Can Anti-Müllerian Hormone Predict the Diagnosis of Polycystic Ovary Syndrome? A Systematic Review and Meta-Analysis of Extracted Data

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Context: Existing biochemical tests for polycystic ovary syndrome (PCOS) have poor sensitivity and specificity. Many women with PCOS have high anti-Müllerian hormone (AMH) concentrations; thus, this may be a useful addition to the diagnostic criteria.

Objective: A systematic literature review was performed to assess the true accuracy of AMH in the prediction of PCOS and to determine the optimal diagnostic threshold.

Data Sources: Published and gray literature were searched for all years until January 2013.

Study Selection: Observational studies defining PCOS according to the Rotterdam criteria and assessing the value of AMH in diagnosing PCOS were selected. Ten studies of the initial 314 hits reporting AMH values in the diagnosis of PCOS were included in the meta-analysis and the construction of the summary receiver-operating characteristic curve. Four studies that plotted individual AMH serum levels of women with PCOS and controls on graphs were selected for individual data extraction.

Data Extraction: Two researchers independently assessed the abstracts resulted from the initial search against the inclusion criteria, graded the papers for selection and verification biases, and selected the papers that assessed the value of AMH in diagnosing PCOS. Data were extracted from 4 studies with the plotted individual data on graphs with the help of computer software.

Data Synthesis: The meta-analysis of the extracted data demonstrated the specificity and sensitivity in diagnosing PCOS in the symptomatic women of 79.4% and 82.8%, respectively, for a cutoff value of AMH of 4.7 ng/mL. The area under the curve was 0.87 (95% confidence interval 0.83–0.92), identical with the area under the curve of 0.87 for the summary receiver-operating characteristic curve involving 10 separate studies.

Conclusions: AMH may be a useful initial diagnostic test for PCOS subject to validation in prospective population cohorts. (J Clin Endocrinol Metab 98: 3332–3340, 2013)

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women of reproductive age and the most frequent cause of hyperandrogenism and oligo-ovulation, both of which have substantial psychological, social, and economic consequences (1). An increased awareness of this disorder in the general population and medical communities has taken place in recent years, with greater understanding of the long-term associations of the condition, including the metabolic syndrome and its associated comorbidities (2), as well as the risk of specific

* S.I. and T.W.K. contributed equally to this work.

Abbreviations: AES, Androgen Excess Society; AFC, antral follicle count; AMH, anti-Müllerian hormone; AUC, area under the curve; CI, confidence interval; OR, odds ratio; PCOS, polycystic ovary syndrome; ROC, receiver-operating characteristic; SROC, summary ROC.
diseases such as endometrial cancer and insulin-resistant diabetes in women with a history of PCOS (3–5). Because of the heterogeneity in its presentation, women with PCOS frequently present to a range of disciplines including primary care, endocrinology, and gynecology. Although there has been considerable debate regarding the preferred diagnostic criteria for this heterogeneous condition, within Europe the 2003 Rotterdam criteria have gained considerable ground across specialties (6). This requires the presence of 2 or more of the following: chronic anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic ovaries on transvaginal ultrasonography. The accurate assessment of hyperandrogenemia in women can be technically challenging with the immunoassays in widespread use, and although the free androgen index is often also used, its validity is uncertain (7–9). Thus, an accurate single diagnostic biochemical test would have advantages.

Anti-Müllerian hormone (AMH) is produced by the granulosa cells of follicles from the time at which follicle growth is initiated (10, 11) and is a regulator of early follicular recruitment from the primordial pool (12). AMH expression continues until follicles reach approximately 8 mm in diameter, and expression is very low in larger antral follicles (13, 14). Consequently, there is a good correlation between AMH and antral follicle count (AFC) (15–20). Women with PCOS have high AMH concentrations (21, 22), and accordingly, AMH has been proposed as a marker of PCOS and as a substitute for AFC in the diagnosis of PCOS (21, 23–26). AMH also correlates with the other criteria of PCOS: oligoamenorrhea and hyperandrogenism (17, 18, 20, 26–28). At present a variety of cutoff values of AMH have been proposed but with varying sensitivity and specificity, and the optimal threshold is unknown (23–25, 29). Whether these thresholds should be age specific is also unclear, given the marked changes in AMH in the normal population across the reproductive life span (30) and the possibility that AMH declines in a less rapid manner in women with World Health Organization class II anovulatory infertility (31).

The aim of the present systematic literature review was to assess the accuracy of AMH in the prediction of PCOS and to perform a data aggregation meta-analysis to determine the optimal diagnostic threshold.

Materials and Methods

Search strategy

The following electronic databases were searched up to January 2013: PubMed, Embase, Medline, Web of Knowledge, and the Cochrane trial register. Search terms for PCOS (MeSH, PCOS, PCOD, hyperandrogenism, hirsutism) and antimüllerian hormone, müllerian-inhibiting substance, or müllerian-inhibiting factor were used. A period including all years through January 2013 was covered by the search. The search was limited to papers published in English and related to humans. The abstracts of all studies identified were graded by 2 researchers (S.I. and S.M.N.). Any article that could possibly be of value for the association between AMH and the PCOS was preselected. For completeness we also identified studies that assessed the diagnostic value of AMH for PCOS according to National Institute of Health (NIH) or Androgen Excess Society (AES) criteria (Supplemental Table 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). Only papers defining PCOS according to the Rotterdam criteria were included in our primary analysis to prevent large heterogeneity by pooling studies defining PCOS based on different criteria. In the next step, 2 researchers (S.I. and T.W.K.) carefully read and judged all preselected articles independently. If it was judged possible to construct 2 × 2 tables, in which the test result at a certain cutoff was related to the outcome parameter of PCOS, the study was selected for final recordings and analysis. In the event of any disagreement between the 2 researchers, the opinion of a third researcher (S.M.N.) was final. In every selected study, the reference list was scanned to identify studies that could possibly be included in the selection and then processed as described.

Each selected study was further scored by the researchers S.I. and T.W.K. on the following study quality characteristics: 1) sampling (consecutive vs other); 2) data collection (prospective vs retrospective); 3) study design (cohort vs case-control study); 4) blinding (present or absent); 5) selection bias; and 6) verification bias. Also, data on the cutoff levels used were recorded, as was the assay used for AMH measurement.

Because this review used only published data from the literature, no approval from an institutional review board was required.

AMH assay

Serum AMH values were standardized to give AMH measurements in nanograms per milliliter using the following conversion formula: 1 ng/ml = 7.143 pmol/L. The included studies either reported AMH according to the Immunotech-Beckman Coulter assay (Immunotech-Beckman Coulter, Marseille, France) or the DSL assay (Diagnostic Systems Laboratories Inc, Webster, Texas) as reported in Table 1. We converted the Diagnostic Systems Laboratories assay data into Immunotech-Beckman Coulter values using the conversion formula, 2.02 * Diagnostic Systems Laboratories = Immunotech-Beckman Coulter, which has a reported $r^2$ of 0.85 and has been used previously for data aggregation studies (32).

Analysis

The data were reported graphically in 4 studies and extracted using Plot Digitizer software (provided by sourceforge.net, found online at http://plotdigitizer.sourceforge.net/) to convert data points on the graphs into numerical data (33). Repeated data points were isolated using nonparametric bootstrap sampling (34) guided by the descriptive statistics provided in the supporting text, and we repeatedly sampled the possible repeated data points until we found the set that matched the descriptive statistics. Initial regression tree analysis (35) of the resulting data set showed that age did not contribute as a factor in an optimal predictive model of PCOS given both age and AMH. We therefore restricted full analysis to AMH alone, performing an ag-
Table 1. Characteristics of the 10 Included Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Study</th>
<th>Diagnosis of PCOS</th>
<th>N (PCOS) per Rotterdam</th>
<th>Age, y</th>
<th>Cutoff, ng/mL</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>AUC, 95% CI</th>
<th>Selection Bias</th>
<th>Verification Bias</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homburg et al (47)</td>
<td>2013</td>
<td>Prospective case-control</td>
<td>Rotterdam</td>
<td>90</td>
<td>32.1 ± 3.3</td>
<td>6.72</td>
<td>60.0</td>
<td>98.2</td>
<td>0.81</td>
<td>Yes</td>
<td>No</td>
<td>DSL</td>
</tr>
<tr>
<td>Woo et al (42)</td>
<td>2012</td>
<td>Prospective cross-sectional</td>
<td>Rotterdam</td>
<td>87</td>
<td>22.0–38.0</td>
<td>7.82</td>
<td>75.9</td>
<td>86.8</td>
<td>0.868–0.919</td>
<td>No</td>
<td>No</td>
<td>IBC</td>
</tr>
<tr>
<td>Chao et al (45)</td>
<td>2012</td>
<td>Case-control</td>
<td>Rotterdam</td>
<td>45</td>
<td>29.0–38.0</td>
<td>3.50</td>
<td>74.0</td>
<td>79.0</td>
<td>0.982–0.999</td>
<td>No</td>
<td>No</td>
<td>DSL</td>
</tr>
<tr>
<td>Eilertsen et al (25)</td>
<td>2012</td>
<td>Case-control</td>
<td>Rotterdam</td>
<td>56</td>
<td>33.3 ± 5.5</td>
<td>2.80</td>
<td>94.6</td>
<td>97.1</td>
<td>0.986–0.999</td>
<td>Yes</td>
<td>No</td>
<td>DSL</td>
</tr>
<tr>
<td>Lin et al (29)</td>
<td>2011</td>
<td>Prospective case-control</td>
<td>Rotterdam</td>
<td>126</td>
<td>27.7 ± 5.8</td>
<td>7.30</td>
<td>76.0</td>
<td>70.0</td>
<td>0.774–0.829</td>
<td>Yes</td>
<td>No</td>
<td>IBC</td>
</tr>
<tr>
<td>Dewally et al (24)</td>
<td>2011</td>
<td>Prospective</td>
<td>Rotterdam</td>
<td>62</td>
<td>20.1–34.0</td>
<td>4.90</td>
<td>92.0</td>
<td>97.0</td>
<td>0.947–0.998</td>
<td>Yes</td>
<td>No</td>
<td>IBC</td>
</tr>
<tr>
<td>Li HWR et al (46)</td>
<td>2011</td>
<td>Retrospective</td>
<td>Rotterdam</td>
<td>33</td>
<td>25.0–31.0</td>
<td>5.88</td>
<td>79.0</td>
<td>96.0</td>
<td>0.913–0.982</td>
<td>Yes</td>
<td>No</td>
<td>IBC</td>
</tr>
<tr>
<td>Li L et al (22)</td>
<td>2010</td>
<td>Cohort</td>
<td>Rotterdam</td>
<td>47</td>
<td>17.0–25.0</td>
<td>8.00</td>
<td>61.7</td>
<td>70.0</td>
<td>0.664–0.782</td>
<td>Yes</td>
<td>No</td>
<td>DSL</td>
</tr>
<tr>
<td>Hart et al (43)</td>
<td>2010</td>
<td>Prospective cohort</td>
<td>Rotterdam</td>
<td>64</td>
<td>14.5–17.6</td>
<td>4.20</td>
<td>53.1</td>
<td>69.8</td>
<td>0.64–0.74</td>
<td>No</td>
<td>No</td>
<td>IBC</td>
</tr>
<tr>
<td>Pigny et al (23)</td>
<td>2006</td>
<td>Prospective cohort</td>
<td>Rotterdam</td>
<td>73</td>
<td>22.0–36.4</td>
<td>8.40</td>
<td>67.0</td>
<td>92.0</td>
<td>0.851–0.905</td>
<td>Yes</td>
<td>No</td>
<td>IBC</td>
</tr>
</tbody>
</table>

Abbreviations: IBC, assay from Immunotech-Beckman Coulter; DSL, assay from Diagnostic Systems Laboratories Inc. Diagnosis of PCOS was determined by the Rotterdam criteria. Age values are reported as range or mean ± SD.

The optimal sensitivity and specificity from the ROC curve for the 4 combined studies was collated with the sensitivities and specificities reported for the 10 studies resulted from the systematic search, forming a data set for summary ROC (SROC) analysis. Publication bias was assessed using the funnel plots of reported sensitivities and specificities against study size, following the methodology described by Delgado-Bolton et al (36). A symmetric plot would provide reassurance, whereas an asymmetric plot would suggest the presence of publication bias.

Initial analysis of the combined diagnostic log-odds ratio (OR) showed that the collected studies were heterogeneous with respect to OR, indicating that studies should be assigned weights inversely proportional to the variance of the log of the diagnostic OR of the study (37). Subsequent analysis followed the standard SROC methodology (38–40) of the following: 1) log-adjusting sensitivities and specificities and separation into variables D (the diagnostic log-OR) and S (a measure of diagnostic threshold); 2) weighting the D and S values by inverse variance; 3) fitting the adjusted data to the affine model \(D = a + bS\); 4) reversing the log adjustment of a and b to obtain a summary ROC curve.

### Results

#### Systematic review

The systematic search of the biomedical databases produced 484 hits; after excluding duplicates, 314 citations were identified (Figure 1). Unpublished literature (gray literature, open gray web site) fulfilling the search indexes was not identified. After excluding articles based on the title or abstract, 55 articles were assessed fully for eligibility. Ten studies reported the capacity of AMH in diagnosing PCOS according to the Rotterdam criteria (6) and were included in the meta-analysis (22–25, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32).
The characteristics of the included studies are listed in Table 1. Most the studies were prospective, but selection biases were apparent in most of the studies, which recruited the participants from gynecological or fertility clinics. We also identified 4 studies defining PCOS according to AES or NIH criteria, which assessed the value of AMH in diagnosing PCOS (Supplemental Table 1) (25, 43, 48, 49). However, we have not conducted a meta-analysis on the studies defining PCOS according to non-Rotterdam criteria because this was not our primary objective and may introduce significant selection bias.

In addition, 4 of the above-mentioned 55 studies included individual serum AMH levels in female participants with PCOS (according to the Rotterdam criteria) and controls plotted in graphs (26, 45, 50, 51). Women with PCOS were recruited from gynecology/infertility clinics, ie, were symptomatic, rather than from the general population. The controls used were similar for all 4 studies and specified the following: 1) had regular menstrual cycles with an interval of 21–35 days; 2) had no medical history of hirsutism or severe acne; 3) had no evidence of endocrine disease; 4) had no history of ovarian abnormalities; 5) had no history of ovarian or uterine surgery; and 6) had no history of taking medicines that contained hormones within the previous 2 months. In the study of Chao et al (45), all controls had at least one natural pregnancy carried to term. The raw data were extracted with the assistance of software and were combined in a single data set of serum AMH levels for 146 females with PCOS (according to the Rotterdam criteria) and 136 control women without PCOS. For 2 of these studies, AMH was plotted relative to age, and therefore, a third data set with AMH and age was created for 110 women with PCOS and 103 controls (26, 45).

**Figure 2.** AMH in the diagnosis of PCOS. Top panel, The summary ROC curve (thick line), reported sensitivity and specificity values of the 10 included studies (circles), and the sensitivity and specificity values for the individual patient data aggregation meta-analysis (square). Bottom panel, ROC curve, optimal cutoff value, and AUC for the individual patient data aggregation meta-analysis.  

**Accuracy of AMH in diagnosing PCOS (Rotterdam criteria)**

Sensitivities and specificities for the diagnosis of PCOS calculated from each study reporting on AMH are summarized in Table 1. The sensitivity varied between 64% and 99%. There was no evidence of publication bias in the studies used to obtain summary statistics (Figure 2), The SROC curve obtained from all studies has high area under the curve (AUC) of 0.87 (Figure 3, top panel).
Meta-analysis of the extracted data for accuracy of AMH in diagnosing PCOS (Rotterdam)

The extracted raw data were used for descriptive statistics. Women with PCOS had a similar age range to women without PCOS [29 years (15–44 years) vs 31 years (12–44 years), mean (range)]. Serum AMH was almost 4-fold higher in women with PCOS compared with non-PCOS women [median (25th to 75th percentile), 8.71 ng/mL (5.29–14.09 ng/mL) vs 2.36 ng/mL (1.52–4.24 ng/mL)].

Specificity and sensitivity in diagnosing PCOS in symptomatic women by using AMH were calculated from the raw data set and were 79.4% and 82.8%, respectively, for a cutoff value of AMH of 4.7 ng/mL. The AUC was high [0.87, 95% confidence interval (CI) 0.83, 0.92] suggesting that AMH is a good diagnostic test of PCOS (Figure 2, bottom panel). Adding age to AMH in the predictive model did not change the cutoff value of AMH: the optimal classification tree using both AMH and age had a single bifurcation at the AMH value 4.7 ng/mL, with age not contributing as a variable.

Clinical value

On the basis of the summary ROC curve depicted in Figure 2A, a range of threshold AMH values for the diagnosis of PCOS with their associated sensitivities and specificities were calculated (Table 2).

Discussion

This systematic review, extracted data meta-analysis, and summary ROC summarize the currently available evidence concerning the accuracy of AMH in the diagnosis of PCOS. It would appear that AMH has good discriminatory capacity in separating normal women and women with PCOS as defined by the Rotterdam criteria (6). Several previous studies have suggested diagnostic thresholds for AMH for PCOS diagnosis (22–25, 42–47). Our summary graphic demonstrates that these may have potentially over- or underestimated the diagnostic performance. This is likely to reflect the variability in study design including sample size, recruitment source, age of participants, and control selection. The composition of PCOS subclasses within each of these studies may also have dif-
AMH concentrations have been shown to differ relative to which of the 3 components of the Rotterdam consensus statement are present (52). The appropriateness of our suggested threshold for the generic consensus definition of PCOS can be confirmed in well-phenotyped prospective population cohorts.

The largest study (24) has provided very similar values to that derived from our aggregated data meta-analysis of 4.7 ng/mL or greater (33.6 pmol/L), suggesting a biological plausibility for this value, even though it did not contribute to our data meta-analysis. However, this study (24) clearly excluded from the control group women with asymptomatic polycystic ovaries who may have increased AMH levels, thereby accentuating any difference, whereas other studies may have included them in the controls resulting in different diagnosing thresholds (53). The impact of different control selection has also been identified by Rosenfield et al (48), who suggested an AMH greater than 6.2 ng/mL for a diagnosis of PCOS according to NIH criteria but a much higher cutoff level of 10.7 ng/mL for specifically discriminating PCOS women from asymptomatic women with polycystic ovary morphology.

Although the meta-analysis and summary ROC curve have focused on diagnosing PCOS according to the Rotterdam criteria, AMH would appear to have a good discriminatory value in diagnosing PCOS according to AES or NIH criteria also, as shown by the high AUC of the ROC analysis of each individual study identified (Supplemental Table 1). The only contradictory study is by Hart et al (43), but the authors acknowledge that by defining PCOS based on the AES criteria, the control group included teenaged girls with irregular cycles, so the study is likely to have included girls who would be otherwise diagnosed as PCOS according to the Rotterdam criteria.

Due to the heterogeneous nature of PCOS, it has been argued that no single value would be capable of defining the disease but rather that AMH can only replace polycystic ovary morphology (24, 25). The correlation of AMH and AFC is well known and strong, and furthermore, AMH correlates weakly but significantly with oligomenorrhea and biochemical hyperandrogenism (17, 18, 20, 26–28). However, that AMH correlates with all of these key features of the PCOS diagnosis would suggest a diagnostic threshold of AMH may be achievable accepting limitations of the sensitivity and specificity. In support of this possibility, a recent analysis of a case-control cohort suggested that 6.7 ng/mL would have a sensitivity of 60% and specificity of 98.2% for the diagnosis of PCOS (47).

AFC is currently a cornerstone of PCOS diagnosis according to the Rotterdam criteria (55). However, the current diagnostic threshold of 12 or more follicles per ovary has been questioned (24, 56). This is primarily due to technical advances in imaging, whereby identification of more follicles leads to a major but artificial increase in the prevalence of polycystic ovary morphology in normal populations and in particular in younger women (57–59).

Obstructive oligoovulation has reported sensitivity and specificity of 81% and 92%, respectively, for the diagnosis of PCOS (24, 59). A higher threshold of 19 follicles has reported sensitivity and specificity of 81% and 92%, respectively, for the diagnosis of PCOS (24), but because this is based in part on the quality of the ultrasound technology rather than a true biological/medical discrimination, there is a significant inherent artificiality to the proposed cutoffs (18, 21, 24). Consistent with this is the even more recent suggestion that this threshold should be raised to 26 follicles (60). Because AMH is produced from these small antral follicles (13), the alternative of measuring it as a stable product, which is not subject to the same ongoing technical advances or operator dependence, would be attractive (61).

In view of the biphasic effect of age on serum AMH values (30, 62–64), some authors have suggested adapting different thresholds according to the patients’ age (25). This may be particularly relevant in adolescents and young adults, in whom AMH levels are rising (30) and when the diagnosis is frequently made (65). However, in our data set, age did not have an effect on the suggested cutoff point. Although there is a need for analysis with larger data sets with age groups younger than 25 years, we therefore at
present suggest that age-specific thresholds with their inherent inconvenience are not required.

Limitations

Although the process of systematic literature review and meta-analysis is a practical way of generating a more powerful estimate of true-effect size with less random error than individual studies, it does come with some limitations. First of all, the heterogeneity of the studies must be addressed because it may affect the justification for pooling the data into one analysis. In the case of the present meta-analysis, heterogeneity was caused by both study quality characteristics and slight differences in study populations. This was overcome by using a growth curve with minimization of the residuals to develop an average/summary ROC curve. Additionally, the definition of included studies was limited to those that defined PCOS according to the Rotterdam criteria (6). However, we appreciate that the derived value and its associated sensitivity and specificity may not be applicable for all PCOS subgroups and confirmation of the optimal threshold for the various PCOS subtypes is warranted.

Many of these methodological problems can be overcome by using individual patient data meta-analysis, or as shown here, using data aggregation approaches, which, although not allowing adjustment for confounders, does provide a large number of values for primary analysis. Recent initiatives in this field include assessment of AMH for the prediction of excessive ovarian response (66). We acknowledge that we included only a fraction of the cumulative data for the estimation of the AMH threshold value, but the ROC resulted from the extracted data was very similar to the summary ROC resulting from the entire data. Thus, the expected variation in the suggested threshold value is likely very small.

Most of the pooled studies assessed women with PCOS who were recruited from fertility clinics. This may have underestimated or overestimated the sensitivity and specificity of AMH as a diagnostic test of PCOS due to the potential differential PCOS case mix seen in that clinical context. Case control studies classically overestimate the performance characteristics of a diagnostic test; however, potentially this effect of selection bias due to recruitment from a fertility clinic may be small because there is no trend of decreased sensitivity or specificity in the studies that involved participants from the general population (Table 1). However, women with elevated AMH due to other rare causes like granulosa cell tumors were clearly not included. Future prospective population cohorts will be able to confirm the utility and performance characteristics of our suggested thresholds for the diagnosis of PCOS in the general population.

Lastly, there are some limitations that apply specifically to the method used to assess AMH levels. The studies in this meta-analysis did not use the same AMH assay. There is a noteworthy difference between the Beckman-Coulter ELISA and the Diagnostic Systems Laboratories ELISA leading to a wide dispersion of values (41, 44). In the current study we have used a previously validated conversion factor to align these assays to the Immunotech standards that now underlie the Beckman Coulter Generation II assay (30, 33). This approach has been used previously to derive a normal range of AMH across the life course, with values equivalent to those observed in prospective cohorts (30, 53, 54). However, the proposed threshold value may change according to the AMH assay technique until the development of an international standard.

Summary

The current systematic review and meta-analysis suggest that AMH is a useful first-line investigation in the identification of women with PCOS. Future assessment of the role of AMH in the diagnosis of the various subcategories of PCOS that inevitably exist with the current classification system is required.

Acknowledgments

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