Sriha, B., Fatma, L. B., Limem, H. B., . . . Miled, A. (2012). Biochemical and morphological differences between CA125 isolated from healthy women and patients with epithelial ovarian cancer from Tunisian population. *Pathology and Oncology Research*, 18, 325–330.
- Chen, K., Gentry-Maharaj, A., Burnell, M., Steentoft, C., Marcos-Silva, L., Mandel, U., . . . Blixt, O. (2013). Microarray glycoprofiling of CA125 improves differential diagnosis of ovarian cancer. *Journal of Proteome Research*, 12, 1408–1418.
- Gelmann, E. P., Chia, D., Pinsky, P. F., Andriole, G. L., Crawford, E. D., & Reding, D., . . . PLCO Screening Trial Investigators. (2001). Relationship of demographic and clinical factors to free and total prostate-specific antigen. *Urology*, 58, 561–566.
- Hamilton, J. A., Iles, R. K., Gunn, L. K., Wilson, C. M., Lower, A. M., & Grudzinskas, J. G. (2002). High concentrations of CA 125 in uterine flushings: Influence of cause of infertility and menstrual cycle day. *Gynecologic Endocrinology*, 16, 19–25.
- Hollingsworth, M. A., & Swanson, B. J. (2004). Mucins in cancer: Protection and control of the cell surface. *Nature Reviews: Cancer*, 4, 45–60.
- Jacobs, I., & Bast, R. C. (1989). The CA125 tumour-associated antigen: A review of the literature. *Human Reproduction*, 4, 1–12.
- Jankovic, M. M., & Milutinovic, B. S. (2008). Glycoforms of CA125 antigen as a possible cancer marker. *Cancer Biomarkers*, 4, 35–42.
- Jankovic, M. M., & Tapuskovic, B. S. (2005). Molecular forms and microheterogeneity of the oligosaccharide chains of pregnancy-associated CA125 antigen. *Human Reproduction*, 20, 2632–2638.
- 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.
- King, R. C., & Stansfield, W. D. (2002). *A dictionary of genetics*. New York, NY: Oxford University Press.
- 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.
- Maeda, T., Inoue, M., Koshiba, S., Yabuki, T., Aoki, M., Nunokawa, E., . . . Yokoyama, S. (2004). Solution structure of the SEA domain from the murine homologue of ovarian cancer antigen CA125 (MUC16). *Journal of Biological Chemistry*, 279, 3174–3182.
- McLemore, M. R., & Aouizerat, B. E. (2005). Introducing the MUC16 gene: Implications for early detection in epithelial ovarian cancer. *Biological Research for Nursing*, 6, 262–267.
- McLemore, M. R., Aouizerat, B. E., Lee, K. A., Chen, L.-M., Tozzi, M., Cooper, B., & Miaskowski, C. (2012). A comparison of the cyclic variation in serum CA125 levels across the menstrual cycle using two commercial assays. *Biological Research for Nursing*, 14, 250–256.
- Meany, D. L., Zhang, Z., Sokoll, L. J., Zhang, H., & Chan, D. W. (2009). Glycoproteomics for prostate cancer detection: Changes in serum PSA glycosylation patterns. *Journal of Proteomics Research*, 8, 613–619.
- Nustad, K., Onsrud, M., Jansson, B., & Warren, D. (1998). CA125-epitopes and molecular size. *International Journal of Biological Markers*, 13, 196–199.
- Nustad, K., Lebedin, Y., Lloyd, K. O., Shigemasa, K., de Bruijn, H. W. A., Jansson, B., . . . O'Brien, T. J. (2002). Epitopes on CA 125 from cervical mucus and ascites fluid and characterization of six new antibodies. Third report from the ISOBM TD-1 workshop. *Tumour Biology*, 23, 303–314.
- O'Brien, T. J., Beard, J. B., Underwood, L. J., Dennis, R. A., Santin, A. D., & York, L. (2001). The CA 125 gene: An extracellular superstructure dominated by repeat sequences. *Tumour Biology*, 22, 348–366.
- O'Brien, T. J., Beard, J. B., Underwood, L. J., & Shigemasa, K. (2002). The CA 125 gene: A newly discovered extension of the glycosylated N-terminal domain doubles the size of this extracellular superstructure. *Tumour Biology*, 23, 154–169.
- O'Brien, T. J., Hanimoto, H., Konishi, I., & Gee, M. (1998). More than 15 years of CA125: What is known about the antigen, its

- structure and its function. *International Journal of Biological Markers*, 13, 188–195.
- Salдова, R., Struwe, W. B., Wynne, K., Elia, G., Duffy, M. J., & Rudd, P. M. (2013). Exploring the glycosylation of serum CA125. *International Journal of Molecular Science*, 14, 15636–15654.
- Stewart, W. E., De Somer, P., Edy, V. G., Paucker, K., Berg, K., & Ogburn, C. A. (1975). Distinct molecular species of human interferons: Requirements for stabilization and reactivation of human leukocyte and fibroblast interferons. *Journal of General Virology*, 26, 327–331.
- Tiollais, P., Galibert, F., & Boiron, M. (1971). Evidence for the existence of several molecular species in the “45S fraction” of mammalian ribosomal precursor RNA. *Proceedings from the National Academy of Sciences*, 68, 1117–1120.
- Tuzun, Y., Celik, Y., Bayan, K., Yilmaz, S., Dursun, M., & Canoruc, F. (2009). Correlations of tumour markers in ascitic fluid and serum: Are measurements of ascitic tumor markers a futile attempt? *Journal of Medical Research*, 37, 79–86.
- Weiland, F., Martin, K., Oehler, M. K., & Hoffmann, P. (2012). Deciphering the molecular nature of ovarian biomarker CA 125. *International Journal of Molecular Sciences*, 13, 10568–10582.
- Whitehouse, C., & Solomon, E. (2003). Current status of the molecular characterization of the ovarian cancer antigen CA125 and implications for its use in clinical screening. *Gynecologic Oncology*, 88, S152–S157.
- Wong, N. K., Easton, R. L., Panico, M., Sutton-Smith, M., Morrison, J. C., & Patankar, S. (2003). Characterization of the oligosaccharides associated with the human ovarian tumor marker CA125. *Journal of Biological Chemistry*, 278, 28619–28634.
- Yin, B. W., & Lloyd, K. O. (2001). Molecular cloning of the CA125 ovarian cancer antigen: Identification as a new mucin, MUC16. *Journal of Biological Chemistry*, 276, 27371–27375.