Antimüllerian hormone to determine polycystic ovarian morphology

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Objective: To determine a cutoff for the Elecsys AMH Plus immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) to identify polycystic ovarian morphology (PCOM), a polycystic ovary syndrome (PCOS) criterion.
Design: The AMH Protein in Humans for polycystic ovaRian mOrphology DIagnostic TEsting (APHRODITE) study was a retrospective, multicenter, case-control study. The serum antimüllerian hormone (AMH) level was measured using the Elecsys AMH Plus immunoassay. The antral follicle count was determined using transvaginal ultrasound. An AMH cutoff was derived and validated in separate cohorts with cases of PCOS with full phenotype A (oligo/anovulation, hyperandrogenism, and PCOM) versus that with controls. Exploratory analyses of age and PCOS phenotype were performed.
Setting: Not applicable.
Patient(s): Polycystic ovary syndrome-positive (PCOS A–D per the Rotterdam criteria) and PCOS-negative women aged 25–45 years.
Intervention(s): None.
Main Outcome Measure(s): A validated cutoff for AMH using the Elecsys AMH Plus assay for PCOM.
Result(s): In the validation cohort (455 cases and 500 controls), an AMH cutoff of 3.2 ng/mL (23 pmol/L) resulted in a sensitivity of 88.6% (95% confidence interval [CI] 85.3–91.3) and specificity of 84.6% (95% CI 81.1–87.7) for PCOM diagnosis as well as an area under the receiver-operator characteristic curve of 93.6% (95% CI 92.2–95.1). In women aged 25–35 years, the sensitivity and specificity for the cutoff were 88.5% and 80.3%, respectively, versus 77.8% and 90.1%, respectively, in women aged 36–45 years. The results were consistent across PCOS phenotypes A–D.
Conclusion(s): The Elecsys AMH Plus immunoassay, with a cutoff of 3.2 ng/mL (23 pmol/L), is a robust method for identifying PCOM to aid in PCOS diagnosis. (Fertil Steril® 2021;116:1149–57. ©2021 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Antimüllerian hormone, polycystic ovarian morphology, polycystic ovary syndrome, diagnosis

Polycystic ovary syndrome (PCOS) affects approximately 8%–13% of women of reproductive age, and despite being 1 of the most common endocrine pathologies in this age group, up to 70% of women remain undiagnosed (1–3). The clinical features of PCOS include anovulation, hyperandrogenism (HA), and associated clinical features (e.g., hirsutism, acne, and androgenic alopecia) as well as polycystic ovarian morphology (PCOM; i.e., an excess number of follicles in 1 ovary or both ovaries) (4–7).

Polycystic ovarian morphology is 1 of the 3 diagnostic criteria for PCOS according to the Rotterdam criteria and is present in most women with PCOS (2). The Rotterdam criteria have defined PCOM as the presence of ≥12 follicles in each ovary measuring 2–9 mm in diameter and/or an increased ovarian volume (>10 mL), in the absence of follicles measuring >10 mm, assessed using transvaginal ultrasound (TVUS) with a frequency of 4–8 MHz (8). More recently, because of the use of higher-resolution TVUS equipment, the current international guidelines have defined PCOM as an antral

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follicle count (AFC) of >20 per ovary and/or ovarian volume of ≥10 mL (6).

The replacement of TVUS measurement with a simple blood test for assessing PCOM would be clinically advantageous. Many women presenting with PCOS symptoms are treated at the primary care level by general practitioners, and the lack of easy access to TVUS contributes to a delayed diagnosis or underdiagnosis (5). Further limitations of TVUS are that not all gynecologists are well trained in assessing AFC, and the determination of PCOM using TVUS is not standardized (9). The level of antimüllerian hormone (AMH) and the number of ovarian follicles measuring 2–9 mm (in both ovaries) both correlate with ovarian primordial follicle number (10), and there is a good correlation between circulating AMH values and follicle count per ovary in women of reproductive age (11, 12). The adoption of AMH as a biomarker for PCOM requires the derivation and validation of AMH cutoffs/thresholds in large populations of women with different ages (6, 13, 14).

We conducted a large case-control study to derive and validate a cutoff for AMH level using the Elecsys AMH Plus immunoassay (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) to identify PCOM and support the diagnosis of PCOS in women of reproductive age (25–45 years).

MATERIALS AND METHODS

Trial Design

The AMH Protein in Humans for polycystic ovaRian mOrphology DIagnostic TEsting (APHRODITE) study was a retrospective, noninterventional, multicenter, case-control study conducted between October 2018 and November 2019.

A statistical analysis plan for the study was designed and written by the employees of the study's sponsor (Roche Diagnostics) in collaboration with investigators from the Erasmus University Medical Center (Rotterdam). Data were combined and analyzed by the employees of Roche Diagnostics. All investigators vouch for the accuracy and completeness of the reported data and fidelity of the statistical analysis plan. A third party was hired by the sponsor to provide writing assistance for the manuscript; all the investigators reviewed and commented on each draft of the manuscript and decided to submit the manuscript for publication. A confidentiality clause was included in the signed research agreement between the Erasmus University Medical Center and Roche Diagnostics. Ethics committee approval was previously obtained for the studies from which residual serum samples were used. The study was conducted according to the principles of the Declaration of Helsinki.

The primary study objective was to derive and validate an AMH cutoff, using the Elecsys AMH Plus immunoassay, for PCOM in women with PCOS (full phenotype A) versus that for PCOS-negative controls. The exploratory objectives are listed in the Supplemental Material (available online).

Participants

Participants (women with PCOS, cases) were selected from a local database at the Erasmus University Medical Center, which included information from Cycle disturbances, OLigo or Amenorrhea (COLA) screening. Controls were selected from 2 studies previously conducted by Roche Diagnostics (11, 15). Further details are provided in the Supplemental Material.

The inclusion criteria for the PCOS cases were women aged 25–45 years diagnosed with PCOS per the Rotterdam criteria, who met at least 2 of the following 3 criteria: ovulatory dysfunction (OD; defined as oligo/amenorrhea), HA, and PCOM (AFC ≥12 per ovary; measured using Philips EnVisor ultrasound [Koninklijke Philips N.V., Amsterdam, the Netherlands] with a frequency of 4–8 MHz); and/or women with an ovarian volume of >10 mL in at least 1 ovary (8). After inclusion, the PCOS cases were classified into phenotype A (HA + OD + PCOM), B (HA + OD), C (HA + PCOM), or D (OD + PCOM). The exclusion criteria were congenital adrenal hyperplasia, Cushing syndrome, androgen-secreting tumors, and the use of oral contraceptives.

The inclusion criteria for the controls were women aged 25–45 years with a regular menstrual cycle (average 25–35 days) no major uterine or ovarian abnormalities detected using TVUS, no previous in vitro fertilization cycles, and an AFC of ≤20 per ovary (per current international guidelines; TVUS frequency bandwidth includes 8 MHz or higher) (6). The exclusion criteria for the controls were PCOS diagnosis (per the Rotterdam criteria), body mass index (BMI) of ≥40 kg/m^2, the use of hormonal contraceptives within 3 months of enrolment, ongoing pregnancy, presence of serum human chorionic gonadotrophin, major ovarian abnormalities, endocrine or metabolic abnormalities, and current treatment for malignancy.

After the identification of the overall cohort (all PCOS cases with phenotypes A–D and controls), PCOS cases with phenotype A and controls were selected for a primary analysis population, with the participants blindly randomized to either the development or validation cohort. The details of sample size calculations are provided in the Supplemental Material.

Assessments

Blood samples were drawn on days 2–4 of the menstrual cycle where possible (11). The serum AMH levels were measured on the cobas e 411 analyzer (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) using the Elecsys AMH Plus for the cases and Elecsys AMH assay for the controls (measuring range, 0.01–23 ng/mL) (12, 13). In a subset of control samples, the levels of testosterone and sex hormone-binding globulin were measured for exploratory analyses (further details are provided in Supplemental Material).

Statistical Analyses

Participant characteristics and biomarker levels were reported as descriptive statistics. The correlation between serum AMH levels and AFC was described using pairwise Spearman rho correlation coefficients. The serum AMH cutoff was initially determined in the "development cohort" using a concordance analysis (i.e., equal sensitivity and specificity) and visualized using a cumulative distribution plot. The performance of the derived AMH cutoff was subsequently validated in the "validation cohort." The sensitivity and specificity, with 95%

confidence intervals (CIs), of case-control status, with classification based on the AMH cutoff, were calculated and receiver-operator characteristic (ROC) curves generated. The prespecified acceptance criteria for specificity and sensitivity were >75% and >70%, respectively, and statistical significance was assessed using a 1-sided binomial test (significance level, $\alpha = 0.05$). Statistical analyses were conducted using R (Version 3.4.0; R Foundation for Statistical Computing, Vienna, Austria).

An exploratory multivariate regression analysis was used to evaluate the performance of the derived AMH cutoff in age groups 25–35 and 36–45 years. Further details of the key exploratory and sensitivity analyses are provided in the Supplemental Material.

Role of the Funding Source

This study was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland). M.H., K.B., and J.S. are employees of the study's funder and were involved in the study design, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had the final responsibility of the decision to submit for publication.

RESULTS

Patient Disposition and Characteristics

Participant disposition is presented in Supplemental Figure 1 (available online). The primary analysis included 2,014 individuals, including 484 cases of PCOS with phenotype A and 575 controls in the development cohort as well as 455 cases of PCOS with phenotype A and 500 controls in the validation cohort. The PCOS patients were younger than the controls (median age, 29.0 vs. 36.0 years) and had a higher BMI in both the development (median 28.3 vs. 23.6 kg/m^2) and validation (median 28.1 vs. 23.7 kg/m^2) cohorts. The PCOS cases had higher AMH levels than the controls in both the development (median 6.13 vs. 1.59 ng/mL) and validation (median 6.32 vs. 1.58 ng/mL) cohorts (Table 1). A strong positive correlation was observed between AMH level and AFC in the development and validation cohorts (Spearman $\rho = 0.84$ and 0.85, respectively; Supplemental Fig. 2, available online).

Supplemental Table 1 (available online) shows baseline characteristics according to PCOS phenotype in the overall population. A significant difference was observed in the AMH levels across PCOM-positive individuals with PCOS with phenotypes A, C, and D (Kruskal-Wallis test $P<.01$). However, there was no significant difference in the AMH levels between PCOM-negative individuals with PCOS with phenotype B and controls (Mann-Whitney U test $P = .659$). Supplemental Figure 3 (available online) shows the distribution of AMH levels across the PCOS phenotypes. Individuals with phenotype B (i.e., PCOM-negative individuals detected using TVUS) had lower AMH levels than individuals with phenotypes A, C, and D (median 1.90 vs. ≥4.84 ng/mL) and had levels similar to those of the controls (2.13 ng/mL).

Development and Validation of the AMH Cutoff (Primary Objective; Primary Analysis Population)

A serum AMH cutoff of 3.2 ng/mL (23 pmol/L) was determined in the development cohort (phenotype A vs. controls), providing a sensitivity of 86.2% and specificity of 86.1% for the determination of PCOM (Fig. 1). The serum AMH cutoffs corresponding to the sensitivity ranging between 70%–95% were also determined in the development cohort and are

TABLE 1

Baseline characteristics of the primary analysis population from the APHRODITE study.

	Development Cohort		Validation Cohort	
Characteristic	**Case (n = 484)**	**Control (n = 575)**	**Case (n = 455)**	**Control (n = 500)**
Median age, y (IQR)	29 (27–32)	36 (32–39)	29 (27–32)	36 (32–39)
Age group, y—no. (%)				
25–29	274 (56.6)	61 (10.6)	256 (56.3)	53 (10.6)
30–34	159 (32.9)	186 (32.3)	150 (33.0)	160 (32.0)
35–39	48 (9.9)	211 (36.7)	46 (10.1)	185 (37.0)
40–45	3 (0.6)	117 (20.3)	3 (0.7)	102 (20.4)
Race—no. (%)				
White	315 (69.8)	458 (79.7)	285 (69.7)	404 (80.8)
Asian	70 (15.5)	59 (10.3)	65 (15.9)	51 (10.2)
Black	52 (11.5)	36 (6.3)	39 (9.5)	23 (4.6)
Multiple	14 (3.1)	10 (1.7)	16 (3.9)	11 (2.2)
Other	0	12 (2.1)	4 (1.0)	11 (2.2)
Missing	33	0	46	0
Median BMI, kg/m^2 (IQR)	28.3 (23.8–32.8)	23.6 (21.7–27.5)	28.1 (23.7–33.1)	23.7 (21.4–27.5)
Median AMH level, ng/mL (IQR)	6.13 (4.06–9.25)	1.59 (0.837–2.50)	6.32 (4.24–9.30)	1.58 (0.760–2.56)
Median number of follicles—no. (IQR)	44.0 (32.0–58.0)	12.0 (8.0–15.0)	42.5 (30.0–60.0)	12.0 (8.0–16.0)
AFC ≤ 20 (%; controls) or AFC ≥ 12 (cases)—no. (%)	13 (2.8)	575 (100)	9 (2.1)	500 (100)
Missing—no.	24	0	21	0

Note: AFC = antral follicle count; AMH = antimüllerian hormone; APHRODITE = AMH Protein in Humans for Polycystic Ovarian Morphology Diagnostic Testing; BMI = body mass index; IQR = interquartile range.

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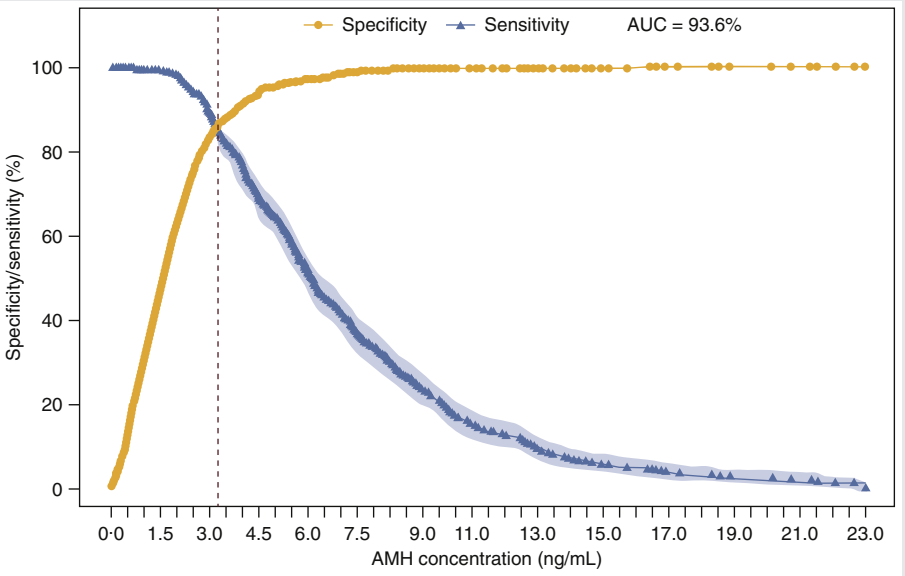
FIGURE 1



A cumulative distribution plot for the determination of AMH cutoff for PCOM in the development cohort of the APHRODITE study. The *red dashed line* indicates the cutoff with the minimum absolute difference between sensitivity and specificity (cases, n = 484; controls, n = 575). AMH = antimüllerian hormone; APHRODITE = AMH Protein in Humans for Polycystic Ovarian Morphology Diagnostic Testing; AUC = area under the curve; PCOM = polycystic ovarian morphology.

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summarized in Supplemental Table 2 (available online). A serum AMH cutoff of 2.40 ng/mL provided a sensitivity of 95.0% and specificity of 73.0%, whereas a cutoff of 4.44 ng/mL only provided a sensitivity of 70.2% and specificity of 93.2%.

The serum AMH distribution clearly differed between the cases and controls in both the development and validation cohorts, although some overlap was observed (Fig. 2A; Supplemental Fig. 4, available online). In the validation cohort (n = 955), 403 (42.0%) cases and 77 (8.1%) controls had a serum AMH level of >3.2 ng/mL, and 52 (5.4%) cases and 423 (44.3%) controls had an AMH level of ≤3.2 ng/mL. Based on these data, the 3.2 ng/mL cutoff resulted in a sensitivity of 88.6% (95% CI 85.3–91.3) and specificity of 84.6% (95% CI 81.1–87.7) for the determination of PCOM, and the overall percentage agreement (OPA) was 86.5% (95% CI 84.2–88.6).

Based on an analysis of ROC curves, the AMH cutoff of 3.2 ng/mL was found to be associated with an area under the curve (AUC) of 93.6% (95% CI 92.2–95.0) in the development cohort (Fig. 2B) and 93.6% (95% CI 92.2–95.1) in the validation cohort (Fig. 2C).

Effect of Age on the Performance of the AMH Cutoff (Primary Analysis Population; Exploratory Analysis)

The AMH levels decreased with age among the cases and controls (Fig. 2D). In women aged 25–35 years (n = 1,394, including 867 cases), the AMH cutoff of 3.2 ng/mL showed a sensitivity of 88.5% and specificity of 80.3%. The OPA was 85.4%, and the AUC was 92.2% (Fig. 2E). In women aged 36–45 years (n = 620; including 72 cases), the sensitivity was 77.8% and specificity was 90.1%. The OPA was 88.7%, and the AUC was 90.9% (Fig. 2F).

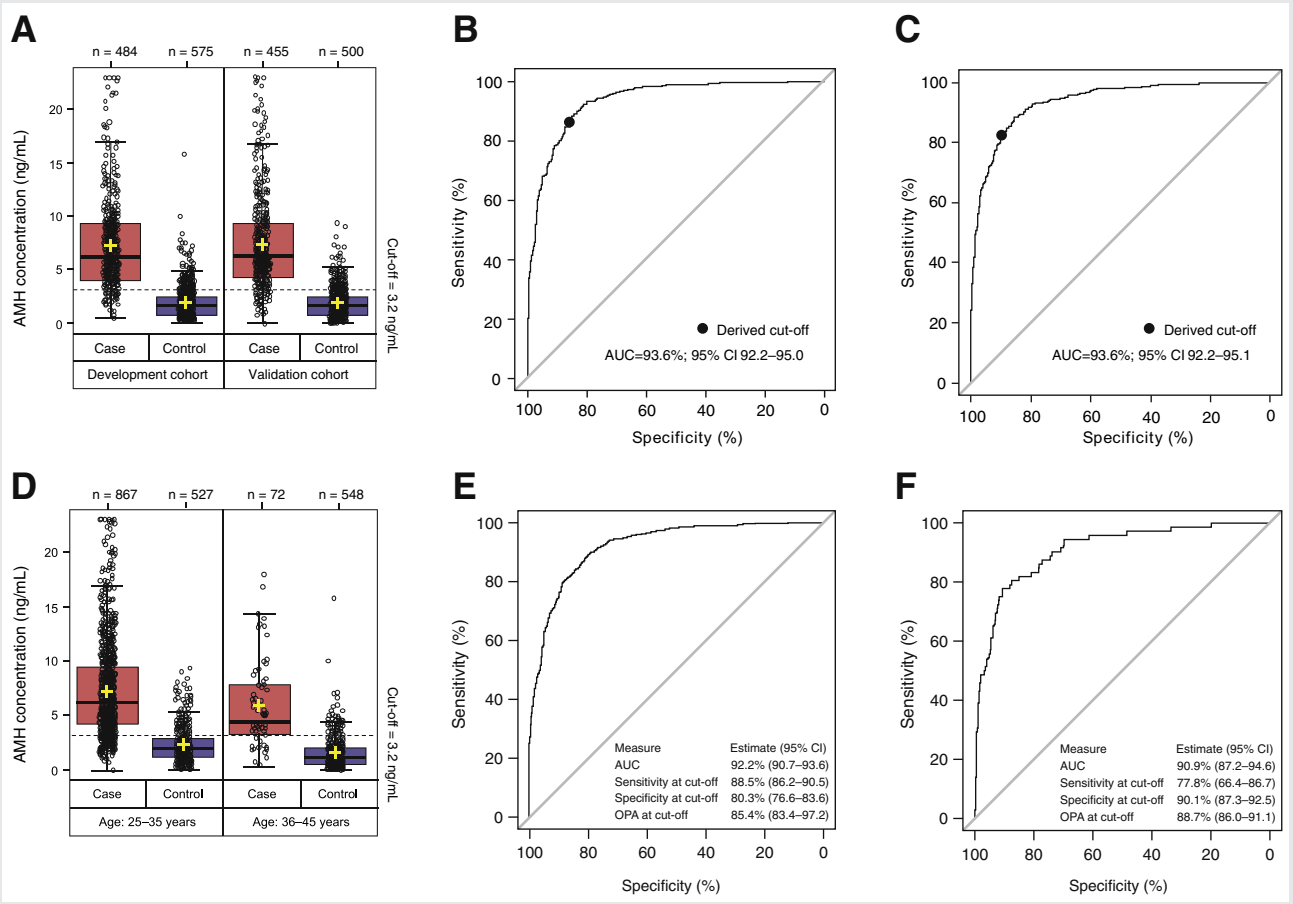
Effect of PCOS Phenotype on the AMH Cutoff (Overall Population)

The AMH cutoff of 3.2 ng/mL showed similar results across phenotypes A–D (Fig. 3). At a specificity of 85.3% (95% CI 83.0–87.4) in the control group, the sensitivity of the AMH cutoff for the determination of PCOM was 75.7% (95% CI 72.0–79.2) for phenotype D, 80.5% (95% CI 78.5–82.4) for all PCOS phenotypes A–D, and 82.9% (95% CI 80.9–84.7) for PCOS phenotypes A, C, and D combined. The corresponding OPA was 81.9% (95% CI 80.0–83.8), 82.4% (95% CI 80.9–83.8), and 83.8% (95% CI 82.4–85.2), respectively.

Relationship Between AFC and AMH Level for PCOS Classification

An analysis of women with confirmed PCOS (including TVUS) showed a positive percentage agreement of 91.0% (95% CI 89.5–92.3) between PCOM defined according to the AMH cutoff of 3.2 ng/mL and PCOS status (Supplemental Table 3, available online). The discordants were primarily from phenotype D (141/148 discordants), with a median BMI of 23.4 kg/m^2 and median AMH level of 2.6 ng/mL.

FIGURE 2



Boxplots of anti-Müllerian hormone (AMH) distribution by (A) case-control status and cohort and by (D) age group or control status; receiver-operator characteristic curves for prediction of case or control status by AMH in the (B) development and (C) validation cohorts, and in the age groups (E) 25–35 years and (F) 36–45 years from the APHRODITE study. Boxes represent the first and third quartiles and the median value. The whiskers represent values that are 1.5× the interquartile range (IQR) or less in cases where the minimal/maximal value lies within 1.5× the IQR. Yellow crosses indicate the mean value. AUC = area under the curve; CI = confidence interval; OPA = overall percentage agreement.

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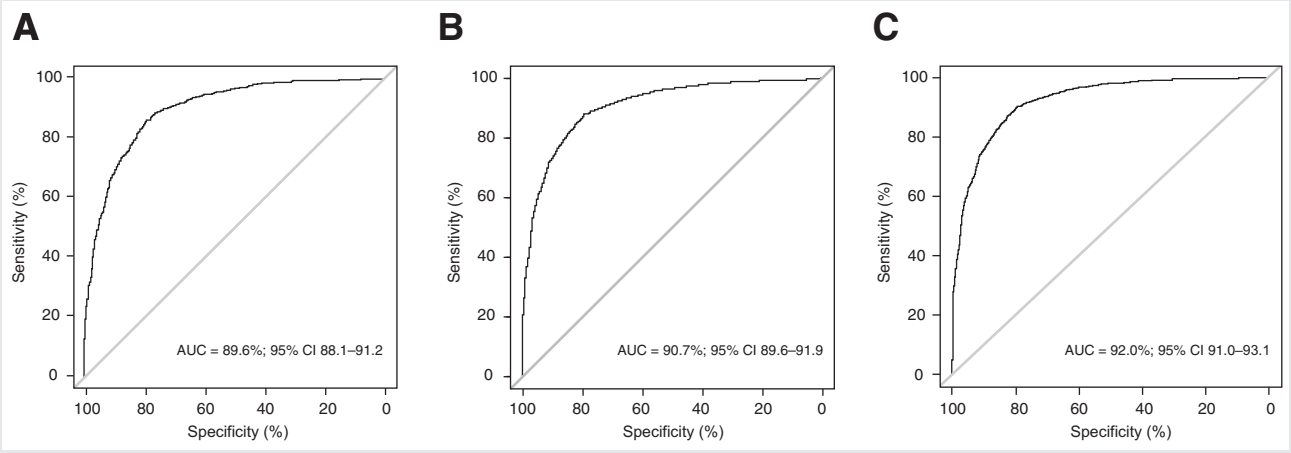
Relationship Between BMI, Race, AMH, and PCOS Classification

The multivariable logistic regression modeling demonstrated that there was a significant difference in the BMI between the cases and controls ($P = .044$). However, the distribution of AMH levels between the cases and controls was similar when categorized by case-control status and BMI group (underweight-to-normal women <25 kg/m^2 vs. overweight-to-obese women ≥ 25 kg/m^2; $P = .457$; Supplemental Fig. 5A, available online).

In cases and controls with a BMI of <25 kg/m^2, the cutoff of 3.2 ng/mL resulted in a sensitivity of 93.5% (95% CI 90.1–96.1) and specificity of 82.7% (95% CI 79.6–85.6) for the determination of PCOM, and the OPA was 86.1% (95% CI 83.7–88.2). In cases and controls with a BMI of ≥ 25 kg/m^2, the cutoff of 3.2 ng/mL resulted in a sensitivity of 85.1% (95% CI 82.1–87.7) and specificity of 89.0% (95% CI 85.7–91.9) for the determination of PCOM, and the OPA was 86.6% (95% CI 84.4–88.6). Based on the analysis of the ROC curves, the AMH cutoff of 3.2 ng/mL was found to be associated with an AUC of 95.3% (95% CI 93.9–96.7) in underweight-to-normal women (Supplemental Fig. 5B) and 93.9% (95% CI 92.5–95.4) in overweight-to-obese women (Supplemental Fig. 5C).

The multivariable logistic regression modeling also showed that race had no significant effect on case-control status (black, $P = .523$; multiple, $P = .638$; other, $P = .799$; and white, $P = .464$). Based on the analysis of the ROC curves, the AMH cutoff of 3.2 ng/mL was found to be associated with an AUC of 94.4% (95% CI 93.3–95.5) in white women (Supplemental Fig. 6A, available online), 89.1% (95% CI 85.0–93.1) in Asian women (Supplemental Fig. 6B), and 96.0% (95% CI 92.6–99.4) in black women (Supplemental Fig. 6C).

FIGURE 3



Receiver-operator characteristic curves for prediction of case or control status by AMH in populations comprising (A) phenotype D PCOS cases and controls, (B) phenotype A–D PCOS cases and controls, and (C) phenotype A, C, and D PCOS cases and controls from the APHRODITE study. AMH = anti-Müllerian hormone; AUC = area under the curve; CI = confidence interval; PCOS = polycystic ovarian syndrome.

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Sensitivity Analysis—Sample Stability (Overall Population)

The serum samples collected from patients with PCOS were of various ages at the time of AMH measurement (range 1–18 years). Therefore, a statistical model was used to assess the effect of sample age on AMH concentration. The model showed no significant difference in the AMH concentrations over time (Supplemental Fig. 7, available online).

Derivation of a Free Androgen Index (FAI) Cutoff for Biochemical Hyperandrogenism

A method comparison of FAI measurements taken using Elecsys FAI and other platforms (n = 157, PCOS phenotypes A–D) demonstrated that an Elecsys FAI of >2.5 can be used to define biochemical HA (Supplemental Fig. 8, available online).

DISCUSSION

This study demonstrates, in the largest cohort investigated to date, a good correlation between serum AMH levels and the current gold standard for assessing PCOM in support of PCOS diagnosis, TVUS-determined AFC. Our results show that a cutoff of 3.2 ng/mL (23 pmol/L) for serum AMH level using the Elecsys AMH Plus immunoassay provides a high sensitivity and specificity for identifying PCOM, irrespective of the age or PCOS phenotype, with a high diagnostic performance; the AUC was 93.6% in the validation cohort.

Nicholas et al. (16) identified an AMH cutoff of 3.15 ng/mL (22.5 pmol/L) for PCOM using the AMH Gen II Beckman Coulter enzyme-linked immunosorbent assay (ELISA; Beckman Coulter, Brea, CA) similar to the cutoff identified in our larger, controlled cohort. Several studies have previously validated AMH cutoffs for the diagnosis of PCOS in various populations; their derived cutoffs were slightly higher than those identified in the current study (17–21). Based on the calculations performed in the development cohort, increasing our AMH cutoff to 3.69 ng/mL would have resulted in a higher specificity (89.0% vs. 86.1%) and lower sensitivity (80.2% vs. 86.2%) compared with the validated serum AMH cutoff of 3.2 ng/mL in the primary analysis population (PCOS phenotype A vs. controls). As such, selecting a cutoff with a higher specificity would have resulted in a substantial loss of sensitivity and performed insufficiently in women with PCOS phenotype D and a mean AMH level of 4.84 ng/mL. The diagnostic performance of an immunoassay is always a compromise between sensitivity and specificity, both of which are comparable for the AMH cutoff of 3.2 ng/mL for the Elecsys AMH Plus immunoassay.

Differences in the diagnostic performance of AMH assays and in the study cohorts/designs used may also explain the higher cutoffs derived in previous studies. For example, an AMH cutoff of 4.7 ng/mL provided a specificity of 77.8% and sensitivity of 80.0% for the diagnosis of PCOS in Taiwanese patients, using Elecsys AMH immunoassay (20). Wongwananuruk et al. (20) noted that European populations usually have a higher BMI compared with the Taiwanese population used in their study, which could have contributed to the discrepancy between the cutoffs observed across studies. However, the exploratory analysis of the present study population found that the AMH levels were similarly distributed between the cases and controls, regardless of a significant difference in the BMI between these 2 groups ($P = .044$), and race had no significant effect on case-control status (20).

Lie Fong et al. (17) used an in-house ELISA (commercially available as AMH Gen II) and showed that an AMH cutoff of 5.5 ng/mL provided a specificity of 82.0% and sensitivity of 84.1% for diagnosing PCOS in younger European patients. In contrast to the present study, no significant difference was observed in the BMI between women with a regular cycle

and women with PCOS. However, the AMH cutoff determined by Lie Fong et al. (17) was intended for the diagnosis of PCOS rather than for the diagnosis of PCOM. This explains why the AMH cutoff determined in this study was higher than that of our study (5.5 vs. 3.2 ng/mL).

Dewailly et al. (18) reported an AMH cutoff of 5 ng/mL, with a specificity of 97% and sensitivity of 92%, for the diagnosis of PCOM in European patients (AMH Immunotech, Beckman Coulter, Brea, CA). The variance in the AMH cutoff values observed between studies can perhaps be attributed to lower measurements provided by automated assays compared with those by previous ELISAs. Indeed, Nelson et al. (22) previously observed substantially lower AMH measurements when using the automated Beckman Coulter Access AMH and Elecsys AMH assays compared with that when using Ansh Labs UltraSensitive AMH (Ansh Labs, Webster, TX) and AMH Gen II Beckman Coulter ELISAs. Differences in AMH measurements have also been observed within automated immunoassays, with the Beckman Coulter Access AMH assay systematically measuring 10% higher AMH values compared with the Elecsys AMH immunoassay (23). This suggests a need for an assay-specific interpretation of AMH measurements in routine clinical practice.

Although most studies have validated an AMH cutoff for the diagnosis of PCOS, we would emphasize that it is currently not possible to replace the relatively complex diagnosis of PCOS with a single AMH measurement. Although the present findings contribute to the evidence required for the adoption of a single AMH measurement for PCOM diagnosis, AMH should be used in conjunction with at least 1 other clinical symptom (e.g., HA or OD) for PCOS diagnosis (6, 14).

Recent guidelines have shown that women with PCOS are underdiagnosed (5, 6). Antimüllerian hormone testing might reduce the number of women with PCOS who undergo a delayed diagnosis or are undiagnosed. For example, the availability of AMH testing in primary care using central laboratories can facilitate earlier diagnosis without the need for a referral to secondary/specialist centers and without the need for TVUS (24). From a clinical perspective, the AMH assay validated here showed both a high sensitivity for diagnosing PCOM and high specificity for avoiding a high rate of false-positive diagnoses. Because patients typically present with clinical symptoms suggestive of PCOS (e.g., HA or menstrual irregularity), the determination of PCOM using a sensitive and specific assay could simplify the diagnosis of PCOS.

Cutoff performance was consistent between the age groups, offering the possibility of a single determinant for PCOM, which would be valuable for clinical decision making. However, it should be noted that these conclusions are drawn from an analysis comprising a relatively small number of PCOS cases aged 36–45 years (n = 72) versus 867 cases aged 25–35 years. The AMH cutoff using the Elecsys AMH Plus immunoassay had a lower sensitivity and higher specificity in older women (i.e., aged >35 vs. ≤35 years).

The AMH cutoff performed consistently well across the PCOS phenotypes. A significant difference was observed in the AMH levels across PCOM-positive individuals with PCOS phenotypes A, C, and D, although the distribution of AMH levels appeared similar across the groups when presented in a boxplot. This could be attributed to the large sample size and imbalanced groups. Polycystic ovarian morphology is not associated with PCOS phenotype B (oligo-/anovulation plus HA without PCOM), which shows levels of AMH similar to those of non-PCOS controls (25, 26). The inclusion/omission of the small number of individuals with phenotype B (n = 65; 4% of PCOS cases; i.e., individuals without PCOM) from the analyses in the overall population did not impact the sensitivity of the cutoff or the AUC. Similar results have been reported by Lie Fong et al. (17) in a similar cohort.

The study's strengths include well-characterized PCOS case-control cohorts and blinded randomization of the study population into the development or validation cohorts. Polycystic ovarian morphology was defined according to the international guidelines for PCOS to allow for comparison with AMH levels. The fully automated Elecsys AMH immunoassay was used for measurements in this study; this is a standardized, robust assay, which has been approved and is globally accessible (including in the United States, Europe, and Asia Pacific countries). Furthermore, the APHRODITE study includes the largest cohort ever used to determine an AMH cutoff for PCOM in women with PCOS reported to date. The derived cutoff was validated in a second adequately powered validation cohort, and sample stability did not appear to affect the results. The study's limitations include the collection of cases and controls from different studies (cases from a single cohort; controls from 2 separate studies), resulting in some differences between the cases and controls (e.g., age and ethnicity distribution) due to differences in study design (e.g. different inclusion criteria and study sites). The samples from the PCOS cases were also collected over a longer period of time compared with those from the controls. Thus, the AMH cutoff was tested in different subgroups according to age, race, and BMI, and similar performance was observed between these subgroups. The observed difference in the BMI between the cases and controls was expected because women with PCOS are more often obese (27). Very few women aged >40 years were enrolled; thus, the sample size was too small for complete development and validation of the AMH cutoff in this subgroup. Similarly, women aged <25 years were excluded because of continually rising AMH levels in this age group (28). However, the age range of the enrolled participants reflects the population presenting to fertility clinics. Thus, our findings are generalizable to European women aged 25–45 years and with symptoms suggestive of PCOS.

CONCLUSIONS

The measurement of serum AMH level using the Elecsys AMH Plus immunoassay provides a robust method for identifying PCOM as part of PCOS diagnosis in women aged 25–45 years. A serum AMH cutoff of 3.2 ng/mL (23 pmol/L) to detect PCOM was validated.

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REFERENCES

1. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod 2010;25:544–51.
2. Bozdag G, Mumusoglu S, Zengin D, Karabulut E, Yildiz BO. The prevalence and phenotypic features of polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod 2016;31:2841–55.
3. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JS, Legro RS, et al. Polycystic ovary syndrome. Nat Revs Dis Primers 2016;2:1–8.
4. Witchel SF, Overfield SE, Peña AS. Polycystic ovary syndrome: pathophysiology, presentation, and treatment with emphasis on adolescent girls. J Endocr Soc 2019;3:1545–73.
5. Gibson-Helm M, Teede H, Dunaif A, Dokras A. Delayed diagnosis and a lack of information associated with dissatisfaction in women with polycystic ovary syndrome. J Clin Endocrinol Metab 2017;102:604–12.
6. Teede H, Misso M, Costello M, Dokras A, Laven J, Moran L, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. Hum Reprod 2018;33:1602–18.
7. American College of Obstetricians and Gynecologists. ACOG practice bulletin no. 194: polycystic ovary syndrome. Obstet Gynecol 2018;131:e157–71.
8. ESHRE TR, ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. Fertil Steril 2004;81:19–25.
9. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. Fertil Steril 2010;94:1044–51.
10. Hansen KR, Hodnett GM, Knowlton N, Craig LB. Correlation of ovarian reserve tests with histologically determined primordial follicle number. Fertil Steril 2011;95:170–5.
11. Anderson RA, Anckaert E, Bosch E, Dewailly D, Dunlop CE, Fehr D, et al. Prospective study into the value of the automated Elecsys antimullerian hormone assay for the assessment of the ovarian growing follicle pool. Fertil Steril 2015;103:1074–80.
12. Dumont A, Robin G, Dewailly D. Anti-müllerian hormone in the pathophysiology and diagnosis of polycystic ovarian syndrome. Curr Opin Endocrinol Diabetes Obes 2018;25:377–84.
13. Bhide P, Homburg R. Anti-Mullerian hormone and polycystic ovary syndrome. Best Pract Res Clin Obstet Gynaecol 2016;37:38–45.
14. Teede H, Misso M, Tassone EC, Dewailly D, Ng EN, Azziz R, et al. Anti-Müllerian hormone in PCOS: a review informing international guidelines. Trends Endocrinol Metab 2019;30:467–78.
15. Jacobs MH, Reuter LM, Baker VL, Craig LB, Sakkas D, Surrey E, et al. A multicentre evaluation of the Elecsys® anti-Mullerian hormone immunoassay for prediction of antral follicle count. Reprod Biomed Online 2019;38:845–52.
16. Nicholas SL, Calder N, Field HP, Balen AH, Barth JH. The utility of Anti-Müllerian hormone in diagnosing polycystic ovary syndrome amongst women presenting to an infertility clinic. In: European Society of Human Reproduction and Embryology; 2014 June 29–2014 July 2; Munich, Germany.
17. Lie Fong S, Laven JS, Duhamel A, Dewailly D. Polycystic ovarian morphology and the diagnosis of polycystic ovary syndrome: redefining threshold levels for follicle count and serum anti-Mullerian hormone using cluster analysis. Hum Reprod 2017;32:1723–31.
18. Dewailly D, Gronier H, Poncelet E, Robin G, Leroy M, Pigny P, et al. Diagnosis of polycystic ovary syndrome (PCOS): revisiting the threshold values of follicle count on ultrasound and of the serum AMH level for the definition of polycystic ovaries. Hum Reprod 2011;26:3123–9.
19. Pigny P, Jonard S, Robert Y, Dewailly D. Serum anti-Mullerian hormone as a surrogate for antral follicle count for definition of the polycystic ovary syndrome. J Clin Endocrinol Metab 2006;91:941–5.
20. Wongwananuruk T, Panichyawat N, Indhavivadhana S, Rattanachaiyanont M, Angsuwathana S, Techatraisak K, et al. Accuracy of anti-Mullerian hormone and total follicles count to diagnose polycystic ovary syndrome in reproductive women. Taiwan J Obstet Gynecol 2018;57:499–506.
21. Yue CY, Lu LK, Li M, Zhang QL, Ying CM. Threshold value of anti-Mullerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. PLoS One 2018;13:e0203129.
22. Nelson SM, Pastuszek E, Kloss G, Malinowska I, Liss J, Lukaszuk A, et al. Two new automated, compared with two enzyme-linked immunosorbent, antimüllerian hormone assays. Fertil Steril 2015;104:1016–23.
23. Iliodromiti S, Salje B, Dewailly D, Fairburn C, Fanchin R, Fleming R, et al. Non-equivalence of anti-Müllerian hormone automated assays—clinical implications for use as a companion diagnostic for individualised gonadotrophin dosing. Hum Reprod 2017;32:1710–5.
24. Peña AS, Witchel SF, Hoeger KM, Oberfield SE, Vogiatzi MG, Misso M, et al. Adolescent polycystic ovary syndrome according to the international evidence-based guideline. BMC Medicine 2020;18:1–6.
25. Jamil AS, Alalaf SK, Al-Tawil NG, Al-Shawaf T. Comparison of clinical and hormonal characteristics among four phenotypes of polycystic ovary syndrome based on the Rotterdam criteria. Arch Gynecol Obstet 2016;293:447–56.
26. Bozdag G, Mumusoglu S, Coskun ZY, Yarali H, Yildiz BO. Anti-Müllerian hormone as a diagnostic tool for PCOS under different diagnostic criteria in an unselected population. Reprod Biomed Online 2019;39:522–9.
27. Lim SS, Davies MJ, Norman RJ, Moran LJ. Overweight, obesity and central obesity in women with polycystic ovary syndrome: a systematic review and meta-analysis. Human reproduction update 2012;18:618–37.
28. Lie Fong S, Schipper I, Valkenburg O, de Jong FH, Visser JA, Laven JSE. The role of anti-Müllerian hormone in the classification of anovulatory infertility. Eur J Obstet Gynecol Reprod Biol 2015;186:75–9.

Hormona antimülleriana para determinar la morfología del ovario poliquístico.

Objetivo: Determinar un punto de corte para el inmunoensayo Elecsys AMH Plus (Roche Diagnostics, Basel, Suiza) para identificar la morfología del ovario poliquístico (PCOM), un criterio de síndrome de ovario poliquístico (PCOS).

Diseño: El estudio de la Proteína AMH en Humanos para Prueba Diagnóstica de la Morfología del Ovario Poliquístico fue un estudio retrospectivo, multicéntrico, caso-control. El nivel sérico de hormona antimülleriana (AMH) fue medido utilizando el inmunoensayo Elecsys AMH Plus. El conteo de folículos antrales fue determinado utilizando ecografía transvaginal. El punto de corte de AMH fue derivado y validado en cohortes separadas con casos de PCOS con fenotipo completo (oligo/ anovulación, hiperandrogenismo, y PCOM) versus controles. Se realizaron análisis exploratorios de edad y fenotipo PCOS.

Escenario: No aplica.

Paciente(s): Mujeres síndrome de ovario poliquístico-positivas (PCOS A-D por criterios de Rotterdam) y PCOS-negativas de 25-45 años.

Intervención(es): Ninguna.

Medida(s) de Resultado Principal: Un punto de corte validado para AMH utilizando el ensayo Elecsys AMH Plus para PCOM.

Resultado(s): En la cohorte de validación (455 casos y 500 controles) un punto de corte de AMH de 3.2 ng/mL (23 pmol/L) resultó en una sensibilidad de 88.6% (intervalo de confianza 95% [CI] 85.3-91.3) y una especificidad de 84.6% (CI 95% 81.1-87.7) para el diagnóstico de PCOM así como un área bajo la curva característica operador-receptor de 93.6% (CI 95% 92.2-95.1). En mujeres de 25-35 años, la sensibilidad y especificidad para el punto de corte fueron 88.5% y 80.3%, respectivamente, versus 77.8% y 90.1%, respectivamente, en mujeres de 36-45 años. Los resultados fueron consistentes en los fenotipos PCOS A-D.

Conclusión(es): El inmunoensayo Elecsys AMH Plus, con un punto de corte de 3.2 ng/mL (23 pmol/L), es un método robusto para identificar PCOM para ayudar en el diagnóstico de PCOS.