Original Article

AMH MEASUREMENT VERSUS OVARIAN ULTRASOUND IN THE DIAGNOSIS OF POLYCYSTIC OVARY SYNDROME IN DIFFERENT PHENOTYPES

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ABSTRACT

Objective: This study was designed to assess the value of serum anti-Müllerian hormone (AMH) in the diagnosis of polycystic ovary syndrome (PCOS) in various phenotypes and to assess ovarian ultrasound parameters.

Methods: We performed a retrospective matched controlled study of 113 females with various PCOS phenotypes and 47 matched controls. The diagnostic utility of AMH measurement and ovarian ultrasound were compared. Using receiver operating characteristic (ROC) curve analyses, the threshold for AMH (>4.7 ng/mL) and ultrasound parameters (follicle number per ovary [FNPO] >22 and ovarian volume [OV] >8 cc) were established.

Results: In the entire cohort, AMH had a low sensitivity of 79%; while FNPO and OV were 93% and 68%, respectively. Specificities ranged from 85 to 96%. In classic anovulatory PCOS, AMH exhibited a sensitivity of 91%, and for FNPO and OV the corresponding sensitivities were 92% and 72%. In the ovulatory phenotype, AMH sensitivity was only 50%, while FNPO and OV were 95% and 50%, respectively. In the nonhyperandrogenic phenotype, the sensitivity of AMH was 53% while those for FNPO and OV were 93% and 67%.

Conclusion: AMH does not appear to be helpful for all subjects with PCOS but may be of some value in those who are anovulatory. However, FNPO was highly sensitive in all phenotypes, and was the single best criterion assessed for all subjects, suggesting the important role of ultrasound. **(Endocr Pract. 2016;22:287-293)**

Abbreviations:
AEPCOS = Androgen Excess and PCOS Society; **AMH** = anti-Müllerian hormone; **BMI** = body mass index; **DHEAS** = dehydroepiandrosterone; **FNPO** = follicle number per ovary; **OV** = ovarian volume; **PCO** = polycystic ovaries; **PCOS** = polycystic ovary syndrome; **ROC** = receiver operating characteristic.

INTRODUCTION

The diagnosis of polycystic ovary syndrome (PCOS) has remained somewhat controversial for several years, and 3 different diagnostic criteria have been proposed (1-3). In December 2012, a National Institutes of Health workshop endorsed the "Rotterdam" criteria as a working diagnosis, as did the Endocrine Society guidelines (4). In using the Rotterdam diagnosis, the evaluation of menstrual function is fairly clear, but marked variability occurs in the assessment of the presence or absence of hyperandrogenism (clinical or biochemical) and/or the ultrasound findings of polycystic ovaries (PCO). Specifically, lack of precision and accuracy in clinical assays for testosterone may lead to erroneous results in the hyperandrogenism evaluation (5), and ultrasound ovarian findings may be heterogeneous. Moreover, various criteria have been used for the diagnosis of PCO (6-9).

According to the Rotterdam or Androgen Excess and PCOS Society (AEPCOS) diagnostic guidelines, the ultrasound diagnosis of PCO should be based on the increased number of small ovarian follicles, with many terms used such as antral follicle count and follicle number per ovary (FNPO, which is our preferred terminology) (7), and/or

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increased ovarian volume (OV) (2). However, using more sensitive ultrasound probes and the vaginal route, it has been determined that the ovary contains many more small follicles than previously observed. In response to this, the new AEPCOS guidelines for assessing ovarian ultrasound characteristics in PCOS have recommended setting the threshold of FNPO (both ovaries) for the diagnosis of polycystic ovaries at ≥25, but only when using newer technologies that afford maximal resolution of ovarian follicles (i.e., using a transducer with a frequency ≥8 MHz) (9). If such technology is not available, it is recommended to use increased OV rather than FNPO for the diagnosis of PCO (9). However, the use of OV as an alternative has not been adequately validated and may have a relatively low sensitivity (9,10). Ovarian size may also be different depending on the particular phenotype of the woman presenting with the presumptive diagnosis of PCOS (11).

There is a need to consider new diagnostic tools to replace or enhance the somewhat subjective and heterogeneous ovarian findings used for PCOS diagnosis. In addition, diagnostic ultrasound may not be available or could be impractical and/or costly for certain types of practices. The measurement of anti-Müllerian hormone (AMH) in blood may offer an attractive and simple alternative. AMH is the product of granulosa cells from small preantral follicles, which are abundant in subjects with PCOS, and may have a role in suppressing follicle-stimulating hormone (FSH) action contributing to the anovulation. It is well known that subjects with PCOS have increased serum AMH values (5,12-18), and several studies have demonstrated that serum AMH positively correlates with ovarian follicle counts, androgen levels (5,12,17), and PCOS symptom severity (18).

While several different thresholds for increased AMH values have been proposed (from 3 to 5 ng/mL), serum AMH alone has been considered to have a relatively low diagnostic sensitivity, being increased in only 70 to 80% of subjects with PCOS (using Rotterdam or AEPCOS criteria). In a recent paper, the sensitivity of an increased AMH value for the diagnosis of PCOS was 76% (17), and a meta-analysis using a cutoff value of 4.7 ng/mL reported a sensitivity of 82% and a specificity of 79% (14). There has been some concern regarding AMH assay reliability due to technical issues and the absence of an international standard (19). Accordingly, at this time, AMH is not considered part of the routine assessment in the diagnosis of PCOS.

Here we wished to re-assess the potential value of AMH as a diagnostic criterion for ovarian findings in subjects with an established diagnosis of PCOS using standardized ultrasound and assay methodologies. Specifically, we wished to assess its usefulness in various PCOS phenotypes using the Rotterdam criteria. We were interested in determining whether an AMH value may assist in diagnosing PCOS if ultrasound were not available or if limited ultrasound data were assessed. A secondary goal was identify which ultrasound findings would be most diagnostic for the various phenotypes.

METHODS

Patients and Controls

This was a retrospective matched control study. A total of 113 subjects with an established diagnosis of PCOS were evaluated to investigate the diagnostic value of serum AMH and the sensitivity of various ovarian ultrasound parameters. These patients, aged 19 to 35 years (mean age 23 ± 4.3 years, mean body mass index [BMI] 27.9 ± 7.3 kg/m^2) were referred between 2013 and 2014 to the Endocrine Unit of the University of Palermo and the department of Obstetrics and Gynecology of the University of Pisa, Italy for the possible diagnosis of PCOS and associated symptoms. Most but not all of the subjects were referred for hyperandrogenism. The diagnosis of PCOS was made according to conventional Rotterdam criteria, including the original ultrasound criteria (2) with the exclusion of pelvic pathology, hyperprolactinemia, and congenital adrenal hyperplasia.

Some patients had been treated previously with various therapies for menstrual irregularity (oligomenorrhea or amenorrhea, defined by absence of menses for 6 or more months) and/or hyperandrogenism (acne or hirsutism) but had not received any treatment for at least 3 months before evaluation in this study. Menstrual cycles were recorded for ≥3 months, and oligomenorrhea was defined as irregular menstrual cycles at intervals of ≥35 days. The subjects with oligomenorrhea or amenorrhea were considered anovulatory. In patients reporting normal menses, at least 2 consecutive menstrual cycles were evaluated, and the finding of low levels of serum progesterone (<3 ng/mL, <9.54 nmol/L) in both cycles suggested the presence of chronic anovulation despite fairly regular withdrawal bleeding. In those with regular cycles and elevated serum progesterone, the diagnosis of ovulatory function was confirmed. Therefore, the study population included both anovulatory (n = 93) and ovulatory (n = 20) patients.

For controls, we selected a group of 47 age- and BMI-matched healthy females from Palermo using the same exclusion criteria described above. The controls were family members of hospital workers and had to have regular menses, no symptoms of hyperandrogenism (acne or hirsutism), and normal androgen levels. No control had any serious diseases. Normal menses were defined as cycles lasting 25 to 34 days. Height and weight were recorded, and BMI was calculated as kg/m^2.

All subjects underwent a complete history and physical examination, biochemical analyses, and transvaginal ultrasonography using an 8- to 10-MHz transducer. None of patients or controls was taking medications for at least 3 months before entering the study. The procedures were in accordance with the Helsinki Declaration of 1975, as

revised in 1983, and this study was approved by the local Ethic Council. All subjects provided informed consent to participate in the study.

Laboratory Analyses

In all patients and controls, serum levels of total testosterone (T), dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone (17OHP), and AMH were determined on cycle day 3 to 5. In nonmenstruating subjects, blood samples were obtained after withdrawal bleeding after progestogen administration. As stated above, controls and normally menstruating patients had serum progesterone measured on cycle day 21 or 22.

Serum hormone levels were quantified by well-established assays that were previously validated in our laboratory in Palermo (20). For AMH measurement, samples were collected into serum tubes with gel separators and centrifuged within 4 hours. We did not pre-mix serum with buffer, but samples were immediately analyzed in all cases, and none of the samples were stored and thawed at a later date for measurements. All samples were immediately assayed by a commercial ELISA provided by Beckman Coulter (AMH Gen II assay, Beckman Coulter, Brea, CA) and was carried out according to the manufacturer's specifications. When performing the examinations in 2013 and 2014, we used the conventional Beckman Coulter assay without the buffer premixing step. The conversion of AMH in ng/mL to pmol/L requires that values be multiplied by 7.143. All steroids were measured by specific radioimmunoassays after extraction using previously described methods (21). The intra- and interassay coefficients of variation did not exceed 6% and 15%, respectively.

Biochemical hyperandrogenism was defined as serum T >60 ng/dL (≥2.08 nmol/L) and/or serum DHEAS ≥3 μg/mL (>7.8 mmol/L). These values for hyperandrogenism have been validated with the use of the previously described assays (21).

Ovarian Ultrasound

In all patients and control subjects, on days 3 to 6 of withdrawal bleeding, ovarian morphology was assessed by transvaginal ultrasound using a transducer frequency of 8- to 10- MHz (MyLab 50 Xvision; Esaote, Genoa, Italy) Ovarian volume (OV) was determined as was the presence, size, and total number of ovarian follicles that were 2 to 10 mm (FNPO).

OV was calculated by the formula $\pi/6$ (D1 × D2 × D3) where the dimensions (D) of length, width, and thickness were used. The sizes of both ovaries were assessed, and mean OV was calculated.

Statistical Analysis

A power analysis had suggested that we would need at least 100 subjects with PCOS (various phenotypes) to identify significant AMH differences from controls.

Statistical analyses were performed using Statview 5.0 (SAS Institute, Cary, NC). Because several values were not normally distributed, a log transformation was necessary to obtain a normal distribution. Mann-Whitney *U* tests were performed to compare parameters between the PCOS and control groups. Analysis of variance (ANOVA) followed by Tukey tests were performed to assess differences in clinical and biochemical parameters between multiple groups. Cutoff values for elevations compared to the control population were determined with receiver operating characteristic (ROC) curve analyses. Here a plot of sensitivity against 1-specificity provides the curve analysis. The area under the curve of this plot provides information about sensitivity and specificity for various threshold values. These analyses were carried out by the Statistics Department of the University of Pisa.

Accuracy of FNPO, OV, and AMH values to discriminate between the various phenotypes of PCOS and controls were evaluated using ROC curve analyses. Differences in reliability between OV and AMH values were assessed by Tukey multiple comparison tests. $P<.05$ was considered statistically significant. All results are reported as mean ± SD.

RESULTS

All controls had normal androgen levels and ovulatory progesterone values. Mean AMH was 2.9 ± 0.8 ng/mL. Patients with PCOS had significantly ($P<.01$) increases in AMH, testosterone, and DHEAS levels and OV and FNPO (Table 1).

The 4 PCOS phenotypes were anovulation, hyperandrogenism, and PCO (phenotype A, n = 73); anovulatory with hyperandrogenism and normal ovaries (phenotype B, n = 5); ovulatory with normal menses, hyperandrogenism, and PCO (phenotype C or OV-PCOS, n = 20); and anovulatory with normal androgen levels and no symptoms of hyperandrogenism and PCO (phenotype D or NH-PCOS, n = 15). Because the number of patients with phenotype B was small (otherwise "classic" patients but with normal ovaries) and because both phenotypes correspond to what has been defined as classic PCOS using NIH criteria, these patients were reported together and indicated as ANOV-PCOS.

The main hormonal and ultrasound data of these different PCOS phenotypes are compared in Table 2. The mean ages of the different phenotypes and controls were similar (ANOV-PCOS: 22.8 ± 4, OV-PCOS: 23.8 ± 5, NH-PCOS: 22.9 ± 5, and controls: 23.1 ± 4 years). The ANOV-PCOS group had higher BMI ($P<.01$), total T ($P<.01$), and AMH ($P<.01$) compared to the OV-PCOS and NH-PCOS groups and higher FNPO ($P<.01$) and OV ($P<.01$) compared to OV-PCOS but not NH-PCOS. Serum DHEAS levels were similar in the 2 hyperandrogenic groups (ANOV-PCOS and OV-PCOS) and significantly higher ($P<.01$) in both

groups compared to the NH-PCOS group. AMH differences remained significant also after adjusting for BMI.

Sensitivity of AMH, OV, and FNPO Thresholds

In normal controls, the upper 95% confidence interval for AMH values was 4.5 ng/mL (32.1 pmol/L). The corresponding upper limit values for FNPO and OV were 22 and 8.8 cc.

Using ROC curves, similar but slightly different threshold values for AMH and OV were obtained. AMH values >4.5 ng/mL had a sensitivity of 79% and a specificity of 91% for PCOS diagnosis. However, the best compromise between sensitivity and specificity was observed with a cutoff value of >4.7 ng/mL that presented a similar sensitivity (79%) but a higher specificity (96%) (area under the curve: 0.952, SD 0.014). For the OV value, the best compromise between sensitivity (68%) and specificity (91%) was an OV of 8 cc, while an OV >8.8 cc had a similar specificity but a slightly lower sensitivity (65%). An FNPO count >22 also provided the best compromise between sensitivity (93%) and specificity (85%) for PCOS diagnosis.

The thresholds selected by ROC curves in the entire cohort exhibited markedly different sensitivities in the different phenotypes (Table 3). In ANOV PCOS, AMH >4.7 ng/mL had a sensitivity of 91% and a specificity of 96% (area under the curve: 0.982, SD 0.002). An FNPO count >22 had the best sensitivity (92.3%) but a relatively low specificity (85%). In same patients, OV >8 cc had a sensitivity of only 72%, although the specificity was 91%. In OV PCOS, AMH >4.7 ng/mL had a sensitivity of only 50%, while an FNPO count >22 had a 95% sensitivity. The sensitivity of an OV value >8 cc was 50%. Similarly, in NH-PCOS, AMH >4.7 ng/mL had a sensitivity of 53%, while an FNPO count >22 had a sensitivity of 95%, and OV values >8 cc had a sensitivity of 67%.

Because of these challenges in OV-PCOS, and NH-PCOS, the efficacy of a combination of the weaker parameters (AMH and OV) was sought. When combining increased AMH (>4.7 ng/mL) and OV (>8 cc), the sensitivity for PCOS diagnosis increased to 85% for OV-PCOS and to 73% for NH-PCOS; the specificity was 95% for both.

DISCUSSION

In this study we assessed the possible use of AMH as a criterion for diagnosing various PCOS phenotypes using the Rotterdam criteria. Specifically, we questioned whether it would be useful if ultrasound were not available. We also compared the utility of serum AMH measurement with the major ultrasound parameters, namely FNPO and OV.

The first step was to use ROC curves to determine the threshold values for serum AMH and for ultrasound FNPO and OV. We carefully selected an age- and BMI-matched control population of normal ovulatory females to determine this. In the past, we have shown that in our population, where there is a relatively low prevalence of obesity (22), mean ovarian size is lower than in other populations. Using the upper 95% confidence intervals of a control population,

Table 1
Hormone Values and Ovarian Ultrasound Findings

	Control	PCOS[a]
n	47	113
Age (years)	23.1 ± 4	23 ± 4.3
BMI (kg/m^2)	27 ± 4	27.6 ± 6
Total T (ng/dL)	34 ± 12	71 ± 20[b]
DHEAS (μg/mL)	1.9 ± 0.4	2.7 ± 1[b]
AMH (ng/mL)	2.9 ± 0.8	9.2 ± 4.7[b]
FNPO	10 ± 4	31.9 ± 7[b]
OV (cc)	4.4 ± 1.8	9.6 ± 3.2[b]

Abbreviations: AMH = anti-Müllerian hormone; BMI = body mass index; DHEAS = dehydroepiandrosterone; FNPO = follicle number per ovary; OV = ovarian volume; PCOS = polycystic ovary syndrome; T = testosterone.
[a] PCOS diagnosed according to the Rotterdam criteria.
[b] *P*<.01 versus controls

Table 2
Hormonal and Ultrasound Data by PCOS Phenotype

	n	BMI	Total T (ng/mL)	DHEAS (μg/mL)	AMH (ng/mL)	FNPO	OV (cc)
Classic anovulatory PCOS	78	28.8 ± 7[a,c]	79 ± 19[a,c]	2.8 ± 1[c]	10.8 ± 4.7[b,c]	33 ± 6[b]	10.1 ± 2.3[b]
Ovulatory PCOS	20	25.5 ± 5	69 ± 18[c]	3 ± 1.2[c]	5.5 ± 1.8	29 ± 5	8.1 ± 2.5
Normoandrogenic PCOS	15	25 ± 6	43 ± 17	1.8 ± 0.6	5.4 ± 2.5	30 ± 6	9.3 ± 3.4

Abbreviations: AMH = anti-Müllerian hormone; BMI = body mass index; DHEAS = dehydroepiandrosterone; FNPO = follicle number per ovary; OV = ovarian volume; PCOS = polycystic ovary syndrome; T = testosterone.
[a] *P*<.05 versus ovulatory PCOS
[b] *P*<.01 versus ovulatory PCOS
[c] *P*<.01 versus normoandrogenic PCOS

Table 3
Sensitivity of Increased Circulating Values of AMH, FNPO Count, and OV[a]

	Anovulatory PCOS (n = 78)	Ovulatory PCOS (n = 20)	Normoandrogenic PCOS (n = 15)	Specificity for diagnosis of PCOS
AMH > 4.7 ng/mL	91%	50%	53%	96%
FNPO >22	92.3%	95%	93%	85%
OV >8 cc	72%	50%	67%	91%

Abbreviations: AMH = anti-Müllerian hormone; FNPO = follicle number per ovary; OV = ovarian volume; PCOS = polycystic ovary syndrome.
[a] Sensitivity and specificity were calculated with receiver operating characteristic analyses.

we considered that an ovarian volume of >7.5 cc indicated enlarged ovaries (10). In this study, we used a different statistical approach (ROC curves) and obtained similar although slightly higher values of ovarian size (threshold of 8 cc). The FNPO threshold was also slightly lower (>22) than that reported in a recent AEPCOS consensus review (16). Interestingly, the threshold of AMH levels in blood (>4.7 ng/mL) was the same that was recently reported in a meta-analysis (14).

Using these thresholds for AMH and ultrasound data in the entire cohort of PCOS patients diagnosed by Rotterdam criteria, AMH only had a sensitivity of 79% and therefore was deemed not useful. Interestingly, our results are very similar to those found in studies involving PCOS patients with larger body mass (17) and a recent meta-analysis of AMH values in PCOS (14).

While theoretically AMH should reflect the same information as the FNPO, we found that FNPO count had the highest sensitivity (93%), while OV had a sensitivity of only 72%. Differences in specificity were relatively small, and while the FNPO count had a lower specificity (85%), an FNPO count >22 was the best compromise between sensitivity and specificity. These data suggest that if reliable ultrasound technology is available, the FNPO count is the best single criterion for PCOS diagnosis and is preferable to serum AMH.

While we feel that PCOS is a clinical diagnosis that should be based on the characteristic criteria of menstrual function and evidence of hyperandrogenism, the ovarian criteria for the diagnosis as required by Rotterdam, are most aided by obtaining an accurate FNPO count. In this study, the FNPO was the single best criterion for all PCOS phenotypes, but we only assessed ultrasound criteria and AMH values for these comparisons. If serum AMH were to be used as a sole criterion for the diagnosis of PCOS, we would miss the diagnosis 20% of the time, while with the FNPO count only 7% of diagnoses would be missed. However, if an accurate FNPO is not available (as often happens), AMH measurement may be a better criterion than ovarian size. In fact, while recent AEPCOS guidelines for assessing ovarian ultrasound criteria for PCOS have suggested using ovarian size when accurate FNPO is not available (7), increased ovarian size had a lower diagnostic sensitivity than serum AMH.

While many studies have been carried out on the diagnostic value of AMH in entire PCOS cohorts (7,12-17), sparse data are available on the diagnostic value of AMH for different phenotypes (15,23). Li et al showed that AMH values are much lower in PCOS patients with normal androgen levels and concluded that AMH is only suitable for predicting PCOS in subjects with hyperandrogenism (23). Köninger and colleagues studied hyperandrogenic and nonhyperandrogenic females with PCOS and reported that in those without hyperandrogenism, AMH showed high specificity and was comparable to antral follicle counts; in patients with hyperandrogenism, AMH was superior to androgens and comparable to antral follicle counts. (15). In our view, the use of a commercial androgen assay, where there is limited sensitivity, may cause an issue in accurately distinguishing between subjects with truly normal or slightly elevated androgen levels. We do not believe there to be major differences in assay technology between the cited studies, although there is no international standard for AMH.

Our study evaluated the sensitivity and specificity of serum AMH for different PCOS phenotypes (not only those with and without hyperandrogenism) and assessed the values of FNPO and ovarian size. Our data therefore provide evidence that the generally considered low diagnostic sensitivity of serum AMH in subjects with PCOS is likely due to the inclusion of the milder phenotypes of PCOS where the sensitivity of serum AMH is low. In the more classic patients, ANOV PCOS, elevated AMH showed high sensitivity (91%), which was similar to that found for FNPO (92.3%) and with a slightly higher specificity. Conversely, increased OV consistently had low sensitivity (72%). Therefore in ANOV PCOS, serum AMH may be helpful and may preclude the need to perform an ultrasound. It is clear, however, that if hyperandrogenism is well established in anovulatory subjects, there may be no need to carry out AMH or ultrasound measurements to aid in the diagnosis.

The case is very different in anovulatory females with "normal" androgen levels and no signs of hyperandrogenism. Here, serum AMH was found to have low sensitivity (53%), and only FNPO would be valuable in aiding with the diagnosis (sensitivity 93%). In OV PCOS, serum AMH also showed a low sensitivity for the diagnosis of PCOS, while the FNPO count had a high sensitivity.

Because FNPO requires the availability of good ultrasound technology, we assessed the possibility of combining OV and AMH measurements for the diagnosis of milder phenotypes of PCOS. Indeed, in OV PCOS, increased serum AMH and elevated OV had a good sensitivity (85%) and a specificity of 95.6%.

The main limitation of our study is the low number of patients with milder forms of PCOS. Larger studies will be needed for definitive conclusions. In addition, we are keenly aware that this was a retrospective analysis, and our conclusions have to be considered in light of these limitations.

CONCLUSION

In summary, our data suggest that AMH may be a valuable adjunct for the diagnosis of PCOS, but only in anovulatory females. Here an AMH >4.7 ng/mL confirms the diagnosis with a high probability. Our data also suggest that in using ultrasound for the diagnosis of PCO, the simpler measurement of OV alone is insufficient to aid in the diagnosis. FNPO is clearly the most sensitive measurement, and was useful for all PCOS phenotypes. A small additional benefit may be afforded by combining AMH and OV in patients with OV PCOS, although this does not appear to be a very practical solution for making the diagnosis of PCOS in this setting.

AMH is not helpful as a diagnostic criterion in all subjects suspected of having PCOS, but it may be helpful in anovulatory subjects where it has the same diagnostic utility as the FNPO count. The single best criterion for all phenotypes appears to be the FNPO, while enlarged ovarian size alone has limited diagnostic utility.

DISCLOSURE

The authors have no multiplicity of interest to disclose.

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