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Anti-mullerian hormone and inhibin B are hormone measures of ovarian function in late reproductive-aged breast cancer survivors

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Abstract

Background—In late reproductive-aged breast cancer survivors, there is a need for "real-time" biomarkers of post-chemotherapy ovarian function. The objective was to determine if anti-mullerian hormone (AMH) and inhibin B are such biomarkers. We tested if AMH and inhibin B were impacted by breast cancer treatment by comparing cancer survivors to age-matched control women. We determined the association between these hormones and post-chemotherapy menstrual pattern.

Methods—127 breast cancer patients with Stages I–III disease, premenopausal at diagnosis, were enrolled post-chemotherapy and followed. 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—Median age at chemotherapy was 43.2 years (range 26.7–57.8). At enrollment, median follow up since chemotherapy was 2.1 years and 55% of subjects had CRA. Compared to age-matched controls, cancer subjects had significantly lower AMH ($p=0.004$) and inhibin B ($p<0.001$) and higher FSH ($p<0.001$). AMH ($p=0.002$) and inhibin B ($p=0.001$) were significantly associated with risk of CRA even after controlling for FSH. AMH was significantly lower ($p=0.03$) and FSH was significantly higher ($p=0.04$) in menstruating subjects who developed subsequent CRA.

Conclusions—AMH and inhibin B are two additional measures of post-chemotherapy ovarian function in late reproductive-aged breast cancer survivors. With further research and validation,

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these hormones may supplement limited current tools for assessing and predicting post-chemotherapy ovarian function

Keywords

Anti-mullerian hormone; inhibin B; FSH; breast cancer; chemotherapy; ovarian failure; amenorrhea

INTRODUCTION

More than two million American women are breast cancer survivors 1. At diagnosis, one-third are less than 54 years old, and one-tenth are ages 35 to 45 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 post-chemotherapy 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, decisions on surgical oophorectomy and prevention/treatment of menopause-related symptoms. Currently, the primary tool and gold standard for assessing post-chemotherapy 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 post-chemotherapy amenorrhea has occurred. Further, in this population, lack of menses does not always represent ovarian failure, requiring patients to use contraception and risk misclassification for adjuvant endocrine therapy 7. Therefore, there is a significant need for reliable, “real-time” biomarkers of ovarian function.

Anti-mullerian hormone (AMH) and inhibin B are hormone measures of ovarian function with limited data in the breast cancer population 8–11. In adult survivors of childhood cancers, these hormones are putative biomarkers of ovarian function that show decreased ovarian reserve in a population where most survivors continue to have regular menses 12–14. It is difficult to generalize these data to breast cancer survivors, who are older at diagnosis, 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 15. Available data on AMH and inhibin B are limited by small sample size or short follow up, mostly confined to the peri-chemotherapy period 8–11. 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 peri-chemotherapy period.

We performed a cohort study to examine AMH, inhibin B and FSH in post-chemotherapy 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.

METHODS

Study population

We studied a cohort of 127 female, post-chemotherapy breast cancer survivors from the Rena Rowan Breast Center of the University of Pennsylvania. Eligibility criteria included AJCC Stages I–III breast cancer, premenopausal at cancer diagnosis (menstrual periods in the year prior to chemotherapy), subsequent treatment with cyclophosphamide-based adjuvant chemotherapy, presence of a uterus and at least one ovary, and initiation of adjuvant chemotherapy 1– 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 (POAS), an ongoing study of late reproductive aging, by age and race 16. POAS subjects have provided demographics, medical history, exposures, menstrual history, 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 three time points: prior to 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 degrees 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 in the Penn Clinical Translational Research Center. Hormone assays were performed in duplicate; duplicate means were analyzed. AMH was assayed using AMH ELISA kits (Diagnostic Systems, Webster, TX). The lower limit of detection for AMH was 25 pg/mL (SI conversion: AMH*7180), and the intra-assay coefficient of variation (cov) was 2%. Dimeric inhibin B was assayed using Inhibin B ELISA kits (Diagnostic Systems, Webster, TX). The intra- and inter-assay cov 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, CA). The intra- and inter-assay cov were less than 5%. Values below detection thresholds were given half of the threshold value in analyses.

Data Analysis

STATA (Release 9, College Station, TX) 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 paired t-test (normally distributed data), signrank test (non-normally distributed data), and McNemar's test (categorical 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 ≥12 months of amenorrhea occurring after start of chemotherapy. Categorical variables were compared using Chi-square or exact methods, while continuous variables were compared using Student's 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 $p \leq 0.1$ based on the Wald test from univariate associations were included in multivariable 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 Student's 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's t-test. All statistical tests were two-sided, and p-values of ≤0.05 were considered to be statistically significant.

Pre-study power calculations were based on POAS AMH data from normal women of late reproductive age, with a mean (SD) AMH level of 0.65 (1.06) ng/mL 17. 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

127 post-chemotherapy 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 (range) of 43.2 (26.7–57.8). At Assessment 1, median time since chemotherapy (range) was 2.1 years (1.0–4.9). Overall, participants were followed for a median of 5.2 years since chemotherapy (range 1.0–7.6). No subject was on hormonal contraceptives or hormone replacement therapy.

Comparison of hormones between cancer and control subjects

One hundred ten cancer subjects were age- and race-matched to 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 ($p=0.01$) and inhibin B ($p=0.001$) and higher FSH ($p<0.001$) 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% (70/127 subjects). Subjects with CRA had significantly lower AMH and inhibin B and higher FSH compared to women without CRA (Table 4). Univariate comparisons also demonstrated that subjects with CRA were significantly older at chemotherapy than subjects without CRA. A multivariable regression

model was developed to examine the relationship between CRA and all three 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 two hormones and clinical confounders (Table 3).

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 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 subjects (13%) who had CRA at Assessment 1 and completed Assessment 2. By clinical factors, women with CRA reversal were younger (mean age [range] 41.7 [38.4–44.8] versus 47.3 [40.3–56], p=<0.001) and more likely to have received dose dense therapy (RR 6.4, p=0.03) than women who continued to be amenorrheic. Levels of Assessment 1AMH (p=0.92), inhibin B (p=0.27) and FSH (p=0.73) did not differ between subjects who underwent CRA reversal compared to subjects who remained amenorrheic.

Four subjects who were menstruating at Assessment 1 subsequently experienced CRA by Assessment 2. Compared to subjects who did not have CRA through the entire follow up, these subjects were of similar age but had lower AMH (25.2 [2.7–233.5] versus 179.4 [96.2–334.1], p=0.03) and higher FSH (48.1 [13.3–173.7] versus 17.4 [12.2–24.7], p=0.04) in Assessment 1. Inhibin B was higher in subjects with CRA progression (134.3[7.1–2532.1] versus 24.5[13.6–44.1], p=0.05), but this occurred due to a single high inhibin B level.

Effect of tamoxifen exposure on hormones

Eighty-seven subjects (71%) were on tamoxifen at Assessment 1. AMH, inhibin B and estradiol levels were similar between users and non-users. AMH levels (geometric mean [95% CI]) were 69.6 (48.5–99.7) in users versus 60.6 (39.8–92.1) in non-users (p=0.63). Inhibin B levels were 12.2 (7.3–20.4) in tamoxifen users and 12.3(8.6–17.4) in non-users (p=0.99). FSH levels were significantly lower in tamoxifen users (35.5[19.2–65.6]) than non-users (42.8 [26.2–69.8]) (p=0.04).

DISCUSSION

We examined three 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 to breast cancer survivors who continued to menstruate. While our numbers are limited, the results suggest decreased AMH and increased FSH precede development of CRA and 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 to 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 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 two markers in addition to FSH are able

to measure ovarian function after gonadotoxic chemotherapy in late reproductive-aged breast cancer survivors.

As hypothesized, all three reproductive hormone levels in survivors with CRA reflected decreased ovarian function compared to 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. While higher FSH levels in amenorrheic survivors is 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, 10, 11, 18–21. Our data are consistent with these smaller reports, but we are able to extend the observation of decreased hormone measures of ovarian reserve beyond the peri-chemotherapy period. With lengthy follow up, the present study is generalizable to the large survivor population that is not immediately post-chemotherapy. Moreover, long follow up enabled the study to capture menstrual pattern changes over time and decreased misclassification by menstrual status.

Finally, the study showed 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 22. FSH was lower in subjects on tamoxifen, consistent with the literature 23, 24. 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 three of these biomarkers have been useful in delineating specific stages in the natural transition to menopause 16, 25–29. Changes in AMH and inhibin B appear to occur earlier than FSH in the natural menopausal transition, seem to reflect subtle changes in ovarian reserve compared to FSH, and may predict time to final menstrual period better than FSH 28, 30–33. 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 about 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 post-chemotherapy for the purpose of evaluating post-chemotherapy ovarian function. Therefore, our results do not apply to using these hormones pre-chemotherapy to predict post-chemotherapy 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 menstrual cycle hormone levels can be higher or lower than the early follicular phase levels of these hormones 34, the result of non-cycle specific bloods 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 are likely real and not from differential bias. Third, we recognize that tamoxifen may independently impact FSH and menstrual pattern 24, but we did not restrict our analyses to subjects who are not on endocrine therapy. Instead, our approach was to control for tamoxifen exposure, after which we continued to show significant associations between CRA and all three hormones.

Further, in including both subjects on and off of tamoxifen, we present more generalizable data as most women with breast cancer are hormone receptor positive 35. 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, e.g. 1-year, 2-year intervals, from chemotherapy.

In conclusion, our study demonstrates AMH and inhibin B to be two additional measures of post-chemotherapy 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 post-chemotherapy ovarian function.

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Table 1

Characteristics of breast cancer cohort at Assessments 1 and 2

Characteristic	Assessment 1 (n=127)	Assessment 2 (n=111)
Median age at start of chemotherapy (range)	43.2 (26.7 – 57.8)	43.4 (28.1–56)
Median age at Assessment (range)	45.3 (28.9–60.6)	48.7 (32.0–62.4)
Race		
Caucasian	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 (cm) (range)	2 (0–8.5)	2 (0–8.5)
Median lymph node + (range)	1 (0–20)	1 (0–20)
Chemotherapy regimen[1]		
AC	48 (38%)	41 (37%)
AC/T	69 (54%)	62 (56%)
FAC	4 (3%)	3 (3%)
Other[2]	4 (3%)	4 (4%)
Median years of follow-up from chemotherapy start to Assessment 1	2.1 (0.4–4.9)	5.3 (2.7–7.6)
Surgical menopause or ovarian suppression at enrollment	5 (4%)	16 (14%)
Chemotherapy related amenorrhea	70 (55%)	62 (56%)

[1] A=doxorubicin, C=cyclophosphamide, T=taxane, F=fluorouracil, N=Vinorelbine, M=methotrexate

[2] AC/N, AC/T/N, CMF, CMF/T

Table 2

Unadjusted pairwise comparison of breast cancer and control subjects

	Breast cancer subjects (n = 110)	Control subjects (n = 110)	p-value
Age at blood draw (mean, range)	46.1 (30.4–59.3)	46.1 (35.4–57.2)	0.59[1]
BMI (mean, 95% CI)	25.8 (24.8–26.8)	27.4 (26.1–28.7)	0.06[1]
Gravidity (median, range)	2 (0–7)	3 (0–8)	0.06[2]
Ever smoked			<0.001[3]
Yes	7 (18%)	31 (82%)	
No	103 (56%)	79 (44%)	
Current alcohol use			<0.001[3]
Yes	80 (82%)	18 (18%)	
No	30 (25%)	92 (75%)	
AMH (pg/mL)[4]	53.1 (40.2–70.2)	99.5 (66.4–149.1)	0.004[1]
Inhibin B (pg/mL)[4]	12.7 (0.93–17.5)	38.5 (29.6–50.1)	<0.001[1]
FSH (IU/L)[4]	35.6 (30.0–42.2)	13.3 (10.9–16.2)	<0.001[1]
Estradiol (pg/mL)[4]	38.7 (30.1–48.6)	30.6 (25.8–36.2)	0.07[1]

[1] Paired t-test

[2] Sign-rank test

[3] McNemar's test

[4] Geometric mean (95% CI). Geometric mean is back-transformed from the group mean of the log hormone levels.

Table 3

Conditional logistic regression models comparing breast cancer to controls by hormone levels and other factors

	AMH		Inhibin B		FSH	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Hormone[1]	0.68 (0.51–0.92)	0.01	0.63 (0.47–0.83)	0.001	4.11(1.91–8.02)	<0.001
Smoking	0.48 (0.14–1.71)	0.15	0.47 (0.12–1.90)	0.29	1.00 (0.22–4.50)	1.00
Alcohol use	10.50 (3.73–29.52)	<0.001	10.50 (3.64–30.30)	<0.001	11.77 (3.66–37.88)	<0.001
BMI	0.98 (0.92–1.04)	0.48	0.98 (0.92–1.04)	0.48	1.02 (0.96–1.10)	0.42
Gravidity	0.82 (0.62–1.07)	0.14	0.74 (0.55–1.01)	0.06	0.82 (0.60–1.11)	0.20

[1]For each log unit increase

Table 4

Univariate associations between subject characteristics and CRA status at Assessment [1]

	CRA (n = 70)	No CRA (n = 52)	p-value
Age at chemotherapy (median, range)	46.5 (38.4–56)	39.1 (26.7–57.8)	< 0.001[2]
Race			0.14[3]
Caucasian	65 (60%)	44 (40%)	
Other	5 (38%)	8 (62%)	
Ever smoked			0.56[3]
Yes	36 (60%)	24 (40%)	
No	34 (55%)	28 (45%)	
BMI (mean, 95% CI)	25.9 ± 5.1	26.3 ± 6.0	0.70[2]
Cumulative cyclophosphamide dose (mg) (median, interquartile range)	4080 (2880–4960)	4160 (3552–5304)	0.39[4]
Cyclophosphamide cycles			0.64[3]
≤ 4	66 (57%)	50 (43%)	
> 4	4 (67%)	2 (33%)	
Chemotherapy regimen			0.07[3]
Taxane-containing	35 (53%)	35 (50%)	
Non-taxane containing	34 (67%)	17 (33%)	
Chemotherapy Schedule			0.07[3]
Every 2 weeks (“dose dense”)	30 (49%)	31 (51%)	
Every 3 weeks	37 (66%)	19 (34%)	
Tamoxifen therapy			0.07[3]
Yes	54 (62%)	33 (38%)	
No	15 (44%)	19 (56%)	
AMH (pg/mL)[5]	39.1 (28.0–54.6)	131.6 (86.3–200.7)	<0.001[2]
Inhibin B (pg/mL)[5]	7.7 (5.3–11.1)	25.3 (16.5–39.0)	<0.001[2]
FSH (IU/L)[5]	52.9 (45.8–61.0)	17.4 (13.4–22.5)	<0.001[2]
Estradiol (pg/mL)[5]	22.0 (17.7–27.2)	92.3 (70.7–120.5)	<0.001[2]

[1] Excludes 5 subjects with surgical menopause or ovarian suppression

[2] Student’s t-test

[3] Chi-square test

[4] Wilcoxon ranksum test

[5] Geometric mean (95% CI). Geometric mean is back-transformed from the group mean of the log hormone levels.

Table 5

Adjusted associations between clinical factors, hormone levels and CRA at Assessment 1

	CRA IRR[1] (95% CI)	p-value
AMH[2]	0.86 (0.78–0.95)	0.003
Inhibin B[2]	0.86 (0.79–0.94)	0.001
FSH[2]	1.85 (1.46–2.32)	<0.001
Age at chemotherapy > 40	2.35 (1.30–4.26)	0.005
Dose dense therapy	1.22 (0.92–1.60)	0.16
Taxane exposure	1.07 (0.82–1.39)	0.64
Tamoxifen exposure	2.04 (1.54–2.71)	<0.001

[1] Incident rate ratio

[2] For each log unit increase