Efficacy of predictive models for polycystic ovary syndrome using serum levels of two antimüllerian hormone isoforms (proAMH and $AMH_{N,C}$)

Michael W. Pankhurst, Ph.D.,[a] Soulmaz Shorakae, M.D.,[c,d] Raymond J. Rodgers, Ph.D.,[b] Helena J. Teede, Ph.D.,[c,d] and Lisa J. Moran, Ph.D.[b,c]

[a] Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand; [b] The Robinson Research Institute, School of Medicine, University of Adelaide, Adelaide, South Australia, Australia; [c] Monash Centre for Health Research Implementation, School of Public Health and Preventive Medicine, Monash University, Melbourne, Victoria, Australia; and [d] Diabetes and Vascular Medicine Unit, Monash Health, Melbourne, Victoria, Australia

Objective: To compare total antimüllerian hormone (AMH), proAMH, $AMH_{N,C}$, and the ratio of the two forms in predictive models for polycystic ovary syndrome (PCOS) diagnosis. Total AMH consists of proAMH (inactive precursor) and $AMH_{N,C}$ (receptor-competent), but neither isoform has been tested individually for their ability to predict PCOS diagnosis.
Design: Cross-sectional study using biobanked samples collected between July 2008 and January 2010.
Setting: Not applicable.
Patient(s): Overweight, premenopausal women aged 18–45 years with PCOS (n = 45, with 21 fulfilling National Institutes of Health diagnostic criteria and 24 fulfilling European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE) criteria, but not National Institutes of Health criteria) and without PCOS (n = 23 controls).
Intervention(s): None.
Main Outcome Measure(s): Serum concentrations of proAMH and total AMH (proAMH and $AMH_{N,C}$ combined) were determined by immunoassay. The $AMH_{N,C}$ concentrations were calculated by subtraction ($[AMH_{N,C}]$ = [total AMH] – [proAMH]). Relative levels of proAMH were expressed as the AMH prohormone index (API = [ProAMH]/[Total AMH] × 100).
Result(s): In women with PCOS, total AMH, proAMH, and $AMH_{N,C}$ levels were higher, and the API was lower (P=.010), than in controls indicating increased conversion of proAMH to $AMH_{N,C}$. Receiver-operating characteristic analysis for proAMH (area under the curve [AUC] = 0.82), $AMH_{N,C}$ (AUC = 0.86), and API (AUC = 0.70) did not improve the prediction for PCOS when compared with total AMH (AUC = 0.86).
Conclusion(s): The proAMH and $AMH_{N,C}$ do not appear to improve the ability to predict a diagnosis of PCOS beyond total AMH assays. However, the ratio of inactive proAMH precursor to receptor-competent $AMH_{N,C}$ (API) differs in women with PCOS relative to unaffected controls indicating that AMH signaling mechanisms may be altered in women with PCOS. (Fertil Steril® 2017;108: 851–7. ©2017 by American Society for Reproductive Medicine.)
Key Words: Predictive models, receiver operator characteristic analysis, proprotein, prohormone, testosterone

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Reprint requests: Michael W. Pankhurst, Ph.D., Department of Anatomy, University of Otago, PO Box 913, Dunedin, 9054, New Zealand (E-mail: michael.pankhurst@otago.ac.nz).

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Polycystic ovary syndrome (PCOS) is a common condition affecting 9%–18% of women of reproductive age (1). The PCOS is associated with reproductive features including menstrual irregularity and infertility and metabolic features including insulin resistance and elevated risk factors for type 2 diabetes mellitus (2). Abnormal ovarian function in women with PCOS is characterized by impaired selection of a dominant follicle and increased numbers of primary and preantral follicles, potentially due to elevated intraovarian androgen production (3).

Women with PCOS tend to exhibit increased serum levels of antimüllerian hormone (AMH) (4–6). The AMH is a transforming growth factor β (TGF-β) superfamily member produced by granulosa cells (GCs) in developing ovarian follicles (7). The functions of AMH include inhibition of primordial follicle activation and regulation of GC sensitivity to FSH (8–10). The increased levels of circulating AMH in women with PCOS are thought to come from the greater number of follicles in polycystic ovaries (PCOs) and increases in AMH production per follicle (11, 12). It is still unclear whether alterations in AMH regulation exacerbate the reproductive features of PCOS, but there are positive associations between serum AMH levels and the degree of abnormal ovarian function in PCOS (13). The possibility that serum AMH levels could replace ultrasound in diagnosing polycystic ovary (PCO) morphology is currently being evaluated (14), but sensitivity and specificity can vary between study centers (15).

The AMH has two protein isoforms in circulation (16). It is synthesized as a cysteine-linked homodimer of the 560-amino acid preproprotein sequence that generates a 140-kDa proprotein precursor (proAMH) after removal of the signal sequence peptides (17). ProAMH is incapable of binding to the AMH type-2 receptor and generation of the form that can bind to the receptor requires proteolytic cleavage of proAMH between amino acids 451 and 452 (18, 19). The cleavage produces a 25-kDa carboxy-terminal dimer (AMH_C) and a 120-kDa amino-terminal dimer (AMH_N), which remain associated in a noncovalent complex ($AMH_{N,C}$) (20, 21) and both AMH_C and $AMH_{N,C}$ can bind to the AMH type-2 receptor (18). The proAMH is the predominant isoform secreted from GCs in the ovary (22), but $AMH_{N,C}$ is the predominant isoform in blood, with little or no free AMH_C detectable (16). Studies in mice indicate that proAMH and $AMH_{N,C}$ are cleared from blood at the same rate and proAMH is not converted to $AMH_{N,C}$ while in circulation (23). This strongly suggests proAMH to $AMH_{N,C}$ ratios in blood are indicative of extracellular proAMH cleavage rates within the ovary.

Circulating concentrations of proAMH and $AMH_{N,C}$ have not been quantified in women with PCOS, as clinical AMH assays measure total AMH, a combined measure of the two forms (24). However, analysis of follicular fluid (FF) from women with PCOS suggests that more proAMH produced is converted to $AMH_{N,C}$, when compared with FF from healthy women (22). The aim of the present study was to determine whether the ratios of proAMH or $AMH_{N,C}$ in serum are altered in women with PCOS relative to women without PCOS and to determine the ability of different AMH-related isoforms to predict a PCOS diagnosis.

MATERIALS AND METHODS

Participants

Study participants were women aged 18-45 years who were recruited between July 2008 and January 2010 as part of a previously published investigation (25). Participants were recruited through community advertisement. The PCOS was defined according to either National Institutes of Health criteria (two features of clinical or biochemical hyperandrogenism and chronic anovulation, and exclusion of other etiologies) (26) or the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) criteria (two of the three features of clinical or biochemical hyperandrogenism, PCO morphology, and oligoovulation or anovulation, and exclusion of other etiologies) (27). Control group participants were eligible if they had no history of diagnosed PCOS, no oligomenorrhea, or clinical or biochemical hyperandrogenism. Ultrasound was only requested for diagnostic purposes in women with only one other PCOS diagnostic feature. Ultrasound data were not collected as an experimental variable for analysis. Prospective participants were excluded if they were pregnant, smoking, had diabetes mellitus type 2, uncontrolled hypertension, had nonstable use of antihypertensives, were taking lipid-lowering or fish oil medications, or using hormonal (e.g., oral contraceptive [OC] pill), or insulin-sensitizing medication unless willing to cease medications for 3 months before study measurements. One woman with PCOS was using stable antihypertensive medication during the study. The study population consisted of 23 controls and 45 women with PCOS (21 qualifying for National Institutes of Health criteria and 24 diagnosed by Rotterdam criteria only). A priori power calculations indicated that this sample size was large enough to detect differences in the mean of one SD or more with β set to 0.95.

Institutional review board ethical approval was obtained from the Standing Committee on Ethics in Research Involving Humans for Monash University, the Human Research Ethics Committee of Monash Health, and the University of Otago Human Ethics Committee (Health). The study was conducted in accordance with the principles set out in the Declaration of Helsinki and all participants provided written, informed consent.

Sampling

Blood samples were taken in the morning between 8:00 and 10:00 AM. Blood samples were taken on days 0–14 of the ovarian cycle if the participant was regularly cycling, with the first day of menstrual bleeding recorded as day 0. Whole blood was stored on ice until centrifugation. Serum was collected and was stored at -80°C. Height, weight, and waist circumference data were also collected from each participant. Body mass index (BMI) was calculated by weight divided by height squared.

Hormone Assays

Total AMH was measured with the AMH Gen II ELISA (Beckman Coulter, Cat# A79765, following field safety notice