LETTER

Can androgens be replaced by AMH in initial screening of Polycystic Ovary Syndrome?

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Dear Editor,

Polycystic ovary syndrome (PCOS) is a hormonal disorder that affects roughly 6%–20% of women of reproductive age.[1] To be diagnosed with PCOS, a woman must meet at least two of the three criteria. First, presence of irregular periods: Women with PCOS often have fewer than nine menstrual periods in a year or experience prolonged menstrual cycles that are more than 35 days. Second, high levels of androgens: Women with PCOS may have high levels of androgens, such as testosterone, in their blood. This can cause symptoms such as excess hair growth on the face and body, acne, and male-pattern baldness. Third, presence of polycystic ovary (PCO) phenotype: Women with PCOS may have enlarged ovaries with multiple small cysts, as seen on an ultrasound. At the same time, other conditions that can cause similar symptoms must be ruled out, such as thyroid disorders and adrenal gland disorders.[2]

The accepted diagnostic criteria for PCOS suggest that an androgen test is essential for diagnosis (Figure 1A).[2] The chemiluminescence method is widely used worldwide due to its efficacy and cost-effectiveness. However, its accuracy in detecting low levels of androgens is not satisfactory. Our research indicates that the correlation (r^2) of testosterone (T) and androstenedione (A4) between the chemiluminescence method and the gold standard of mass spectrometry (MS) is only 0.334 and 0.285, respectively. This suggests that the chemiluminescence method can only explain 33.4% and 28.5% of MS-based T and A4 in female androgen detection. As a result, a

A

Rotterdam Consensus 2003 (two out of three required with exclusion)		Non-invasive and user-friendly PCOSt model[5]
Criterion	**Description**	**Predictors**
1. Clinical and/or bilchemical hyperandrogenism	Hisutism, elevated level of total or free testosterone	1. Serum level of Anti-Müllerian Hormone (AMH)
2. Oligo/amenorrhea, annovulation	Prolonged menstrual cycle	2. Upper limit of menstrual cycle length
3. Polycystic ovaries appearance on ultrasound	Defined by either the excessive number of intermediate follicles and /or increased ovarian volume.	3. Body mass index
Exclusion	Including, but not limited to, 21-hydroxylase deficient nonclassic adrenal hyperplasia, thyroid dysfunction, hyperprolactinemia, neoplastic androgen secretion, *et al.*	Without exclusion

B

	Training set		Validation set	
	Model 1 with T	Model 2 without T	Model 1 with T	Model 2 without T
AUC	0.924(0.896,0.962)	0.919 (0.890, 0.948)	0.922 (0.865, 0.979)	0.928 (0.873, 0.983)
True positive rate	0.976(0.952,0.988)	0.952 (0.922, 0.971)	1.000 (0.951, 1.000)	0.973 (0.907, 0.993)
True negative rate	0.431(0.305,0.567)	0.490 (0.359, 0.623)	0.417 (0.193, 0.680)	0.583 (0.320, 0.870)

C

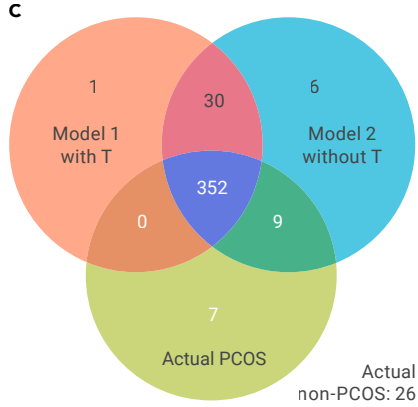

Figure 1. Comparison of Model Performance with and without Androgens (A) Description of the Rotterdam criterion used for diagnosing Polycystic Ovary Syndrome (PCOS) and introducing PCOSt, a PCOS screening tool that does not consider androgens. (B) The performances of models 1 and 2 were assessed using the AUC, sensitivity (true positive rate), and specificity (true negative rate), along with a 95% confidence interval (CI). (C) The Venn diagram illustrates the performance of the two models, indicating the number of predicted negative and positive PCOS cases for both models, as well as the actual PCOS and non-PCOS cases.

significant number of chemiluminescence measurements on female androgens are inaccurate, impairing the precise diagnosis of PCOS patients.

Excessive AMH can disrupt the hypothalamus-pituitary-ovary axis and lead to overproduction of androgen, particularly testosterone (T).[3,4] Based on this observation, we have previously developed the PCOSt model, which uses only three indicators - AMH, menstrual cycle, and BMI - to predict the probability of PCOS occurrence (Figure 1A).[5] By assessing the predicted risk of PCOS, we can stratify the population and implement targeted management approaches. However, the androgen data used to develop the PCOSt model relied on chemiluminescence-based androgens, including testosterone (T) and androstenedione (A4), which may not be accurate in detecting female androgens. Therefore, we developed a MS-based androgen panel comprising T, A4, dehydroepiandrosterone (DHEA), 11-hydroxyandrostenedione (11OHA4), 11-hydroxytestosterone (11OHT), 11-ketoandrostenedione (11KA4), 11KT, and 17-hydroxyprogesterone (17OHP).[6]

To sum up, we have developed a non-invasive PCOS screening model without androgens, named PCOSt, which is non-invasive and user-friendly. In this study, we plan to combine MS-based androgens with other commonly used clinical indicators associated with PCOS. By utilizing data modeling techniques, we aim to explore the feasibility of screening for PCOS without relying on androgens.

PREDICTING PCOS WITH AND WITHOUT ANDROGENS

We conducted a retrospective observational cohort study at Peking University Third Hospital, with ethics approval obtained from the Institutional Review Board of Peking University Third Hospital (approval number 2019-041-02). The same samples used in a previous study by Zhang et al. (2022) were analyzed,[6] which comprised of 432 patients who underwent oral glucose tolerance tests at our reproductive center. The objective of this study was to predict if the patient had PCOS or not, with age, BMI, MS-T, MS-A4, MS-DHEA, MS-11OHA4, MS-11KA4, MS-11OHT, MS-11KT, 17OHP, serum AMH level, serum inhibin B level, homeostatic model assessment of insulin resistance (HOMA-IR), and upper limit of menstrual cycle length (UML) used as predicting variables.

Table 1. The contributions of each predictor in model 1 and model 2

	Main Effect (%)		Total Effect (%)	
	model 1	model 2	model 1	model 2
AMH (ng/ml)	59.9	57.6	75.7	77.2
UML (days)	9.2	12.7	18.1	26.0
Age (years)	6.8	5.3	11.9	10.5
MS-T (nmol/L)	4.6		9.2	
inhibin B (pg/ml)	2.0	3.8	3.9	8.5

UML, upple limit of menstrual cycle length; MS-T, mass spectrum based testosterone.

Model 1: building a PCOS model using all independent predictors

For each independent variable, we conducted univariable logistic regression and eliminated those with an AUC (Area under ROC curve) less than 0.6 and P-value greater than 0.1 from the model building process. Six variables remained after this step, namely AMH, MS-T, MS-A4, inhibin B, age, and UML. Through preliminary exploration, we discovered that these six variables displayed a rough linear relationship with the outcome variable. As a result, we included them as continuous variables in the model building process.

To screen variables for this model, we utilized LASSO logistic regression and implemented a five-fold cross-validation method to establish the predicting model, as previously described.[7,8] Five variables, namely AMH, UML, age, MS-T, and inhibin B, were ultimately included in our model, which we referred to as model 1. The AUCs for the training and validation sets were 0.924 (0.896, 0.952) and 0.922 (0.865, 0.979), respectively. Table 1 presents the contributions of each predicting variable in model 1, displayed by main effect and total effect. The main effect shows the relative contribution of each variable alone, while the total effect demonstrates the relative contribution of each variable alone and in combination with others. In terms of the MS-based androgen panel, only MS-T was included in model 1, with a main effect contribution of only 4.6%. Given that MS-T' s contribution to model 1 was relatively small, we explored the possibility of excluding it during the initial PCOS screening.

Model 2: building a PCOS model without MS-T

We trained and validated another model, referred to as model 2, by refitting the same training and validation sets without MS-T, using only the other four variables. The contributions of each predictor in model 2 are also presented in Table 1. After removing MS-T, the contribution of AMH, in terms of both main and total effect, changed from 59.9% and 75.7% to 57.6% and 77.2%, respectively. The main and total effect of UML also increased from 9.2% and 18.1% to 12.7% and 26.0%, respectively, after excluding MS-T. The contribution of age and inhibin B also exhibited slight variation before and after excluding MS-T.

Figure 1B displays a comparison of the performances of the two models. The figure reveals that the 95% confidence intervals (CIs) of the AUC, true positive rates (sensitivity), and true negative rates (specificity) for models 1 and 2 overlapped, indicating no significant statistical difference between the two models. Therefore, model 2 without MS-T was not inferior to model 1.

We utilized the net reclassification improvement (NRI) to compare models 1 and 2. NRI is a simple summary measure that quantifies the improvement in performance caused by the addition of new risk markers to a prediction model. The NRIs were 0.035 (Z = 0.782, P = 0.434) and 0.140 (Z = 1.170, P = 0.242) for the training and validation sets, respectively, indicating no significant difference in prediction performance between the two models. Figure 1C presents a Venn diagram of the model prediction results for models 1 and 2.

LIMITATION

The data used in this letter primarily came from infertility patients who received oral glucose tolerance test (OGTT) at our reproductive center. These patients either had at least one of the three symptoms mentioned in the PCOS diagnostic criteria or metabolic diseases or high-risk factors for PCOS such as obesity. Whilst this group is different from the general infertile cohort, they still represent a natural cohort population. In the future, we plan to verify and clarify our conclusions in the general infertile cohort. We speculate that the conclusion that PCOS does not require androgens in the initial screening process for different cohort populations should remain the same, but the parameter estimates of the model may vary. This could result in differences in the predicted PCOS probability of the same subject using different models, which we will verify in future research.

PROSPECTIVE

Firstly, we constructed model 1 with five predictors to predict PCOS. Among the eight steroids in the MS-based androgen panel, only T had a small contribution of 4.6% and was included in the model. Subsequently, we excluded T and rebuilt model 2 using the remaining four variables. We found no significant difference between the performances of model 1 and model 2. Since AMH had the largest contribution in both models and has a good correlation with androgens (correlation coefficients between AMH and T and between AMH and A4 were 0.57 and 0.51, respectively[6]), it is possible that AMH could replace androgens for initial PCOS screening. However, for androgen-oriented PCOS subtyping and corresponding treatment, multiple androgen tests based on MS will still be required.

PCOS may lead to infertility, pregnancy complications, metabolic disorders, sleep apnea, emotional disorders, cardiovascular disease, and endometrial cancer. Given the potential risks involved, it is crucial to screen women for PCOS. We have developed a non-invasive screening tool for PCOS, utilizing AMH, menstrual cycle length, and body mass index to assess the likelihood of a subject developing PCOS and further classify the risk. Targeted intervention measures are then aimed at medium and high-risk populations accordingly. This study' s conclusion further reinforces the rationale of our prior PCOS screening model without the use of androgens, suggesting that for the initial diagnosis or screening of PCOS, AMH can effectively replace androgens and produce satisfactory results, as shown in the comparable model

performance. However, does this mean that androgens are meaningless in diagnosing PCOS? We disagree. Another study of ours illustrated the distinct contribution of various androgens to different PCOS phenotypes, implying that MS-based androgen detection may serve as a potential means for subtype diagnoses of PCOS. However, further researches are required to substantiate this possibility.

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DECLARATION OF INTERESTS

The authors declare no competing interests.