Published in final edited form as:
Fertil Steril. 2011 April ; 95(5): 1857–1859. doi:10.1016/j.fertnstert.2010.12.005.

Antral follicle count provides additive information to hormone measures in determining ovarian function in breast cancer survivors

H. Irene Su, MD, MSCE[1], **Karine Chung, MD, MSCE**[2], **Mary D. Sammel, ScD**[3], **Clarisa R. Gracia, MD, MSCE**[3,4], and **Angela DeMichele, MD, MSCE**[3,5]

[1] Division of Reproductive Endocrinology and Infertility, University of California, San Diego

[2] Division of Reproductive Endocrinology and Infertility, University of Southern California

[3] Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine

[4] Division of Reproductive Endocrinology and Infertility, University of Pennsylvania School of Medicine

[5] Rowan Breast Center, Abramson Cancer Center, University of Pennsylvania School of Medicine

Abstract

In breast cancer survivors, AFC appears to provide data on ovarian function that is independent of AMH, FSH and inhibin B. Hence, ultrasound appears to be a tool that may not only corroborate, but also add to the accuracy of hormone measures in determining ovarian function in breast cancer survivors.

Young breast cancer survivors constitute a large population to whom post-chemotherapy ovarian function is important. Currently, the gold standard for measuring ovarian function after exposure to gonadotoxic chemotherapy remains long-term menstrual pattern, which requires watchful waiting. Recently, hormone measures of ovarian reserve including follicle stimulating hormone, anti-mullerian hormone and inhibin B have been associated with post-chemotherapy ovarian function in breast cancer survivors (1–5). Ovarian morphometry is another measure of ovarian reserve in women undergoing fertility treatment (6), but there are limited data in breast cancer patients (4,7). The objective of this study was to determine if antral follicle count (AFC) and ovarian volume (OV) are associated with chemotherapy-related ovarian failure (CROF) after breast cancer treatment. We hypothesized that these measures would provide additive information to AMH, FSH and inhibin B in this population.

Corresponding author H. Irene Su, MD MSCE, Moores UCSD Cancer Center, 3855 Health Sciences Drive, Dept. 0901, La Jolla, CA 92093-0901, Telephone: 858-822-5986, Fax: 858-822-2399, hisu@ucsd.edu.
Requests for reprints should be sent to the corresponding author.

Conflicts of interest
None

We performed a cross-sectional study assessing hormonal and ultrasound measures of ovarian reserve in 56 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 at least 1 year before enrollment. We selected this recruitment window to obtain adequate follow up time for events (CROF) to occur. Tamoxifen for breast cancer was not an exclusion criterion; no subject was on a GnRH agonist. The subjects in this study are a subset of a larger longitudinal cohort of ovarian aging in breast cancer survivors (5). This study was approved by the University of Pennsylvania Institutional Review Board.

At enrollment, subjects provided self-reported menstrual pattern data and underwent a blood draw and pelvic ultrasound. The study enrollment visit was timed with oncology follow up and was therefore not specific to menstrual cycle day. Sera were extracted and frozen at −80 degrees C. Clinical data were abstracted from medical charts.

OV and AFC were determined by transvaginal pelvic ultrasonography performed by two trained gynecologists using a standardized protocol. The maximum transverse, anterior-posterior and longitudinal diameters for all ovaries were measured and the volume was estimated as $\pi/6 \times 3$ diameters. All ovarian follicles between 2 and 10 millimeter in diameter were counted. Antral follicle count for each subject was the sum of antral follicles from both ovaries.

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, 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 (8).

STATA (Release 9, College Station, TX) software was used for analyses. Summary statistics were performed for all variables. The primary outcome was CROF, determined by self-reported menstrual history and defined as ≥12 months of amenorrhea occurring after start of chemotherapy. We determined the association between CROF status and measures of ovarian reserve (AFC, OV, FSH, AMH, inhibin B) using Wilcoxon rank-sum test (non-normally distributed variables). Correlation coefficients among measures of ovarian reserve were measured and expressed as Spearman's rho.

For each measure of ovarian reserve, a cutpoint was selected to optimize the positive predictive value for CROF (the probability that the subject who has an abnormal ovarian reserve test truly has CROF). Poisson regression methods were used to model the cumulative incidence of CROF and its association with individual and combinations of measures of ovarian reserve. Receiver-operating characteristic (ROC) curves were generated for each model, and the areas under the curve (AUC) among models were compared using the Chi-square test. A p-value of ≤ 0.05 was considered significant.

56 breast cancer survivors from the longitudinal cohort underwent a pelvic ultrasound and blood draw and provided menstrual data. Median age (range) at chemotherapy was 43.6 (30–56) years. Median age at study assessment was 48 (35–62). All subjects underwent

cyclophosphamide-based chemotherapy regimens; 38 (67%) of subjects had hormone-receptor positive breast cancer. Thirty-four subjects (60%) met criteria for CROF. The median (range) for each measure of ovarian reserve were as follows: AFC 0 (0–17), OV 2.2 (0.5–11.8) ml, FSH 44.2 (4.6–200.9) IU/L, AMH <25 (<25–1780) pg/mL, and inhibin <5 (<5–134.4) pg/mL.

Compared to menstruating subjects, CROF subjects had significantly lower median AFC (0 vs. 2) and OV (1.9 vs. 4.6 ml), along with lower AMH (<25 vs. 229 pg/mL) and inhibin B (<5 vs. 24.9 pg/mL) and higher FSH (79 vs. 19 IU/L) (all $p<0.001$). AFC was moderately correlated with OV (0.54), FSH (−0.39), AMH (0.54) and inhibin B (0.27). The cutpoints that optimized PPV for each measure of ovarian reserve were AFC <1, FSH ≥ 40, AMH ≤ 25, and inhibin B ≤ 5. AFC had the highest AUC compared to all other measures (Table). In addition, models incorporating AFC in addition to FSH or in addition to AMH had significantly higher AUC than FSH or AMH alone ($p=0.002$). Tamoxifen exposure was not associated with AFC ($p=0.43$).

In this cohort of late-reproductive aged breast cancer survivors, AFC appears to provide data on ovarian function that is additive to hormone measures of ovarian reserve. Ultrasound appears to be a tool that will not only corroborate, but also improve the discriminatory ability of hormone measures in determining ovarian failure in breast cancer survivors.

Ovarian function after breast cancer treatment is a clinically important survivorship issue with implications on fertility potential, menopausal concerns and breast cancer prognosis (9–11). In late-reproductive aged breast cancer patients, accurately identifying residual ovarian function after chemotherapy is especially critical for selection of adjuvant hormonal therapy (tamoxifen in premenopausal women and aromatase inhibitors in postmenopausal women). Because amenorrhea may not always represent a permanent cessation of ovarian function in these patients (12), surrogate measures of ovarian function are needed, even in patients who do not have fertility concerns. Therefore, the current study sought to test these potential measures of post-chemotherapy ovarian function in late reproductive-aged breast cancer patients. Moreover, to decrease misclassification by menstrual pattern, we sought to assess these measures at time points distant from chemotherapy.

To date, two other studies have examined AFC in breast cancer survivors, both of which compared AFC between regularly menstruating survivors and controls who did not have breast cancer and undergo chemotherapy (4,7). By demonstrating lower AFC in breast cancer survivors than in controls, the studies support the potential use of AFC as a surrogate measure of ovarian reserve in this population. In this context, the present study identified clinically useful cutpoints for both AFC and hormone measures. In addition, in comparing AFC to hormone measures, there is a suggestion that AFC captures additional data, implying the potential utility of pelvic ultrasounds in addition to blood assessment.

The study has several limitations. First, the sample size is small and results, especially cutpoints in measures of ovarian reserve, need validation in larger cohorts of breast cancer survivors. Second, assessment of ovarian function occurred post-chemotherapy and results are not meant to represent pre-chemotherapy predictors of post-chemotherapy function. An additional limitation is that ultrasounds were timed with oncology visits and therefore performed throughout the menstrual cycle for the 22 menstruating subjects. Recent data have demonstrated intra-cycle variability AFC in infertile women, but no significant trend in this variability by menstrual cycle phase (13). In light of these data, it is unlikely that our ultrasound data were differentially biased. While ultrasounds were performed using a standardized protocol on the same ultrasound machine to minimize inter-observer variation, intra- and inter-observer variability were not measured. Finally, because positive predictive

value differs based on the prevalence of disease, the cutpoints identified in this study are meant to be extrapolated to populations with a similar prevalence of CROF.

In conclusion, this study suggests a role for pelvic ultrasounds in assessing ovarian function in late-reproductive aged breast cancer survivors. AFC may be an independent, useful marker for ovarian failure.

Acknowledgments

Funding sources

Commonwealth of Pennsylvania Health Research Formula Funds (A.D.), ACS MRSG-08-110-01-CCE (I.S.), NIH HD058799 (I.S.)

References

1. Anders C, Marcom PK, Peterson B, Gu L, Unruhe S, Welch R, et al. A pilot study of predictive markers of chemotherapy-related amenorrhea among premenopausal women with early stage breast cancer. Cancer Invest. 2008; 26:286–95. [PubMed: 18317970]
2. Anderson RA, Themmen AP, Al-Qahtani A, Groome NP, Cameron DA. The effects of chemotherapy and long-term gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. Hum Reprod. 2006; 21:2583–92. [PubMed: 16820385]
3. Lutchman Singh K, Muttukrishna S, Stein RC, McGarrigle HH, Patel A, Parikh B, et al. Predictors of ovarian reserve in young women with breast cancer. Br J Cancer. 2007; 96:1808–16. [PubMed: 17533402]
4. Partridge AH, Ruddy KJ, Gelber S, Schapira L, Abusief M, Meyer M, et al. Ovarian reserve in women who remain premenopausal after chemotherapy for early stage breast cancer. Fertil Steril. 2009
5. Su HSMD, Green JA, Velders L, Stankiewicz C, Matro J, Freeman EW, Gracia CR, DeMichele A. Anti-mullerian hormone and inhibin B are hormone measures of ovarian function in late reproductive-aged breast cancer survivors. Cancer. 2009 in press.
6. Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update. 2006; 12:685–718. [PubMed: 16891297]
7. Lutchman Singh K, Davies M, Chatterjee R. Fertility in female cancer survivors: pathophysiology, preservation and the role of ovarian reserve testing. Hum Reprod Update. 2005; 11:69–89. [PubMed: 15569700]
8. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect. 2004; 112:1691–6. [PubMed: 15579415]
9. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. Lancet. 2005; 365:1687–717. [PubMed: 15894097]
10. Ganz PA, Greendale GA, Petersen L, Kahn B, Bower JE. Breast cancer in younger women: reproductive and late health effects of treatment. J Clin Oncol. 2003; 21:4184–93. [PubMed: 14615446]
11. Partridge AH, Gelber S, Peppercorn J, Sampson E, Knudsen K, Laufer M, et al. Web-based survey of fertility issues in young women with breast cancer. J Clin Oncol. 2004; 22:4174–83. [PubMed: 15483028]
12. Petrek JA, Naughton MJ, Case LD, Paskett ED, Naftalis EZ, Singletary SE, et al. Incidence, time course, and determinants of menstrual bleeding after breast cancer treatment: a prospective study. J Clin Oncol. 2006; 24:1045–51. [PubMed: 16476708]
13. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 25:221–7. [PubMed: 19840990]

Table 1

Test characteristics at ovarian reserve cutpoints

	AFC < 1	AMH ≤ 25 pg/mL	FSH ≥ 40IU/L	Inhibin B ≤ 5pg/ml
PPV	90%	79%	81%	76%
NPV	73%	56%	59%	41%
Specificity	89%	60%	64%	64%
Sensitivity	79%	76%	78%	54%

Table 2

Ovarian reserve measures and CROF

Model	AUC
AFC < 1	.82
AMH ≤ 25 pg/ml	.71
FSH ≥ 40IU/L	.72
Inhibin B ≤ 5pg/ml	.63
AFC+AMH	.87*
AFC+FSH	.87*
AMH+FSH	.74

* p=0.002 Vs. AMH or FSH only