Meno√Check®

Assay sensitivity does matter in the determination of menopausal status

Menopause is a woman's status after her final menstrual period (FMP). It is an ageing phenomenon reflecting the loss of ovarian follicular function. Women across the globe experience menopause at a similar age with minimal geographical variation. Healthy women reach menopause at a mean age of 52 years with variation in +/- 2 years. But some women reach menopause at 40 years or earlier due to lifestyle changes or medical conditions such as primary ovarian insufficiency (POI), diminished ovarian reserve, surgical menopause, auto immune illness etc. Induced menopause refers to cessation of menstruation due to bilateral oophorectomy iatrogenic ablation of ovarian function (e.g. by chemotherapy or pelvic radiation).[1] During menopausal transition (MT) or climacteric, women experience varying degrees of vasomotor symptoms like mood swings, sleep disorders, hot flashes, sweating, headaches, etc. Furthermore, depending on the age at menopause the risk increases for bone loss, Alzheimer's disease, cardiovascular risk and metabolic syndrome varies[2].

Since menopause is a midlife event, screening and evaluation of a woman's health during MT is an essential part of health evaluation. The North American Menopause Society (NAMS) guidelines suggest that the health evaluation at the time of the menopause transition should be tailored to the individual woman based on her medical, social, and family history, as well as on her symptoms and quality-of-life goals. Menstrual cycle changes are the best indicator of menopause stage. Although, hormone measurements to determine menopause status are not routinely indicated, Follicle Stimulating Hormone (FSH) and Anti-Müllerian Hormone (AMH) levels indicate their usefulness in menopause staging. But if menopause symptoms are atypical or occur at an early age, hormonal measurements are even more essential as a part of clinical evaluation[1].

The number and quality of follicles diminish after 40 years causing a decline in estrogen production and fewer ovulations. Estrogen may drop precipitously or spike higher than normal. Furthermore, FSH levels rise to persuade the ovaries into producing more estrogen. But all in vain. Although a high FSH can be a sign that perimenopause has begun, a single FSH reading isn't a reliable indicator because day-to-day hormone levels can fluctuate dramatically. Additionally, variations in FSH and Estradiol (E2) immunoassays may lead to inconsistencies in the reported data. Mass spectrometry-based methods for estradiol measurements are reasonably well suited to the diagnosis and management of infertility but the assays' imprecision and method-to-method differences remain problematic for measurements in perimenopausal women. In the SWAN study, mean E2 levels did not decrease until 2.03 years before the final menstrual period, using a sensitive immunoassay with a lower limit of

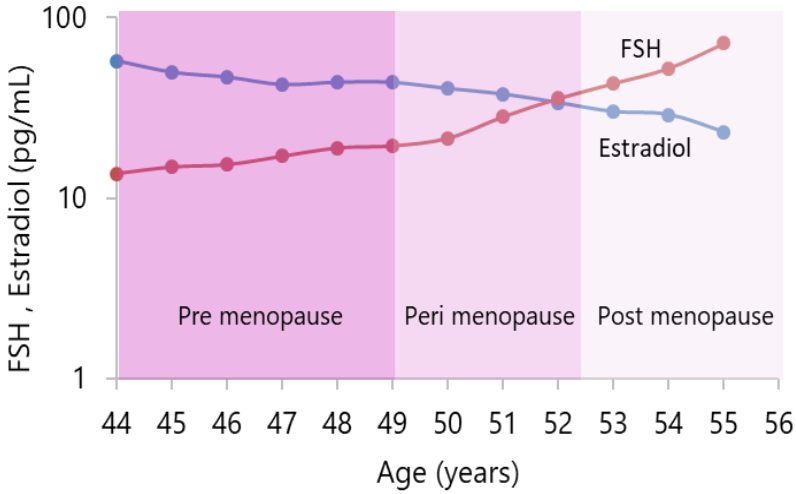

Fig. 1. Decline of FSH and E2 levels over menopausal transition

detection of 1 pg/mL.[4,5] Most commercial Estradiol assays cannot accurately measure E2 levels in postmenopausal women (<20 pg/mL) (Fig.1). Salivary hormone testing of Estradiol is inaccurate and never indicated by NAMS clinical guidelines.

Blood levels of Anti-Müllerian Hormone (AMH), which declines by age, represent one of the key markers to determine the menopausal status of women[2]. Women are born with a finite number of follicles/oocytes that decline over age. Anti-Müllerian Hormone, which is produced from growing follicles, inhibits the growth of primordial follicles to maintain the ovarian reserve. Levels of AMH decline to non-detectable after menopause. AMH has shown promise as a biomarker of ovarian reserve but the current commercially available assays are not sensitive enough to allow assessment of low ovarian reserve or low AMH in women in menopausal transition. Ansh Labs has developed picoAMH, a novel ELISA test with an unequivocal sensitivity of 6 pg/mL.[6] Branded as MenoCheck, the picoAMH ELISA kit has been FDA cleared as an aid in the determination of menopausal status in women between 42 and 62 years. The MenoCheck picoAMH reference ranges and menopause categories were determined in the longitudinal, multi-center SWAN study (Study of Women's Health Across the Nation)[7]. Blood AMH levels fall steadily from 44 years to 55 years over the menopausal transition (Fig. 2). Age-stratified expected values for serum AMH is presented for ostensibly healthy women > 5 years from their final menstrual period (FMP), < 5 years from FMP, and at FMP or later in their menopausal transition (Fig. 3). The final menstrual period (FMP) for each woman in the SWAN Study were assigned retrospectively after 12 months of amenorrhea (the clinical definition of natural menopause). Menopausal categories for assigning status were based on the approximate time to the final menstrual period (FMP). Three menopausal categories were defined based on the time to final menstrual period (TTFMP). Serum AMH measurements, with picogram-level sensitive assays, allow for the categorization of menopausal status and calculation of time to FMP thus providing valuable additional information to support physician's efforts to determine a woman's menopausal status and management[8].

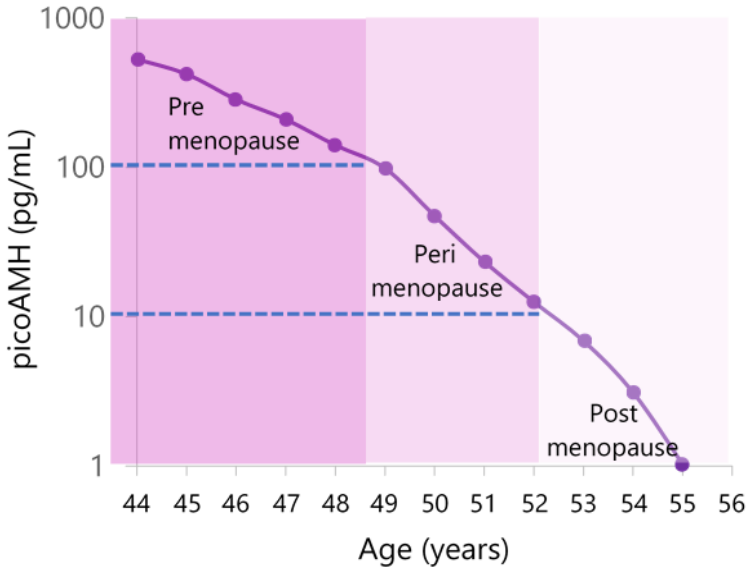

Fig. 2. Steady decline of AMH levels over menopausal transition

Menopause category	Final menstrual period (FMP)	AMH concentration
Pre-Menopause	>5 yrs. from FMP	>100 pg/mL
Peri-Menopause	<5 yrs. from FMP	10-99.9 pg/mL
Post-Menopause	At FMP or later	<10 pg/mL

Fig. 3. Menopausal categories, years from FMP and AMH concentration

Clinical Studies

Clinical Studies	Key Findings	Conclusion
picoAMH & Trans-Menopausal Bone Loss[9]	In multivariable linear regression adjusted for age, BMI, smoking, race/ethnicity, and study site each 75% (or four-fold) decrement in AMH level was associated with 0.15% per year faster decline in spine BMD ($p<0.001$) and 0.13% per year faster decline in femoral neck BMD ($p=0.005$).	Serum levels of picoAMH in women going through the MT can indeed predict the rate of trans-menopausal bone loss and help identify the women at risk of most loss. AMH levels appear to provide information about the rate of bone loss beyond that provided by serum levels of estradiol and FSH.
picoAMH & Onset of Menopausal Vasomotor Symptoms[10]	AMH remained significantly associated ($p<.001$) with incident frequent VMS after adjusting for menopause status (adjusted HR 0.29, 95% CI 0.22 – 0.39), for estradiol (adjusted HR 0.36, 95% CI 0.27 – 0.50), and for FSH (adjusted HR 0.55, 95% CI 0.42 – 0.71).	AMH is useful for predicting incidence of VMS, particularly frequent VMS, independent of other key markers of menopause stage based on menstrual bleeding or other reproductive hormones.
picoAMH & Menopausal Lipids Change[11]	In unadjusted models, lower premenopausal AMH levels and greater declines in AMH over the MT were significantly associated with higher levels of all lipids [per 1 log unit decrease in premenopausal AMH: Cholesterol β(se): 2.76(0.38) , log Triglycerides: 0.01(0.01), LDL-C: 1.86(0.35), apoB: 1.45(0.31), HDL-C: 0.54(0.17), apoA-1: 0.83(0.30), P values <0.006; per 1 log unit decline in AMH over MT: Cholesterol β(se): 2.80(0.15) , log Triglycerides: 0.01(0.002), LDL-C: 2.12(0.13), apoB: 1.09(0.12), HDL-C: 0.46(0.05), apoA-1: 2.44(0.16), P values <0.0001].	Women with less ovarian reserve as reflected by lower premenopausal AMH levels and greater declines in AMH over the MT had a worse lipid profile, with the exception that apoA-1, were unexpectedly higher. Measuring AMH earlier in the MT could help identify premenopausal women at increased risk of later CVD.
picoAMH & New-Onset of Sleep Disturbance[12]	AMH was significantly negatively associated with new-onset sleep disturbance (hazard ratio [HR] 0.90, 95% CI 0.82–0.998, $p=0.047$) and awakenings (HR 0.90, 95% CI 0.81–0.995, $p=0.040$) after adjusting for all covariates except VMS.	AMH predicts the development of sleep disturbance in general, and awakenings, during the menopause transition, but this association is mediated by VMS, which appears to drive the association between declining AMH levels and new-onset sleep problems.
picoAMH & Early Menopause[13]	In multivariable conditional logistic regression models adjusting for matching factors, body mass index, smoking, parity, oral contraceptive use, and other factors, each 0.10 ng/mL decrease in AMH was associated with a 14% higher risk of early menopause (95% confidence interval (CI) 1.10 to 1.18; $P < 0.001$).	This is the first prospective study to evaluate whether AMH levels are associated with early menopause. These findings support the utility of AMH as a clinical marker of early menopause in otherwise healthy women.

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