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Correlation between sonographic and endocrine markers of ovarian aging as predictors for late menopausal transition

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Abstract

Objective: Recent studies suggest that ovarian volume and antral follicle counts (AFCs) may be useful indicators of menopause status. In this study, we examined several sonographic and endocrine markers of ovarian aging for their ability to discriminate between premenopausal and late menopausal transition (LMT) status.

Methods: A total of 40 women aged 40 to 55 years were enrolled in this cross-sectional study. Premenopausal women (n = 21) were required to have regular menstrual cycles (24 to 35 days), and women in LMT (n = 19) must have experienced 3 to 11 months of amenorrhea. Participants underwent a transvaginal ultrasound to determine ovarian volume and AFCs; provided blood for the measurement of antimüllerian hormone (AMH), follicle-stimulating hormone (FSH), luteinizing hormone, and estradiol; and completed a questionnaire. The correlation between ovarian aging markers and AFCs was investigated. The area under the receiver operating characteristic curve (ROC_{AUC}) was calculated as a measure of diagnostic accuracy.

Results: Serum AMH levels were more strongly correlated with AFCs than were serum levels of FSH, luteinizing hormone, and estradiol. Serum levels of AMH and FSH had the highest diagnostic accuracy (ROC_{AUC}, 0.893 and 0.890, respectively) for LMT. The inclusion of FSH to AMH in a multivariable model improved the diagnostic accuracy (ROC_{AUC}, 0.932); however, FSH did not have a statistically significant relationship with LMT, whereas AMH tended to be significant ($P = 0.017$). The ROC curves for sonographic makers (AFC and ovarian volume) and AMH in determining LMT differed significantly ($z = 1.76$, $P < 0.05$; $z = 1.86$, $P < 0.05$, respectively).

Conclusions: AMH alone or in combination with FSH may be a useful indicator of LMT. These data suggest that sonographic markers cannot be substituted for AMH in determining LMT. However, we cannot definitively say that endocrine markers (especially AMH as a single indicator) are better than sonographic markers for determining LMT because serum AMH levels have a strong correlation with AFCs.

Key Words: Ovarian volume – Antral follicle counts – Antimüllerian hormone – Follicle-stimulating hormone – Late menopausal transition.

The quantitative aspect of ovarian aging is reflected by a decline in the size of the primordial follicle pool. Direct measurement of the primordial follicle pool is impossible. The pattern of age-related decline in the number of antral follicles is similar to the decline in total follicle numbers, as described in the study of Faddy and Gosden.[1] So far, assessment of the number of antral follicles by ultrasonography, the antral follicle count (AFC), best predicts the quantitative aspect of ovarian reserve.[2] The loss of primordial follicles and the corresponding changes in hormone levels lead to the reduction of ovarian volume.[3] Recent reports suggest that ovarian volume and AFCs may be sensitive and specific markers of reproductive aging or menopause status.[4,5] AFC and ovarian volume, which are compared with follicle-stimulating hormone (FSH) levels to detect postmenopause status,[6] have been proposed as markers of the menopausal transition.[7,8] During the transition, hormone levels often vary markedly; hence, FSH and estradiol (E_2) levels are unreliable indicators of menopause status.[9]

Guidelines for classifying the stages of reproductive aging were proposed in 2001 at the Stages of Reproductive Aging Workshop (STRAW).[10] Menopausal transition was divided into early menopausal transition (EMT) and late menopausal transition (LMT) stages. A recent study suggested that antimüllerian hormone (AMH) level is a promising predictor of the menopausal transition (characterized by cycle irregularity).[11] This study led to the conclusion that AMH is a better predictor of EMT than are conventional markers, such as FSH,

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ovarian volume, and AFC. However, recent publications have recommended the use of ovarian volume and AFC as markers for determining menopause status.[6,12] AFC and ovarian volume, which are compared with FSH levels, were proposed as markers of postmenopause status.[6] In addition, ovarian volume and AFCs may be early indicators of the menopausal transition because they are thought to change before FSH levels.[7,8,13]

The question of whether sonographic measurement predicts LMT better than serum FSH and AMH measurement does remains unanswered. Hence, the present study focused on the usefulness of sonographic markers, such as ovarian volume and AFCs, to predict LMT. If sonographic markers can predict LMT status as accurately as FSH and AMH can, clinicians can determine LMT status as soon as they visualize the ovaries with ultrasound without having to send blood samples to a laboratory for hormone level analysis. However, once women reached the late transition, with more than 3 months of amenorrhea, marked decreases in E_2 and inhibin α levels and significant increases in FSH levels were observed.[14] With respect to other known markers, AMH seems to better reflect the continuous decline of the oocyte/follicle pool with age.[11] The decrease in AMH with advancing age may comparably occur before the changes in other recognized aging-related variables, indicating that serum AMH levels may be the best marker for ovarian aging and menopausal transition.

Therefore, the purpose of this study was to test our hypothesis that endocrine markers, such as FSH and AMH levels, are more effective than sonographic markers in determining LMT.

To our knowledge, these markers' accuracy in determining LMT has not been directly compared. Moreover, no existing studies have compared sonographic and endocrine markers in Asian women.

METHODS

Study sample

This observational, cross-sectional study included 40 women who were recruited through local advertisements. The preliminary sample consisted of 43 women; however, 3 women were excluded from the database because ultrasound examinations revealed that they had only one ovary. The women were premenopausal and LMT women between 40 and 55 years of age who were instructed to call the clinic office if they were interested in participating in a research study on hormones, ovary size, and menopause. Women who called the office were screened for eligibility. Women were eligible if they were between 40 and 55 years old, had not undergone ovarian surgical operation/removal or hysterectomy, did not receive hormone therapy (HT), did not have ovarian cysts or follicles larger than 10 mm, did not have cancer of the reproductive organs, and did not receive any chemotherapy or radiation treatment in their lifetime. No participant had any evidence of endocrine disorders; all had normal prolactin and thyroid-stimulating hormone levels and no evidence of polycystic ovarian syndrome. This study was approved by the institutional review board of the Eulji University Hospital, and an informed consent form was obtained from all patients and controls before they were included in the study.

Data collection

Each participant made one brief visit to the gynecology division of Eulji University Hospital in Daejeon, South Korea. They were interviewed about their demographic, social, and medical conditions between August 2008 and April 2009, and then underwent a transvaginal ultrasound to evaluate their genital internal organs. A single observer performed all examinations. This clinic visit was scheduled on day 1, 2, or 3 of the menstrual cycle for premenopausal women. All amenorrheic women received didrogesterone (10 mg/d for 7 d). If uterine bleeding occurred, blood samples were taken 1 to 3 days after the bleeding began. In the absence of bleeding, blood samples were taken 7 days after didrogesterone withdrawal. During the clinic visit, each participant underwent a transvaginal ultrasound, provided a blood sample, and completed a detailed questionnaire that asked about each woman's age, body mass index (BMI), smoking and medication history, menstrual history, and reproductive history. Questionnaire data were also used to categorize each participant as either premenopausal or LMT. Menopausal status was based on the women's response to an interview about the characteristics of their menses and its cessation. Premenopausal women were defined as those who had not yet experienced any change in menstrual frequency or flow, and women in the LMT were defined by 3 to 11 months of amenorrhea. BMI was calculated by dividing weight in kilograms by height squared (meters squared).

Hormone assays

Blood samples were obtained by venipuncture on cycle days 1 through 3 for regularly menstruating women or between 1 and 3 days after the beginning of didrogesterone withdrawal bleeding for nonmenstruating women, to measure the serum levels of AMH, FSH, luteinizing hormone (LH), and E_2. Serum was separated from the blood samples and stored at −20°C until assayed. Samples from a given participant were analyzed for each hormone in the same assay to avoid interassay variation. Serum levels of E_2, LH, and FSH were measured by chemiluminescent immunoassay using commercial kits (ADVIA Centaur, Bayer Corporation, Tarrytown, New York). The detection limits of the assay were 10 pg/mL for E_2, 0.2 mIU/mL for LH, and 0.1 mIU/mL for FSH. Intra-assay and interassay coefficients of variation, respectively, were 4.9% and 5.7% for E_2, 3.2% and 6.9% for LH, and 3.3% and 7.8% for FSH. Serum levels of AMH were determined with enzyme-linked immunosorbent assays, using commercial kits from Diagnostic Systems Laboratories Inc. (Webster, TX). The detection limits of this assay were 0.006 ng/mL for AMH, and its intra-assay and interassay coefficients of variations were 4.6% and 8.2%, respectively, for AMH.

Ultrasound examination

All ultrasound examinations were performed by a single observer who was blinded to the results of the hormone assays, using SSD 1000 Aloka ultrasound equipment and a transvaginal 5-MHz frequency probe. These transvaginal ultrasound examinations were used to obtain the ovarian volume and AFCs of both ovaries on days 1 through 3 of the menstrual cycle or of progesterone withdrawal bleeding. Some parameters were applied to exclude participants with conditions that could have impaired an accurate estimate of ovarian volume and AFCs, including unilateral oophorectomy, cysts or ovarian masses larger than 20 mm, pregnancy, inflammatory pelvic disease, gonadal dysgenesis, undetermined menopause status, or secondary amenorrhea. Cases in which only one ovary could be located during transvaginal ultrasounds were excluded from the analysis. Consequently, 40 women were included in the statistical analysis.

Ovarian volume was calculated as the product of the longitudinal, transverse, and anteroposterior dimensions × 0.526.[15] Data for ovarian volume were based on the mean values of all available measurements (cm^3) for each woman. For each ovary, ovarian volume was measured two times by the same observer. The left and right ovarian volumes were pooled into a single volume. Mean ovarian volume was calculated when both right and left ovaries could be measured with ultrasound. Ovarian volume showed excellent intraobserver agreement, with an intraclass correlation coefficient of 0.984. There was no significant difference between right ovarian volume and left ovarian volume, as indicated by intraobserver agreement and an intraclass correlation coefficient of 0.878.

Right and left ovarian AFCs were also averaged to create a single follicle count for each participant. The ovary was examined by scanning from the outer to the inner margin. All round or oval sonolucent structures within the contour of the ovary were considered follicles and were measured and counted as such. For the data analysis, only follicles 2 to 10 mm in size were included because follicles smaller than 2 mm were not visible in the ultrasound and none of the eligible participants had follicles larger than 10 mm.

Data analysis

All data were analyzed using SPSS statistical software (SPSS, Inc., Chicago, IL). Premenopausal and LMT women's mean FSH, LH, E_2, and AMH levels; age; ovarian volume; and AFCs were compared using *t* tests, with the significance level set at $P \leq 0.05$. To assess the relationships between ovarian aging markers and AFC in premenopause and LMT, Pearson's correlation coefficients were calculated. We drew scatter plots for the correlations between ovarian aging markers and AFCs. Univariate and multivariate logistic regression analyses were used to assess the relationships among ovarian aging markers in premenopause and LMT. For the multiple analysis, a backward stepwise selection with *P* less than 0.05 for entry was applied. All variables with statistically significant associations with LMT in the univariate analysis were included in the multivariate logistic regression. The area under the receiver operating characteristic curve (ROC_{AUC}) was calculated to assess the ability to discriminate between premenopausal and LMT status. These curves were produced by plotting the relationship between the proportions of true positives (sensitivity) against the proportion of false-positives (1 − specificity). The ROC_{AUC} may vary between 0.5 (no discriminative power) and 1.0 (perfect discrimination). The areas under two ROC curves were compared using a *Z* test, with the significance set at less than 0.05.

RESULTS

Sample characteristics are shown in Table 1. The mean ages were significantly different for premenopausal (46.1 ± 3.0 y) and LMT (49.0 ± 3.1 y) women ($P = 0.006$). However, there were no statistically significant differences in BMI (23.1 ± 2.7 vs 22.7 ± 2.6 kg/m^2; $P = 0.10$), parity (1.7 ± 0. 7 vs 1.9 ± 0.5; $P = 0.25$), and E_2 (57.4 ± 28.5 vs 42.8 ± 29.3 pg/mL, $P = 0.117$) between the premenopause and LMT groups. Endocrine markers of ovarian aging (serum levels of FSH, LH, and AMH) other than serum E_2 levels were significantly different in the premenopause and LMT groups ($P < 0.001$). The women in LMT had higher FSH and LH levels as well as lower AMH levels than the women in premenopause. Sonographic markers of ovarian aging, such as ovarian volume and AFCs, were also significantly different for premenopause and LMT women ($P < 0.05$). The women in premenopause had a mean ovarian volume of 2.5 ± 1.4 cm^3 and mean AFCs of 3.3 ± 1.3, whereas the women in LMT had a mean ovarian volume of 1.6 ± 0.7 cm^3 and mean AFCs of 1.2 ± 0.9. As expected, AFCs and ovarian volume in the women in LMT were significantly lower than those of the women in premenopause.

Relationships between AFCs and age, ovarian volume, and serum levels of FSH, LH, E_2, and AMH on cycle day 3 (for premenopausal women) or progesterone withdrawal bleeding day 3 (LMT) are shown in Figure 1. Ovarian volume was highly correlated with AFCs ($r = 0.779$, $P < 0.0001$). AMH,

TABLE 1. *Characteristics of the study participants and ovarian aging markers in premenopause and late menopausal transition*

	Premenopause (n = 21)	Late menopausal transition (n = 19)	*P*
Age, y	46.1 ± 3.0	49.0 ± 3.1	0.006
BMI, kg/m^2	23.1 ± 2.7	22.7 ± 2.6	0.10
Parity	1.7 ± 0.7	1.9 ± 0.5	0.25
Antral follicle count	3.3 ± 1.3	1.2 ± 0.9	0.002
Ovarian volume, cm^3	2.5 ± 1.4	1.6 ± 0.7	0.015
FSH, mIU/mL	13.3 ± 10.8	55.0 ± 39.5	<0.0001
LH, mIU/mL	8.7 ± 3.9	24.5 ± 18.0	<0.0001
Estradiol, pg/mL	57.4 ± 28.5	42.8 ± 29.3	0.117
AMH, ng/mL	1.2 ± 1.6	0.1 ± 0.3	0.008

Values are means ± SD.
BMI, body mass index; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, antimüllerian hormone.

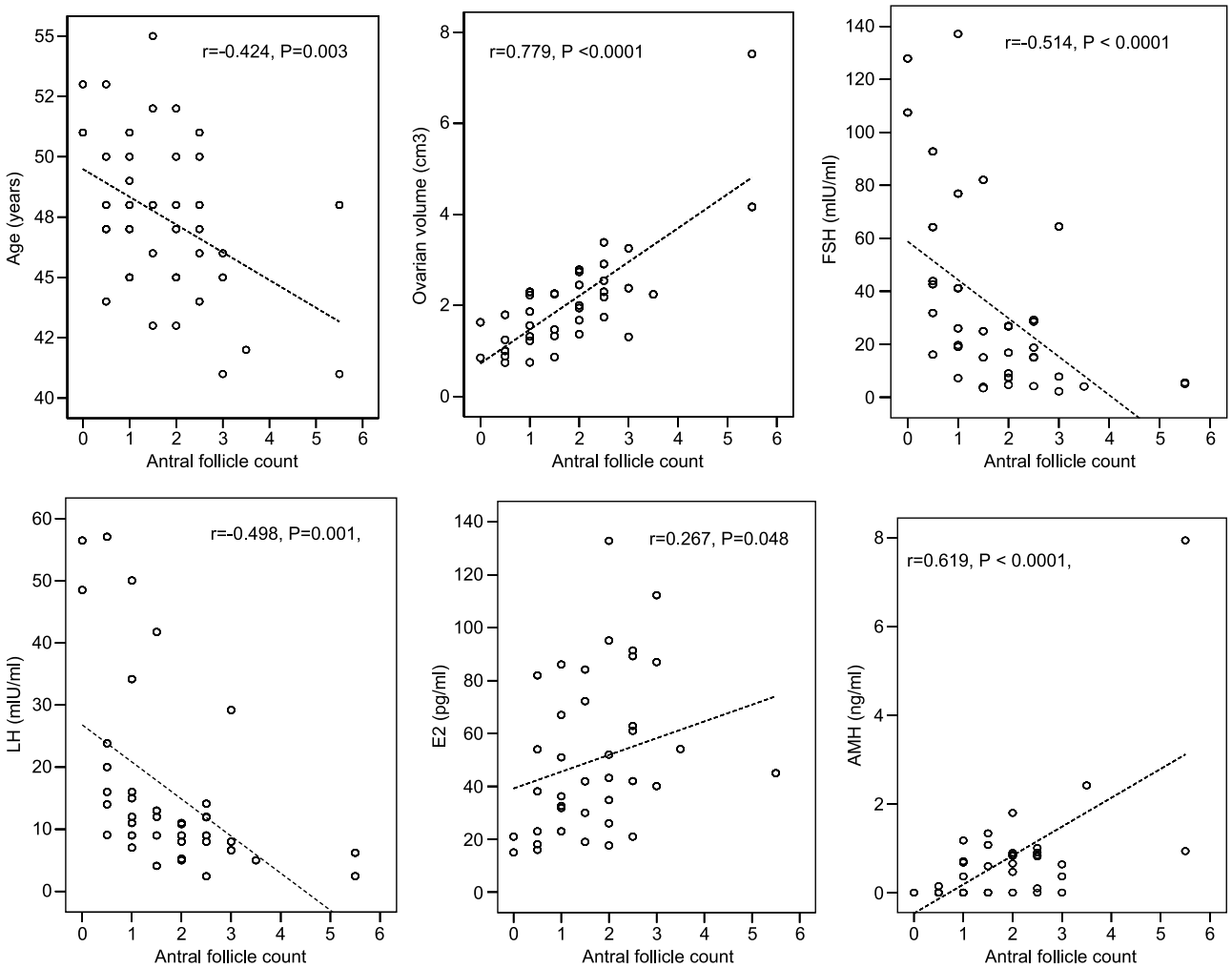

FIG. 1. Relationships between the antral follicle count and age, ovarian volume, and serum levels of FSH, LH, E_2, and AMH on cycle day 3 (for premenopausal women) and progesterone withdrawal bleeding day 3 (for late menopausal transition women). Pearson's correlation coefficient (r) is followed by the P value. Ovarian volume and serum AMH levels were strongly correlated with antral follicle count ($P < 0.0001$) than were other endocrine markers of ovarian aging, such as FSH, LH, and E_2. FSH, follicle-stimulating hormone; LH, luteinizing hormone; E_2, estradiol; AMH, antimüllerian hormone.

FSH, LH, and E_2 levels were significantly correlated with AFCs. A good correlation was observed between AMH and AFCs, comparable with the correlation between ovarian volume and AFCs. Notably, the correlation between serum AMH levels and AFCs ($r = 0.619$, $P < 0.0001$) was stronger than the correlations between serum levels of FSH, LH, and E_2

TABLE 2. *Logistic regression for prediction of late menopausal transition*

	Odds ratio (95% CI)	P	ROC_{AUC} (95% CI)
Univariate analysis			
Age, y	1.36 (1.07-1.74)	0.013	0.73 (0.58-0.89)
Antral follicle count	0.29 (0.12-0.70)	0.006	0.79 (0.65-0.93)
Ovarian volume, cm^3	0.28 (0.10-0.80)	0.018	0.75 (0.60-0.91)
AFC + OV	0.66 (0.47-0.91)	0.012	0.81 (0.67-0.94)
FSH, mIU/mL	1.10 (1.03-1.18)	0.005	0.89 (0.79-0.99)
LH, mIU/mL	1.21 (1.03-1.42)	0.022	0.80 (0.66-0.94)
Estradiol, pg/mL	0.98 (0.96-1.01)	0.123	0.50 (0.32-0.68)
AMH, ng/mL	0.01 (0.001-0.13)	<0.0001	0.89 (0.79-0.99)
Multivariate analysis			
FSH, mIU/mL	1.07 (0.99-1.16)	0.094	
AMH, ng/mL	0.042 (0.003-0.573)	0.017	0.93 (0.85-1.01)

Logistic regression model: $P = \frac{e(0.79 + 1.07 \times \text{FSH} + 0.042 \times \text{AMH})}{1 + e(0.79 + 1.07 \times \text{FSH} + 0.042 \times \text{AMH})}$, where P = probability of menopausal transition.

ROC_{AUC}, area under the receiver operating characteristic curve; AFC, antral follicle count; OV, ovarian volume; FSH, follicle-stimulating hormone; LH, luteinizing hormone; AMH, antimüllerian hormone.

and AFCs ($r = -0.514$, $P < 0.0001$; $r = -0.498$, $P = 0.001$; $r = 0.267$, $P = 0.48$, respectively).

The univariate logistic regression analysis results are presented in Table 2. Age, AFCs, ovarian volume, AFCs plus ovarian volume, and serum FSH, LH, and AMH levels were significantly associated with LMT, whereas serum E_2 level was not. Based on the ROC_{AUC}, AMH and FSH had the best discriminative potential for predicting LMT. The AUC values were 0.893 and 0.890 for AMH and FSH, respectively, in the univariate analysis. AFCs plus ovarian volume (ROC_{AUC}, 0.81), AFCs (ROC_{AUC}, 0.79), and LH (ROC_{AUC}, 0.80) had relatively good discriminative potential and were better markers than ovarian volume (ROC_{AUC}, 0.75) and age (ROC_{AUC}, 0.73). E_2 levels (ROC_{AUC}, 0.50) were not significantly associated with the outcome measure. Five variables that showed a statistically significant association with LMT in the univariate analysis were included in the multivariate logistic regression. These variables were as follows: age, AFCs, ovarian volume, FSH, and AMH. In the multivariate stepwise logistic analysis, the variables AMH and FSH were selected in that order. The ROC_{AUC} increased from 0.893 for AMH alone and 0.890 for FSH alone, respectively, to 0.932 in the logistic regression model. Including FSH and AMH together in a multivariate model improved this predictive value; however, FSH did not have a statistically significant relationship with LMT, whereas AMH tended to be significant ($P = 0.017$).

To determine whether endocrine markers (such as AMH and FSH), sonographic markers (such as AFCs, ovarian volume, and AFCs plus ovarian volume), or a logistic regression model has the best discriminative potential, we compared these methods' diagnostic accuracy in differentiating between premenopause and LMT (Fig. 2). AMH showed better diagnostic accuracy than did AFCs or ovarian volume observed by ultrasound ($z = 1.76$, $P < 0.05$; $z = 1.86$, $P < 0.05$, respectively). In addition, the diagnostic accuracy of the logistic regression model including AMH and FSH was significantly better than that of AFCs plus ovarian volume determined by ultrasound ($z = 2.99$, $P < 0.05$). FSH level provided similar accuracy as AFC ($z = 1.55$, $P = 0.06$) in its ability to differentiate between premenopause and LMT.

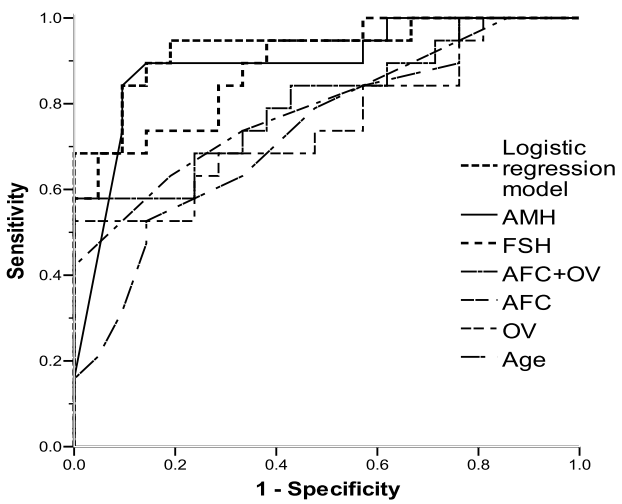

FIG. 2. Receiver operating characteristic curves of endocrine and sonographic markers of ovarian aging for differentiating between premenopause and late menopausal transition. AMH, antimüllerian hormone; FSH, follicle-stimulating hormone; AFC, antral follicle count; OV, ovarian volume.

DISCUSSION

The term menopausal transition describes a time of increasing variability in the menstrual cycle before a woman's final menstrual period. How such variability should be determined has not been explicitly defined. Guidelines for classifying the stages of reproductive aging were proposed in 2001 at the STRAW.[10] The guidelines divide reproductive life into three phases (early, peak, and late) and divide the menopausal transition into early and late stages, with some ambiguity about the onset of EMT. No uniform definition for the transition to menopause (cycle irregularity) is available, but some recent proposals suggest defining transition as an increasing variability in cycle patterns.[12,16] Most women who are symptomatic during the menopausal transition present with amenorrhea, frequent or excessive bleeding, or hot flashes and other symptoms of estrogen deficiency. The prevalence of hot flashes increases as the menopausal transition progresses, reaching a high of about 63% during the LMT.[17,18] Accurate detection of the LMT is critical because it may enable physicians to prescribe treatments or preventive measures that could reduce a woman's risk of vasomotor symptoms and of osteoporosis in later life. The relative safety of HT during the menopausal transition has not been thoroughly investigated. The results of one observational study suggest that women who start HT (estrogen alone or in combination with progestin) near menopause have a decreased risk of coronary heart disease.[19] Ongoing studies are evaluating the safety and efficacy of HT during the menopausal transition and the early postmenopausal years.[20] Therefore, these current studies indicate the increasing importance of accurately detecting the LMT. The LMT was defined in STRAW as two skipped cycles and an interval of amenorrhea of 60 days or more. In the Melbourne Women's Midlife Health Project, a slightly different nomenclature was used,[21] with LMT defined as 3 to 11 months of amenorrhea. In the present study, we applied the Melbourne Women's Midlife Health Project definition. The median age at menopause is 50 years, but individual ages at natural menopause can range from 40 to 60 years.[22,23] When a middle-aged woman has amenorrhea for 3 months or more, clinicians need to evaluate whether she is in the LMT or is experiencing secondary amenorrhea.

A profound decrease in the follicular phase concentrations of inhibin β and slightly increased FSH levels seem to be the first endocrine marker of the EMT; however, they are not statistically significantly different from those in women with regular cycles. Van Rooij et al[11] found that AMH is a powerful predictor of the EMT and that adding the combined measurements of AMH and inhibin β improved its predictive power.[11] They also found that AFC and FSH performed less

well in predicting cycle irregularity (EMT) compared with AMH, inhibin β, and age.[11] However, their study was not conducted with the aim of predicting the LMT. Tehrani et al[24] followed 147 naturally fertile 40- to 50-year-old women with regular menstrual cycles three times at 3-year intervals and measured their blood levels of AMH. They found that a single AMH measurement was a good predictor of continuing normal menstrual cycles for the next 6 years in fertile, late reproductive-aged women. This study has some limitations, however: other ovarian aging markers were not measured, and therefore, comparisons among different aging markers and AMH levels were not possible. Previous longitudinal studies have shown that AMH is a novel test to predict EMT[14] and menopause status in late reproductive–aged women.[24] However, there are no longitudinal data for AMH's ability to predict LMT when combined with other endocrine and sonographic markers of ovarian aging. Our study aimed to verify the results of these previous studies[6,11,12,24] of the predictability of LMT. To our knowledge, our cross-sectional study is the first of its kind to attempt to discriminate between premenopause and LMT using AMH levels in combination with other endocrine and sonographic ovarian aging markers. Because changes in ovarian volume and AFCs are likely to occur before the menses cease, these measures may serve as earlier indicators of postmenopause status than menstrual status.[7,8,13] A recent study suggests that ovarian volume and AFCs had sensitivity and specificity similar to FSH levels and age and that ovarian volume and AFCs may be useful indicators of menopause status.[6] The results of a different study of another population of healthy women confirmed these observations.[12] These authors reported that ovarian volume, AFC, and chronological age are all individually predictive of menopause status, with similar accuracies.[12] Previous studies suggested that sonographic measurements were likely to serve as earlier indicators of postmenopause status. However, these results were limited because endocrine markers of ovarian aging (eg, FSH or AMH levels) were absent. In contrast to previous studies of ovarian aging markers as postmenopause status predictors,[6,12] we compared the diagnostic accuracy of endocrine and sonographic markers in differentiating between premenopause and LMT.

Hormonal changes, such as increased FSH and LH levels, do not indicate perimenopause, and normal hormone levels have been identified during this period.[25] Scheffer et al[2] experimentally evaluated predictors of ovarian aging and found that AFC (diameters of 2-10 mm) was a better predictor than either ovarian volume or biochemical markers such as E_2, inhibin β, and FSH, although a strong correlation was established between all indicators. Thus far, ultrasound assessment of the number of antral follicles (the AFC) best predicts the quantitative aspect of ovarian reserve.[2] However, once women reached the late transition, with more than 3 months of amenorrhea, marked decreases in E_2 and inhibin α and significant increases in FSH level were observed.[14] We expected FSH levels and AMH levels to determine LMT more accurately than sonographic markers would, including AFCs and ovarian volume. However, there were no significant differences between FSH and AFCs. As expected, AMH was more diagnostically accurate than AFCs or ovarian volume observed by ultrasound. In addition, the diagnostic accuracy of the logistic regression model that included AMH and FSH was also better than that of AFCs plus ovarian volume observed by ultrasound, and the difference was statistically significant. Thus, AMH was more diagnostically accurate than age or ultrasound markers such as AFC and ovarian volume.

The present investigation was also designed to evaluate the direct relationship between serum AMH levels and AFCs and to compare the strength of correlations between AFCs and the usual menopause status markers. Previous studies reported that serum AMH levels were closely related to AFC and that this relationship was remarkably more strong than the relationships between AMH and inhibin β, E_2, FSH, or LH.[26-28] Those results agreed with our findings of a significant correlation between serum AMH levels and AFCs and a stronger correlation between AMH and AFC than between AMH and E_2, FSH, or LH. This evidence reinforces the correlation between serum AMH levels and AFCs and suggests that AMH may reflect ovarian follicular status better than the usual hormonal markers do.

AMH and FSH were selected for a multivariate analysis. Including FSH with AMH in a multivariate model increased its predictive value to 0.93. FSH did not have a statistically significant relationship with LMT, whereas AMH tended to be significant ($P = 0.017$). Results of a previous study suggest that the associations between potential predictors did not improve their accuracy for predicting menopause status.[12] In contrast, Van Rooij et al[11] suggested that combining ovarian aging markers such as AMH, inhibin β, and age in a multivariate logistic model seemed to improve its predictive value. The current study also suggests that including inhibin β and FSH concentrations with AMH in a multivariate model could improve its ability to predict ovarian response.[29] In agreement with the studies described previously, our study revealed that combining ovarian aging markers in a multivariate logistic model improved its accuracy in predicting LMT.

Previous data indicated that age, ovarian volume, AFC, and FSH are similarly predictive of postmenopause status.[6,12] Another study found that age was a good predictor of EMT.[11] These studies examined a positively selected study population with proven natural fertility. Therefore, age may have performed better in this study population. Our study did not include a positively selected population. Therefore, our study population was likely to contain some women with unrecognized subfertility. The present study found that chronological age was a poor predictor of LMT. It is probable that AMH and FSH are more important predictors than age. Therefore, the additional value of measuring AMH and FSH may be important.

Currently, FSH is incorporated into the STRAW staging system as an endocrine marker to discern the menopausal transition. FSH was chosen because it can be readily assayed in most laboratories, unlike AMH and inhibin β.[10] Van Rooij

et al[11] have provided evidence that FSH is a poorer predictor of EMT than AMH is and was selected only in a minority of cases. Contrary to previous results,[11] we found that FSH was a relatively useful marker because it has similar accuracy as AMH, which is a better single predictor of the LMT, but AFC has similar accuracy as FSH for LMT. Analyses of early follicular phase serum samples from the Study of Women's Health Across the Nation suggest that although single FSH measure is an independent marker of LMT, it is less predictive than menstrual bleeding criteria such as 60-day intermenstrual interval.[30] Additional analyses on early cycle sonographic marker or AMH measurements from this study are awaited. The present study indicated that the diagnostic value of FSH is comparable with that of AFC, and, therefore, FSH could be replaced by AFC in discriminating between premenopause and LMT. However, the diagnostic accuracy of AMH was better than that of AFCs or ovarian volume determined by ultrasound, and the difference was statistically significant. Therefore, sonographic measurements did not reflect ovarian aging better than AMH did. However, it is not enough that endocrine markers (especially AMH as a single indicator) is better than sonographic markers for determining LMT because two markers (AMH and AFCs) are strongly correlated.

There are advantages to using AMH instead of AFC or ovarian volume to predict LMT because AMH testing allows all predictive information to be obtained with blood sampling and no extra ultrasound is needed. Furthermore, because there is no change in AMH levels in response to gonadotrophins, AMH can be measured throughout the cycle, unlike other parameters that can be determined only during the early follicle phase. Serum AMH demonstrated less individual intracycle and intercycle variation than AFCs did and may therefore be considered a more reliable means of assessing ovarian reserve.[31] In our study, AFCs and ovarian volume were better indicators of LMT than premenopause. However, AFC measurement required an additional transvaginal ultrasound examination during the early follicular phase. Moreover, combining sonographic and endocrine markers did not improve the diagnostic accuracy in our study. There were no benefits or synergic effects from combining sonographic and endocrine markers versus using a single marker, such as AMH. These results indicate that serum AMH is the best single marker for determining LMT than other endocrine and sonographic markers of ovarian aging.

Our research was limited because this was a small, cross-sectional study with a limited number of events. Although not conclusive, our data suggest that endocrine markers, such as AMH or FSH levels, determined LMT more accurately than sonographic markers did. Future large-scale longitudinal studies may be more conclusive.

CONCLUSIONS

The present study provides strong evidence that serum AMH is a better single marker for determining LMT than are other endocrine and sonographic markers of ovarian aging and that adding FSH levels to AMH in a multivariate model improves its diagnostic accuracy. These data suggest that sonographic markers cannot be substituted for AMH as LMT predictors. The diagnostic value of FSH is comparable with that of AFC; therefore, FSH could be replaced by AFC. However, we cannot definitively say that endocrine markers (especially AMH as a single indicator) are better than sonographic markers for determining LMT because the two markers (AMH and AFCs) were strongly correlated. Hence, further large-scale longitudinal studies are necessary to confirm our results.

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REFERENCES

1. Faddy MJ, Gosden RG. A model conforming the decline in follicle numbers to the age of menopause in women. *Hum Reprod* 1996;11: 1484-1486.
2. Scheffer GJ, Broekmans FJ, Looman CW, et al. The number of antral follicles in normal women with proven fertility is the best reflection of reproductive age. *Hum Reprod* 2003;18:700-706.
3. Erdem M, Erdem A, Biberoglu K, Arslam M. Age-related changes in ovarian volume, antral follicle counts and basal follicle stimulating hormone levels: comparison between fertile and infertile women. *Gynecol Endocrinol* 2003;17:199-205.
4. Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER. Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. *Fertil Steril* 1999;72:845-851.
5. Flaws JA, Rhodes JC, Langenberg P, Hirshfield AN, Kjerulff K, Sharara FI. Ovarian volume and menopausal status. *Menopause* 2000;7:53-61.
6. Flaws JA, Langenberg P, Babus J, Hirshfield AN, Sharara FI. Ovarian volume and antral follicle counts as indicators of menopausal status. *Menopause* 2001;8:175-180.
7. Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Accelerated disappearance of ovarian follicles in midlife: implications for forecasting menopause. *Hum Reprod* 1992;7:1342-1346.
8. te Velde ER, Scheffer GJ, Dorland M, Broekmans FJ, Fauser BC. Developmental and endocrine aspects of normal ovarian aging. *Mol Cell Endocrinol* 1998;145:67-73.
9. Burger HG, Dudley EC, Robertson DM, Dennerstein L. Hormonal changes in the menopause transition. *Recent Prog Horm Res* 2002;57: 257-275.
10. Soules MR, Sherman S, Parrott E, et al. Stages of Reproductive Ageing Workshop (STRAW). *J Womens Health Gend Based Med* 2001; 10:843-848.
11. Van Rooij IA, Tonkelaar I, Broekmans FJ, et al. Anti-müllerian hormone is a promising predictor for the occurrence of the menopausal transition. *Menopause* 2004;11:601-606.
12. Giacobbe M, Mendes Pinto-Neto A, Simões Costa-Paiva LH, Martinez EZ. The usefulness of ovarian volume, antral follicle count and age as predictors of menopausal status. *Climacteric* 2004;7:255-260.
13. Burger HG. The endocrinology of the menopause. *J Steroid Biochem Mol Biol* 1999;69:31-35.
14. Burger HG, Cahir N, Robertson DM, et al. Serum inhibins A and B fall differentially as FSH rises in perimenopausal women. *Clin Endocrinol* 1998;48:809-813.
15. Sharara FI, McClamrock HD. The effect of aging on ovarian volume measurements in infertile women. *Obstet Gynecol* 1999;94:57-60.
16. Lenton EA, Sexton L, Lee S, Cooke ID. Progressive changes in LH and FSH and LH: FSH ratio in women throughout reproductive life. *Maturitas* 1988;10:35-43.
17. Mitchell ES, Woods NF, Mariella A. Three stages of the menopausal transition from the Seattle Midlife Women's Health Study: toward a more precise definition. *Menopause* 2000;7:334-349.

18. Dennerstein L, Dudley EC, Hopper JL, Guthrie JR, Burger HG. A prospective population-based study of menopausal symptoms. *Obstet Gynecol* 2000;96:351-358.
19. Grodstein F, Manson JE, Stampfer MJ. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. *J Womens Health (Larchmt)* 2006;5:35-44.
20. Manson JE, Bassuk SS, Harman SM, et al. Postmenopausal hormone therapy: new questions and the case for new clinical trials. *Menopause* 2006;13:139-147.
21. Dennerstein L. Well-being symptoms and the menopausal transition. *Maturitas* 1996;23:147-157.
22. Sowers MR, La Pietra MT. Menopause: its epidemiology and potential association with chronic diseases. *Epidemiol Rev* 1995;17:287-302.
23. Khaw KT. Epidemiology of the menopause. *Br Med Bull* 1992;48:249-261.
24. Tehrani FR, Solaymani-Dodaran M, Azizi F. A single test of anti-mullerian hormone in late reproductive–aged women is a good predictor of menopause. *Menopause* 2009;16:797-802.
25. Prior JC. Perimenopause: the complex endocrinology of the menopausal transition. *Endocr Rev* 1998;19:397-428.
26. Burger HG, Dudley EC, Hopper JL, et al. Prospectively measured levels of serum follicle-stimulating hormone, estradiol, and the dimeric inhibins during the menopausal transition in a population-based cohort of women. *J Clin Endocrinol Metab* 1999;84:4025-4030.
27. Durlinger AL, Visser JA, Themmen AP. Regulation of ovarian function: the role of anti-Mullerian hormone. *Reproduction* 2002;124:601-609.
28. Fanchin R, Schonauer LM, Righini C, Guibourdenche J, Frydman R, Taieb J. Serum anti-Mullerian hormone is more strongly related to ovarian follicular status than serum inhibin B, estradiol, FSH and LH on day 3. *Hum Reprod* 2003;18:323-327.
29. van Rooij IA, Broekmans FJ, te Velde ER, et al. Serum anti-Mullerian hormone levels: a novel measure of ovarian reserve. *Hum Reprod* 2002; 17:3065-3071.
30. Randolf JF, Crawford SJ, Dennerstein L. The value of follicle-stimulating hormone concentration and clinical findings as markers of the late menopause transition. *J Clin Endocrinol Metab* 2006;91:3034-3040.
31. van Disseldorp J, Lambalk CB, Kwee J, et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. *Hum Reprod* 2010;25:221-227.