GYNECOLOGY

# Use of antimüllerian hormone to predict the menopausal transition in HIV-infected women

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**BACKGROUND:** HIV infection has been associated with early menopausal onset, which may have adverse long-term health consequences. Antimüllerian hormone, a biomarker of ovarian reserve and gonadal aging, is reduced in HIV-infected women.

**OBJECTIVE:** We sought to assess the relationship of antimüllerian hormone to age of menopause onset in HIV-infected women.

**STUDY DESIGN:** We used antimüllerian hormone levels measured in plasma in 2461 HIV-infected participants from the Women's Interagency HIV Study to model the age at final menstrual period. Multivariable normal mixture models for censored data were used to identify factors associated with age at final menstrual period.

**RESULTS:** Higher antimüllerian hormone at age 40 years was associated with later age at final menstrual period, even after multivariable adjustment for smoking, CD4 cell count, plasma HIV RNA, hepatitis C infection, and history of clinical AIDS. Each doubling of antimüllerian hormone was associated with a 1.5-year increase in the age at final menstrual period. Median age at final menstrual period ranged from 45 years for those in the 10th percentile of antimüllerian hormone to 52 years for those in the 90th percentile. Other factors independently associated with earlier age at final menstrual period included smoking, hepatitis C infection, higher HIV RNA levels, and history of clinical AIDS.

**CONCLUSION:** Antimüllerian hormone is highly predictive of age at final menstrual period in HIV-infected women. Measuring antimüllerian hormone in HIV-infected women may enable clinicians to predict risk of early menopause, and potentially implement individualized treatment plans to prevent menopause-related comorbidities and to aid in interpretation of symptoms.

**Key words:** AIDS, antimüllerian hormone, hepatitis C virus infection, HIV, menopause, ovarian reserve, viremia

## Introduction

Even among recipients of potent antiretroviral therapy, HIV infection has been reported to be associated with early onset of menopause.[1-3] Women represent about 25% of HIV-infected persons in the United States[4] and over half of all HIV-infected persons globally.[5] Early menopause is a risk factor for bone loss, cardiovascular disease (CVD), and neurological disease.[6-8] This is particularly concerning in the setting of HIV infection, since HIV infection itself has been associated with increased risk of CVD, low bone mass, and other comorbidities. Additionally, menopause is associated with vasomotor symptoms such as hot flashes, night sweats, and sleep disruption that also occur with the progression of HIV illnesses or the adverse effects of antiretroviral medications.[1]

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Ovarian production of sex steroids (ie, progesterone, estradiol, and testosterone) contributes to sex differences in immune responses and CVD.[9,10] While bone demineralization increases with the loss of ovarian steroids after menopause, the impact of menopause on the persistence of other sex differences is unknown, including differences in HIV disease progression and AIDS-defining diseases. In HIV-infected women, menopause affects immune function[11] and thus disease progression, leading to lower CD4 cell counts.[12,13] Menopause also adversely influences the outcome of antiviral therapy for hepatitis C virus (HCV) infection.[14]

We recently reported that plasma levels of antimüllerian hormone (AMH), a biomarker of ovarian reserve and gonadal aging, are lower in HIV-infected women.[15] The relationship of levels of AMH to age of menopause onset has been studied in the general population,[16-19] but is unknown in HIV-infected women. Likewise, factors associated with age at menopause have been well characterized in the general population,[20,21] but few prospective studies in HIV-infected women have been conducted.

The objective of this study was to use levels of AMH measured in plasma to model the age of final menstrual period (FMP) in 2461 ethnically diverse HIV-infected participants from the Women's Interagency HIV Study (WIHS). We also sought to identify other factors associated with age at FMP in HIV-infected women, including lifestyle factors, lymphocyte variables, parity, gravidity, use of sex steroids, age at menarche, and HIV-related variables.

## Materials and Methods

### Study population

The WIHS is a longitudinal observational cohort study of HIV infection and related conditions in women.[22] Participants are interviewed and examined every 6 months. Women who contributed data to this report were enrolled in the first or second expansions of WIHS. In brief, 3766 women (2791 HIV-infected and 975 HIV-uninfected) were enrolled in either 1994 through 1995 (n = 2623, early cohort) or 2001 through 2002 (n = 1143, late cohort) from 6 US sites (Bronx/Manhattan, NY; Brooklyn, NY; Chicago, IL; Los Angeles, CA; San Francisco, CA; and Washington, DC). Enrollment in the

early cohort occurred prior to the broad availability of potent antiretroviral regimens, and thus is a rough indicator of longer duration of untreated HIV infection. HIV-infected WIHS participants are representative of HIV-infected women in the United States, based on contemporaneous national and local surveillance reports regarding demographics and risk factors for prevalent HIV cases among women.[23-25]

For this analysis, participants with a history of cancer chemotherapy were excluded, because previous studies have shown that such treatment can result in a rapid decline in AMH values.[26] There were no exclusions for menstrual characteristics such as cycle length or irregularity. The median number of AMH measurements for each participant was 3 (interquartile range 2-5), and median follow-up between first and last AMH measurements was 7 years (interquartile range 5-11) for those with at least 2 measures. Written informed consent was provided by all participants after approval of the human subjects protocols by internal review committees at each affiliated institution.

## AMH assay

AMH levels were determined using a commercially available enzyme-linked immunosorbent assay (Gen II; Beckman Coulter Inc, Chaska, MN). Plasma samples were frozen at −80°C and not thawed prior to testing, which was conducted blind to HIV status. Interassay coefficients of variations were 8.2% at 2.8 ng/mL and 9.4% at 8.5 ng/mL. The lower limit of detection was 0.08 ng/mL.

The primary predictor in this study was AMH level at age 40 years, which was estimated for all women using the fitted random intercept from a left-censored linear mixed effects regression model of log-transformed AMH, via previously published methods.[15] Of the 2740 HIV-positive participants with measured AMH, 32% had values measured within 1 year of age 40 years and 71% had AMH measured within 5 years of age 40 years. Examination of model fit for the left-censored random effects model showed a substantial proportion of outliers, where accurate estimates of AMH at age 40 years could not be obtained. Women who were older and had AMH value below assay detection at the time of WIHS enrollment tended to have larger SE for estimated AMH at age 40 years. We therefore excluded participants with SE >2 (9% of the total). We also conducted sensitivity analyses to address uncertainty in the estimation of AMH at age 40 years. These analyses included use of an uncertainty weight to downweight observations with large SE (calculated as $1/SE^2$, where SE = the SE of estimated AMH at age 40 years), multiple imputation (using the SAS [SAS Institute Inc, Cary, NC] MMI_IMPUTE macro for multilevel data[27]), and restriction of the cohort to those with AMH measured near age 40 years (defined as ±1 year and as ±5 years).

## Other measurements

Study interviews assessed regularity of menstrual periods, obstetrical history, history of gynecological surgery and medical conditions, use of tobacco and illicit drugs, and use of exogenous steroids and other medications. HIV serology was performed at baseline and prospectively in women with negative results. Quantification of HIV RNA copy numbers (viral load) was performed on plasma, using NucliSens (bioMérieux, Inc, Durham, NC), NASBA (nucleic acid sequence based amplification), Taqman (Thermo Fisher Scientific Inc, North Waltham, MA), and Roche Amplicor (Roche Molecular Systems Inc, Pleasanton, CA) assays with limits of detection ranging from 20-300 copies/mL, depending on testing date. Lymphocyte subsets (including determination of $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ cell counts) were measured in whole blood semiannually using laboratories that participate in the National Institute of Allergy and Infectious Diseases Division of AIDS Virology and Immunology Laboratory Quality Assurance Programs. This analysis used the $CD4^+$ (T-helper cell) counts measured at the time of the WIHS visit closest to age 40 years and the nadir count (lowest $CD4^+$ T-cell count measured prior to age 40 years). HCV infection was identified by second-generation or third-generation enzyme-linked immunoassay serology at WIHS entry. HCV RNA testing methods included the Roche COBAS AMPLICOR HCV MONITOR test (v2.0/Kovacs, w01043; Roche Molecular Systems) and the TAQMAN test (w07007 and w07034; Thermo Fisher Scientific Inc).

## Outcome

The primary outcome of this study was age at FMP, defined using self-reports provided at study visits. The occurrence of FMP was defined as self-reported menstrual period followed by at least 2 consecutive semiannual WIHS visits at which no interval menses was reported. Women reporting recurrence of menses after amenorrhea were not considered to have FMP. Additionally, women who reported amenorrhea during or immediately following pregnancy were not considered to have FMP. Within this cohort, age at FMP could be left censored (occurring prior to the first WIHS visit), interval censored/observed (recorded during WIHS), or right censored (had not yet occurred at last WIHS visit).

## Covariates

For this analysis, covariates were selected using the individual participant's WIHS visit that occurred closest to age 40 years, to correspond to the level of AMH measured at that age. Candidate covariates included demographics (age, race/ethnicity), WIHS enrollment cohort (early vs late), lifestyle factors (smoking and illicit drug use), body mass index (BMI), waist circumference, fertility and menstrual-related factors (parity, gravidity, use of sex steroids, age at menarche), lymphocyte variables (current and nadir levels of $CD4^+$, $CD8^+$, total lymphocytes, and white blood cell [WBC] counts), and HIV-related factors (use of antiretroviral medications, number of HIV RNA copies in plasma [viral load], history of clinical AIDS, hepatitis C serology status, and history of weight loss). Multiple imputation using the Markov chain Monte Carlo method for arbitrary missing multivariate normal data was used to impute missing covariates, with 10 imputations to ensure ~95% relative efficiency.[28] The percentage of missing observations for