Original Article

Antimullerian Hormone and Inhibin B Are Hormone Measures of Ovarian Function in Late Reproductive-Aged Breast Cancer Survivors

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BACKGROUND: In late reproductive-aged breast cancer survivors, there is a need for real-time biomarkers of postchemotherapy ovarian function. The objective was to determine whether antimullerian hormone (AMH) and inhibin B are such biomarkers. The authors tested whether AMH and inhibin B were impacted by breast cancer treatment by comparing cancer survivors to age-matched control women and determined the association between these hormones and postchemotherapy menstrual pattern. **METHODS:** Breast cancer patients (n = 127) with American Joint Committee on Cancer stage I to III disease who were premenopausal at diagnosis were enrolled postchemotherapy and observed. The primary endpoint was chemotherapy-related amenorrhea (CRA) (≥12 months of amenorrhea after chemotherapy). Matched pair analyses compared AMH, inhibin B, and follicle-stimulating hormone (FSH) levels between cancer and age-matched control subjects. Associations between hormones, CRA status, and change in CRA status over time were assessed. **RESULTS:** The median age of the patients at chemotherapy was 43.2 years (range, 26.7-57.8 years). At enrollment, median follow-up since chemotherapy was 2.1 years, and 55% of subjects had CRA. Compared with age-matched controls, cancer subjects had significantly lower AMH ($P = .004$) and inhibin B ($P < .001$) and higher FSH ($P < .001$). AMH ($P = .002$) and inhibin B ($P = .001$) were found to be significantly associated with risk of CRA, even after controlling for FSH. AMH was significantly lower ($P = .03$) and FSH was significantly higher ($P = .04$) in menstruating subjects who developed subsequent CRA. **CONCLUSIONS:** AMH and inhibin B are 2 additional measures of postchemotherapy ovarian function in late reproductive-aged breast cancer survivors. With further research and validation, these hormones may supplement limited current tools for assessing and predicting postchemotherapy ovarian function. ***Cancer* 2010;116:592–9.** *© 2009 American Cancer Society.*

KEYWORDS: antimullerian hormone, inhibin B, follicle-stimulating hormone, breast cancer, chemotherapy, ovarian failure, amenorrhea.

More than 2 million American women are breast cancer survivors.[1] At diagnosis, approximately one-third are younger than 54 years old, and 10% are aged 35 to 45 years.[2] Most breast cancer patients will receive gonadotoxic chemotherapy, commonly including cyclophosphamide.[3] Gonadotoxic chemotherapy accelerates natural ovarian aging, leading to shortened reproductive life and early menopause.[4-6] Assessing postchemotherapy ovarian function in breast cancer survivors of late reproductive age is important to clinical decision making on a range of issues such as choice of adjuvant endocrine therapy, surgical oophorectomy, and prevention/treatment of menopause-related symptoms. Currently, the primary tool and gold standard for assessing postchemotherapy ovarian function is menstrual pattern. However, determining ovarian function by menstrual pattern requires watchful waiting by patients and physicians. The diagnosis of chemotherapy-related amenorrhea (CRA) is made retrospectively after prolonged postchemotherapy amenorrhea has occurred. Furthermore, in this population, lack of menses does not always represent ovarian failure, requiring patients to use contraception and risk

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misclassification for adjuvant endocrine therapy.[7] Therefore, there is a significant need for reliable, real-time biomarkers of ovarian function.

Antimullerian hormone (AMH) and inhibin B are hormone measures of ovarian function with limited data in the breast cancer population.[8-10] In adult survivors of childhood cancers, these hormones are putative biomarkers of ovarian function that demonstrate decreased ovarian reserve in a population in which most survivors continue to have regular menses.[11-13] It is difficult to generalize these data to breast cancer survivors, who are older at diagnosis and exposed to different treatment regimens, and in whom these biomarkers may be useful beyond prediction of fertility. In the breast cancer population, most data on hormone measures of ovarian function report on follicle-stimulating hormone (FSH), which rises with decreased ovarian function.[14] Available data regarding AMH and inhibin B are limited by small sample size or short follow-up, mostly confined to the perichemotherapy period.[8-10] There is a clear shortage of data on AMH and inhibin B as potential measures of ovarian function in late reproductive-aged breast cancer survivors who are beyond the immediate perichemotherapy period.

We performed a cohort study to examine AMH, inhibin B, and FSH in postchemotherapy breast cancer survivors with significant follow-up since chemotherapy. Our first objective was to determine the impact of breast cancer treatment on hormones by comparing cancer survivors to age-matched control women. We hypothesized that we would be able to detect differences in AMH and inhibin B between late reproductive-aged breast cancer survivors and age-matched controls. Our second objective was to determine the association between hormones and CRA in the cancer survivors. Finally, we sought to examine whether hormones can predict subsequent menstrual pattern in the cancer survivors.

MATERIALS AND METHODS

Study Population

We studied a cohort of 127 female, postchemotherapy breast cancer survivors from the Rena Rowan Breast Center of the University of Pennsylvania. Eligibility criteria included American Joint Committee on Cancer stage I to III breast cancer, premenopausal status at cancer diagnosis (menstrual periods in the year before chemotherapy), subsequent treatment with cyclophosphamide-based adjuvant chemotherapy, the presence of a uterus and at least 1 ovary, and initiation of adjuvant chemotherapy 1 to 4 years before enrollment. We selected this recruitment window to obtain adequate follow-up time for events (CRA) to occur. Hormonal therapy for breast cancer was not an exclusion criterion.

We matched breast cancer subjects to normal controls from the Penn Ovarian Aging Study, an ongoing study of late reproductive aging, by age and race.[15] Penn Ovarian Aging Study subjects have provided demographics, medical history, exposures, menstrual history, body mass index (BMI), and blood samples annually since 1995. All participants provided written consent. This study was approved by the University of Pennsylvania Institutional Review Board.

Data Collection

For breast cancer subjects, menstrual pattern data were collected at 3 time points: before chemotherapy, at enrollment (Assessment 1, 1-4 years after chemotherapy), and at a second follow-up (Assessment 2, 2-7 years after chemotherapy). At Assessment 1, breast cancer subjects underwent a blood draw timed with oncology follow-up, and therefore, not specific to menstrual cycle day. Sera were extracted and frozen at −80°C. Clinical data were abstracted from medical charts. For each control, we extracted menstrual pattern data and assayed stored, early follicular phase blood age-matched to the Assessment 1 age of her breast cancer counterpart.

Hormone Measures

Assessment 1 sera were assayed for AMH, inhibin B, FSH, and estradiol. Assays were conducted at the Penn Clinical and Translational Research Center. Hormone assays were performed in duplicate; duplicate means were analyzed. AMH was assayed using AMH enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic Systems, Webster, Tex). The lower limit of detection for AMH was 25 pg/mL (SI conversion: 1 ng/mL = 7.14 pmol/L), and the intra-assay coefficient of variation was 2%. Dimeric inhibin B was assayed using inhibin B ELISA kits (Diagnostic Systems). The intra-assay and inter-assay coefficients of variation were 7.9% and 8.4%, respectively. The lower limit of detection was 5 pg/mL. Estradiol and FSH were measured by radioimmunoassay using Coat-A-Count commercial kits (Diagnostic Products, Los Angeles, Calif). The intra- and interassay coefficients of variation were <5%. Values below detection thresholds were given half of the threshold value in analyses.

Data Analysis

STATA (Release 9, College Station, Tex) software was used for analyses. Summary statistics were performed for all variables. Hormone measures were transformed to natural log values to minimize the impact of skewed distributions.

For the first objective, we compared hormone, menstrual pattern, and demographic data between breast cancer subjects and age-matched controls using the Student *t* test for paired data (normally distributed data), signed-rank test (non-normally distributed data), and McNemar test (categoric data), as appropriate. Hormone and menstrual data were obtained at Assessment 1 in breast cancer subjects and compared with matched data from controls. Conditional logistic regression models compared cancer to control subjects while adjusting for confounders.

Second, we determined the association between Assessment 1 hormone measures and CRA status in breast cancer subjects. CRA was determined by menstrual history and defined as $\geq$12 months of amenorrhea occurring after start of chemotherapy. Categoric variables were compared using chi-square or exact methods, whereas continuous variables were compared using the Student *t* test (normally distributed data) or Wilcoxon rank sum test (ordinal or non-normally distributed variables). Poisson regression methods were used to model the cumulative incidence of CRA and its association with hormone levels while adjusting for confounding. Variables with $P \leq .1$ based on the Wald test for univariate associations were included in multivariate models.

Finally, we examined the association between Assessment 1 hormones and change in CRA status between Assessments 1 and 2 in breast cancer subjects using the Student *t* test. Assessment 2 CRA status was categorized as "no change from Assessment 1 CRA status," "CRA reversal," or "CRA progression." CRA reversal was defined as resumption of menses between Assessments 1 and 2 in subjects with CRA at Assessment 1. CRA reversal was defined as resumption of menses between Assessments 1 and 2 in subjects with CRA at Assessment 1. CRA progression was defined as experiencing at least 12 months of amenorrhea between Assessments 1 and 2 in subjects who did not have CRA at Assessment 1. As secondary analyses, we determined the impact of tamoxifen on AMH, inhibin B, and FSH levels using Student *t* test. All statistical tests were 2-sided, and *P* values of $\leq$.05 were considered to be statistically significant.

Prestudy power calculations were based on Penn Ovarian Aging Study AMH data from normal women of late reproductive age, with a mean $\pm$ standard deviation (SD) AMH level of 0.65 (1.06) ng/mL.[16] With a 5% alpha error, the study had 80% power to detect a difference in mean AMH levels of 0.38 ng/mL between breast cancer subjects and age-matched controls.

RESULTS

A total of 127 postchemotherapy breast cancer survivors were enrolled between 2004 and 2005 (Assessment 1). Assessment 2 was conducted between 2007 and 2008. Cohort characteristics (Table 1) included a median age at start of chemotherapy of 43.2 years (range, 26.7-57.8 years). At Assessment 1, the median time since chemotherapy was 2.1 years (range, 1.0-4.9 years). Overall, participants were observed for a median of 5.2 years since chemotherapy (range, 1.0-7.6 years). No subject was taking hormonal contraceptives or hormone replacement therapy.

Comparison of Hormones Between Cancer and Control Subjects

A total of 110 cancer subjects were age-matched and race-matched with controls. Breast cancer subjects had significantly lower AMH and inhibin B and higher FSH than age-matched controls in pairwise comparisons (Table 2). Cancer status continued to be associated with significantly lower AMH ($P = .01$) and inhibin B ($P = .001$) and higher FSH ($P < .001$) in regression models adjusting for confounders including gravidity, BMI, smoking, and alcohol exposure (Table 3).

Associations Between Hormones and CRA at Assessment 1

Cumulative CRA incidence at Assessment 1 was 55% (70 of 127 subjects). Subjects with CRA had significantly lower AMH and inhibin B and higher FSH compared with women without CRA (Table 4). Univariate comparisons also demonstrated that subjects with CRA were significantly older at chemotherapy than subjects without CRA. A multivariate regression model was developed to examine the relation between CRA and all 3 hormones simultaneously, while controlling for age at chemotherapy, chemotherapy schedule, taxane exposure, and tamoxifen exposure. This model demonstrated that each hormone remains independently associated with CRA risk in the setting of adjusting for the other 2 hormones and clinical confounders (Table 5).

Hormones and Change in CRA Status Between Assessments 1 and 2

At Assessment 2, 87% (n = 111) of subjects provided additional menstrual data. Of 16 women not included in

Table 1. Characteristics of Breast Cancer Cohort at Assessments 1 and 2

Characteristic	Assessment 1, n = 127	Assessment 2, n = 111
Median age at initiation of chemotherapy (range), y	43.2 (26.7-57.8)	43.4 (28.1-56)
Median age at assessment (range), y	45.3 (28.9-60.6)	48.7 (32.0-62.4)
Race		
White	113 (89%)	102 (91%)
African American	5 (4%)	4 (4%)
Other/not reported	9 (7%)	5 (5%)
Breast cancer stage		
I	27 (21%)	27 (25%)
II	80 (63%)	67 (60%)
III	20 (16%)	17 (15%)
Estrogen receptor +	95 (75%)	81 (73%)
Progesterone receptor +	87 (68%)	75 (68%)
HER-2/*neu* +	27 (21%)	23 (21%)
Cyclophosphamide-based chemotherapy	127 (100%)	111 (100%)
Median tumor size (range), cm	2 (0-8.5)	2 (0-8.5)
Median no. of positive lymph nodes (range)	1 (0-20)	1 (0-20)
Chemotherapy regimen		
AC	48 (38%)	41 (37%)
AC/T	69 (54%)	62 (56%)
FAC	4 (3%)	3 (3%)
Other[a]	4 (3%)	4 (4%)
Median y follow-up from initiation of chemotherapy to Assessment 1 (range)	2.1 (1.0-4.9)	5.3 (2.7-7.6)
Surgical menopause or ovarian suppression at enrollment	5 (4%)	16 (14%)
Chemotherapy-related amenorrhea	70 (55%)	62 (56%)

A indicates doxorubicin; C, cyclophosphamide; T, taxane; F, 5-fluorouracil; N, vinorelbine; M, methotrexate.
[a] Other indicates AC/N, AC/T/N, CMF, and CMF/T.

Assessment 2, 2 were deceased, 1 declined follow-up, and 13 were not reached. The baseline characteristics of the Assessment 2 cohort remained unchanged from Assessment 1 (Table 1).

Return of menses, or CRA reversal, occurred in 9 (13%) subjects who had CRA at Assessment 1 and completed Assessment 2. By clinical factors, women with CRA reversal were younger (mean age 41.7 years [range, 38.4-44.8 years] vs 47.3 years [range, 40.3-56 years]; $P = <.001$) and more likely to have received dose-dense therapy (risk ratio, 6.4; $P = .03$) than women who continued to be amenorrheic. Levels of Assessment 1 AMH ($P = .92$), inhibin B ($P = .27$), and FSH ($P = .73$) did not differ between subjects who underwent CRA reversal compared with subjects who remained amenorrheic.

Four subjects who were menstruating at Assessment 1 subsequently experienced CRA by Assessment 2. Compared with subjects who did not have CRA through the entire follow-up, these subjects were of similar age but had lower AMH (25.2 pg/mL [range, <25-233.5 pg/mL] vs 179.4 pg/mL [range, 96.2-334.1 pg/mL]; $P = .03$) and higher FSH (48.1 IU/L [range, 13.3-173.7 IU/L] vs 17.4 IU/L [range, 12.2-24.7 IU/L]; $P = .04$) in Assessment 1. Inhibin B was higher in subjects with CRA progression (134.3 pg/mL [range, 7.1-2532.1 pg/mL] vs 24.5 pg/mL [range, 13.6-44.1 pg/mL]; $P = .05$), but this occurred because of a single high inhibin B level.

Effect of Tamoxifen Exposure on Hormones

Eighty-seven (71%) subjects were on tamoxifen at Assessment 1. AMH, inhibin B, and estradiol levels were similar between users and nonusers. Geometric mean AMH levels) were 69.6 pg/mL (95% confidence interval [95% CI], 48.5-99.7 pg/mL) in users versus 60.6 pg/mL (95% CI, 39.8-92.1 pg/mL) in nonusers ($P = .63$). Inhibin B levels were 12.2 pg/mL (95% CI, 7.3-20.4) in tamoxifen users and 12.3 pg/mL (95% CI, 8.6-17.4) in nonusers ($P =$

Table 2. Unadjusted Pairwise Comparison of Breast Cancer and Control Subjects

Characteristic	Breast Cancer Subjects, n = 110	Control Subjects, n = 110	*P*
Mean age at blood draw (range), y	46.1 (30.4-59.3)	46.1 (35.4-57.2)	.59[a]
Mean BMI (95% CI)	25.8 (24.8-26.8)	27.4 (26.1-28.7)	.06[a]
Median gravidity (range)	2 (0-7)	3 (0-8)	.06[b]
Ever smoked			<.001[c]
Yes	7 (18%)	31 (82%)	
No	103 (56%)	79 (44%)	
Current alcohol use			<.001[c]
Yes	80 (82%)	18 (18%)	
No	30 (25%)	92 (75%)	
AMH, pg/mL[d]	53.1 (40.2-70.2)	99.5 (66.4-149.1)	.004[a]
Inhibin B, pg/mL[d]	12.7 (0.93-17.5)	38.5 (29.6-50.1)	<.001[a]
FSH, IU/L[d]	35.6 (30.0-42.2)	13.3 (10.9-16.2)	<.001[a]
Estradiol, pg/mL[d]	38.7 (30.1-48.6)	30.6 (25.8-36.2)	.07[a]

BMI indicates body mass index; 95% CI, 95% confidence interval; AMH, antimullerian hormone; FSH, follicle-stimulating hormone.
[a] Determined using Student *t* test for paired data.
[b] Determined using signed-rank test.
[c] Determined using McNemar test.
[d] Shown as the geometric mean (95% CI). The geometric mean is back-transformed from the group mean of the log hormone levels.

Table 3. Conditional Logistic Regression Models Comparing Breast Cancer Patients With Controls by Hormone Levels and Other Factors

Characteristic	AMH		Inhibin B		FSH	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Hormone[a]	0.68 (0.51-0.92)	.01	0.63 (0.47-0.83)	.001	4.11 (1.91-8.02)	<.001
Smoking	0.48 (0.14-1.71)	.15	0.47 (0.12-1.90)	.29	1.00 (0.22-4.50)	1.00
Alcohol use	10.50 (3.73-29.52)	<.001	10.50 (3.64-30.30)	<.001	11.77 (3.66-37.88)	<.001
BMI	0.98 (0.92-1.04)	.48	0.98 (0.92-1.04)	.48	1.02 (0.96-1.10)	.42
Gravidity	0.82 (0.62-1.07)	.14	0.74 (0.55-1.01)	.06	0.82 (0.60-1.11)	.20

AMH indicates antimullerian hormone; FSH, follicle-stimulating hormone; OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index.
[a] For each log unit increase.

.99). FSH levels were significantly lower in tamoxifen users (35.5 IU/L [95% CI, 19.2-65.6 IU/L]) than nonusers (42.8 IU/L [95% CI, 26.2-69.8 IU/L]) ($P = .04$).

DISCUSSION

We examined 3 hormone measures of ovarian function in breast cancer survivors of late reproductive age. In addition to FSH, we demonstrated significant differences in AMH and inhibin B between breast cancer survivors and normal controls, and between breast cancer survivors with CRA compared with breast cancer survivors who continued to menstruate. Although our numbers are limited, the results of the current study suggest that decreased AMH and increased FSH precede development of CRA and that there is no association between hormone measures and subsequent resumption of menses. Taken together, these hormones appear to be biomarkers measuring ovarian aging after gonadotoxic chemotherapy exposure in late reproductive-aged women with breast cancer.

Compared with age-matched controls, this cohort of late reproductive-aged cancer survivors had lower AMH and inhibin B levels, as hypothesized, and higher FSH levels. Lower AMH and inhibin B levels (secreted by

Table 4. Univariate Associations Between Subject Characteristics and CRA Status at Assessment[a]

Characteristic	CRA, n = 70	No CRA, n = 52	*P*
Median age at chemotherapy (range), y	46.5 (38.4-56)	39.1 (26.7-57.8)	<.001[b]
Race			.14[c]
Caucasian	65 (60%)	44 (40%)	
Other	5 (38%)	8 (62%)	
Ever smoked			.56[c]
Yes	36 (60%)	24 (40%)	
No	34 (55%)	28 (45%)	
Mean BMI (95% CI)	25.9 ± 5.1	26.3 ± 6.0	.70[b]
Median cumulative cyclophosphamide dose (interquartile range), mg	4080 (2880-4960)	4160 (3552-5304)	.39[d]
No. of cyclophosphamide cycles			.64[c]
≤4	66 (57%)	50 (43%)	
>4	4 (67%)	2 (33%)	
Chemotherapy regimen			.07[c]
Taxane containing	35 (53%)	35 (50%)	
Nontaxane containing	34 (67%)	17 (33%)	
Chemotherapy schedule			.07[c]
Every 2 wk (dose dense)	30 (49%)	31 (51%)	
Every 3 wk	37 (66%)	19 (34%)	
Tamoxifen therapy			.07[c]
Yes	54 (62%)	33 (38%)	
No	15 (44%)	19 (56%)	
AMH, pg/mL[e]	39.1 (28.0-54.6)	131.6 (86.3-200.7)	<.001[b]
Inhibin B, pg/mL[e]	7.7 (5.3-11.1)	25.3 (16.5-39.0)	<.001[b]
FSH, IU/L[e]	52.9 (45.8-61.0)	17.4 (13.4-22.5)	<.001[b]
Estradiol, pg/mL[e]	22.0 (17.7-27.2)	92.3 (70.7-120.5)	<.001[b]

CRA indicates chemotherapy-related amenorrhea; BMI, body mass index; 95% CI, 95% confidence interval; AMH, antimullerian hormone; FSH, follicle-stimulating hormone.
[a] Excludes 5 subjects with surgical menopause or ovarian suppression.
[b] Determined using Student *t* test.
[c] Determined using chi-square test.
[d] Determined using Wilcoxon rank-sum test.
[e] Geometric mean (95% CI). Geometric mean is back-transformed from the group mean of the log hormone levels.

ovaries) are consistent with higher FSH levels (secreted from the pituitary) and reflect the decrease in ovarian function as a result of exposure to gonadotoxic chemotherapy. The results suggest that 2 markers in addition to FSH are able to measure ovarian function after gonadotoxic chemotherapy in late reproductive-aged breast cancer survivors.

As hypothesized, all 3 reproductive hormone levels in survivors with CRA reflected decreased ovarian function compared with survivors who continued menstruating. In addition, our small subset of subjects who developed subsequent CRA suggest that lower levels of AMH and higher levels of FSH precede CRA. Whereas higher FSH levels in amenorrheic survivors are known, lower AMH and inhibin B have only been reported by 3 smaller studies with short follow-up and limited association with menstrual pattern.[8,9,10,17-20] Our data are consistent with these smaller reports, but we are able to

Table 5. Adjusted Associations Between Clinical Factors, Hormone Levels, and CRA at Assessment 1

Characteristic	CRA IRR (95% CI)	*P*
AMH[a]	0.86 (0.78-0.95)	.003
Inhibin B[a]	0.86 (0.79-0.94)	.001
FSH[a]	1.85 (1.46-2.32)	<.001
Age at chemotherapy >40 y	2.35 (1.30-4.26)	.005
Dose-dense therapy	1.22 (0.92-1.60)	.16
Taxane exposure	1.07 (0.82-1.39)	.64
Tamoxifen exposure	2.04 (1.54-2.71)	<.001

CRA indicates chemotherapy-related amenorrhea; IRR, incident rate ratio; 95% CI, 95% confidence interval; AMH, antimullerian hormone; FSH, follicle-stimulating hormone.
[a] For each log unit increase.

extend the observation of decreased hormone measures of ovarian reserve beyond the perichemotherapy period. With lengthy follow-up, the results of the current study are generalizable to the large survivor population that is not immediately postchemotherapy. Moreover, long follow-up enabled the study to capture menstrual pattern changes over time and decreased misclassification by menstrual status.

Finally, the results of the current study demonstrated that AMH and inhibin B levels were not affected by concurrent tamoxifen use. As expected, AMH levels did not differ by tamoxifen exposure, because AMH secretion is gonadotropin-independent, and levels are stable between and throughout menstrual cycles.[21] FSH was lower in subjects on tamoxifen, which is consistent with the literature.[22,23] Because FSH levels may be artificially lowered by tamoxifen, FSH is less reliable in women on tamoxifen. With replication, these are potential advantages of AMH and inhibin over FSH.

There are several reasons to identify additional biomarkers to FSH for measuring ovarian function in late reproductive-aged breast cancer survivors. In addition to the potential advantage of interpreting AMH and inhibin B over FSH in the setting of tamoxifen use, all 3 of these biomarkers have been useful in delineating specific stages in the natural transition to menopause.[15,24-28] Changes in AMH and inhibin B appear to occur earlier than FSH in the natural menopausal transition, appear to reflect subtle changes in ovarian reserve compared with FSH, and may predict time to final menstrual period better than FSH.[27,29-32] They may play a similar role in the transition to menopause for breast cancer patients, a hypothesis that warrants future investigation. Finally, AMH and inhibin B may capture additional information regarding ovarian function independent of FSH, as both AMH and inhibin B had independent associations with CRA after controlling for FSH in this dataset.

Several limitations should be considered. First, hormone levels were obtained postchemotherapy for the purpose of evaluating postchemotherapy ovarian function. Therefore, these results do not apply to using these hormones prechemotherapy to predict postchemotherapy function. A second limitation was that hormone levels were drawn timed to oncology follow-up visits. Therefore, hormone levels were drawn throughout the menstrual cycle, rather than in the early follicular phase, for the 52 menstruating cancer subjects. This limitation affects the precision of FSH and inhibin B levels in menstruating cancer subjects, but would not affect gonadotropin-independent AMH. Importantly, we do not believe that this limitation systematically biased our results for FSH and inhibin B. Because within the menstrual cycle hormone levels can be higher or lower than the early follicular phase levels of these hormones,[33] the result of non–cycle-specific blood samples would be increased variability, which would bias our results toward the null. Therefore, the strong, statistically significant difference in FSH and inhibin B between cancer and control subjects is likely real and not from differential bias. Third, we recognize that tamoxifen may independently impact FSH and menstrual pattern,[23] but we did not restrict our analyses to subjects who are not receiving endocrine therapy. Instead, our approach was to control for tamoxifen exposure, after which we continued to demonstrate significant associations between CRA and all 3 hormones. Furthermore, in including both subjects on and off of tamoxifen, we present more generalizable data, as most women with breast cancer are hormone receptor positive.[34] Finally, Assessment 1 hormone levels varied in length of time from chemotherapy for each cancer survivor. Therefore, we are not powered to provide hormone and menstrual data at defined intervals (eg, 1- or 2-year intervals) from chemotherapy.

In conclusion, the results of the current study demonstrate AMH and inhibin B to be 2 additional measures of postchemotherapy ovarian function in late reproductive-aged breast cancer survivors. With further research and validation, these hormone biomarkers supplement limited current tools for assessing and predicting postchemotherapy ovarian function.

CONFLICT OF INTEREST DISCLOSURES

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