Comparative assessment of five serum antimüllerian hormone assays for the diagnosis of polycystic ovary syndrome

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Objective: To determine whether the different antimüllerian hormone (AMH) immunoassays on the market offer the same performance for the diagnosis of polycystic ovary syndrome (PCOS).
Design: A total of 95 serum AMH samples were retrospectively evaluated for a period of 3 months in the same laboratory.
Setting: Academic center laboratory.
Patient(s): Forty-eight control women with regular menses and no hyperandrogenism and 47 patients with classic PCOS (i.e., hyperandrogenism plus oligoanovulation) attending our department for infertility.
Intervention(s): None.
Main Outcome Measure(s): AMH measurement using five commercial assays. Method comparison and evaluation of the diagnostic performance by receiver operating characteristic analysis.
Result(s): Values obtained with Gen II and AL-105i ELISAs were similar to those provided by EAI AMH/MIS, whereas automatic assays generated lower values. A significant mean difference was observed between Access Dxi (1.35 ng/mL) or Cobas (1.73 ng/mL) and EIA AMH/MIS ELISA. By ROC analysis each assay displayed similar efficiency for PCOS diagnosis. Sensitivities varied from 49% to 74% when setting the specificity at 92%. Cluster analysis run in the control group identified a subgroup of asymptomatic women with polycystic ovary morphology (PCOM). After exclusion of PCOM, the 95th percentile of controls was 4.2 ng/mL (30 pmol/L) with the automatic assays and 5.6 ng/mL (40 pmol/L) with the manual assays.
Conclusion(s): Performance of the different AMH assays for PCOS diagnosis is comparable, providing that different threshold values are used for manual and automatic assays. Measurement of serum AMH level appears as a robust tool for the definition of PCOM. (Fertil Steril® 2016;105:1063–9. ©2016 by American Society for Reproductive Medicine.)
Key Words: AMH, assays, PCOS, diagnosis

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting up to 10% of women of reproductive age (1). The current diagnosis classification uses hyperandrogenism, oligoanovulation, and polycystic ovarian morphology (PCOM) at ultrasound (U/S) (2). Owing to its strong correlation with the number of growing follicles within the ovary (3, 4), we previously proposed to use the serum level of antimüllerian hormone (AMH), a specific product of granulosa cells (3), as a surrogate for follicle number at U/S (5). Using receiver operating characteristic curve (ROC) analysis and the EIA AMH/MIS assay, we recently proposed a revised threshold value of AMH above 35 pmol/L (4.9 ng/mL) as a surrogate for the follicle excess that characterizes PCOM (6). However, it is still unclear whether a switch from a morphological (PCOM) marker to a biochemical one (serum AMH) could translate into an accurate classification of patients with PCOS.

Received September 18, 2015; revised November 23, 2015; accepted December 16, 2015; published online January 6, 2016.
P.P. has nothing to disclose. E.G. has nothing to disclose. A.G. has nothing to disclose. G.R. has nothing to disclose. S.C.-J. has nothing to disclose. A.D. has nothing to disclose. D.D. has nothing to disclose.
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Fertility and Sterility® Vol. 105, No. 4, April 2016 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2015.12.023

Indeed, until recently, the two most widely distributed immunoassays (Gen II and EIA AMH/MIS) used to measure AMH levels in the serum provided different results on the same sample. These differences might be consecutive to [1] the absence of an international standard for AMH, [2] differences in the specificity of the antibodies used in the different kits, [3] the existence of different molecular forms of AMH at the circulating level (7), and [4] variable sensitivity of the immunoassays to interferences such as complement C1q and C3 (8), macroprolactinemia, or heterophilic antibodies (9). For example, until July 2013, complement interference in the Gen II assay resulted in falsely low AMH values around twofold less than those obtained with the EIA AMH/MIS (8, 10). A modified technical procedure was later released by the manufacturer to solve this problem (Beckman Coulter safety notice 20434-4, July 2013). Today the situation seems much more complicated because of the appearance of new products. Indeed, in 2014, Roche Diagnostics and Beckman Coulter launched an automated AMH assay on their analyzers (Cobas Elecsys and Access Dxi, respectively), while the manual immunoassay AL-105i manufactured by AnshLabs became more widely distributed in the United States and Europe.

Therefore we believe that it is time to readdress (reviewed in [11]) whether or not a unique threshold value of serum AMH may be defined to distinguish normal women from patients with PCOS. To reach this goal, we decided to measure AMH levels on serum samples from controls and patients with PCOS with five AMH assays: three manual plate-based enzyme-linked immunosorbent assays (ELISAs)—Gen II, EIA AMH/MIS, and Anshlab AL-105i, using up-to-date technical procedures—and two automated assays (on Access Dxi and Cobas analyzers) to address interkit variability, to determine the diagnostic performance of each kit by ROC analysis, and to propose assay-specific threshold values of AMH to be used for the definition of PCOM.

MATERIALS AND METHODS

Patients

Data from the 95 patients included in this study were obtained from a database including clinical, hormonal, and U/S features that were consecutively recorded between 2012 and 2014. These patients were referred to our department for exploration of hyperandrogenemia, menstrual disorders, and/or infertility due to male factor and/or tubal abnormality. Women with unexplained infertility or endometriosis were excluded. Clinical, hormonal, and U/S examinations were performed in the early follicular phase, between day 2 and 5 of the menstrual cycle. In patients with menstrual disorders, the last menstrual period was either spontaneous or induced by the administration of dydrogesterone (10 mg/d for 7 days). This study was approved by the Institutional Review Board of the University Hospital of Lille. All patients gave their informed consent before inclusion in this study.

Exclusion criteria were the following: age less than 18 or more than 35 years, suspicion of low ovarian reserve (FSH>12 IU/L and/or follicle number per ovary [FNPO] <6 and/or ovarian volume [OV] <2.3 mL), hyperprolactinemia (serum PRL >20 ng/mL on two subsequent determinations), or nonclassic 21-hydroxylase deficiency (basal 17-hydroxyprogesterone [OHP] >5 ng/mL and/or post-ACTH stimulated value >12 ng/mL). Ovarian or adrenal tumors were excluded on the basis of a serum total T or dehydroepiandrosterone-sulfate (DHAS) level lower than 1.5 ng/mL or 15 μmol/L, respectively. Any patient with criteria for hypothalamic amenorrhea was also excluded. Furthermore, any patient with at least one follicle with a diameter greater than 9 mm at U/S or a serum E_2 level above 80 pg/mL was excluded from the study.

Investigations

During the medical examination, patients were specifically asked about their menstrual history. Oligomenorrhea was defined as an average cycle length of more than 35 days and included women with amenorrhea. Clinical hyperandrogenemia was defined by the presence of hirsutism (modified Ferriman-Gallwey score over 6) and/or acne located in more than two areas. Hyperandrogenemia was defined as a serum T level >0.5 ng/mL and/or a serum androstenedione (A) level >1.75 ng/mL, according to our in-house thresholds.

PRL, LH, FSH, E_2, OHP, DHAS and T levels were measured by immunoassays as described elsewhere (12). Serum A was measured by liquid chromatography tandem mass spectrometry (unpublished data). For every patient, U/S determination of FNPO and OV was performed with a Voluson E8 Expert (General Electric Systems) with a 5–9 MHz transvaginal transducer, as described elsewhere (13).

AMH Assays

AMH was measured on the same serum sample stored frozen at −80°C in our biobank using five commercially available assays: three manual ELISAs—EIA AMH/MIS (A11893 Immunotech, Beckman Coulter), Gen II (A79765, Beckman Coulter), and Ultrasensitive AL-105i (Anshlab)—and two fully automated immunoassays—Access Dxi automatic analyzer (B13127, Beckman Coulter) and Cobas e instrument (Roche Diagnostics). All assays measure the proAMH and the cleaved AMH complex ($AMH_{N,C}$). Their functional sensitivity varies from 0.21 pmol/L (Cobas e; [14]) to 3.0 pmol/L (EIA AMH/MIS [J. Taieb, personal communication]). The detectable range of each assay was 0–150 pmol/L (six calibrators) for EIA AMH/MIS, 0–178 pmol/L (seven calibrators) for Gen II, 0–139.9 pmol/L (six calibrators) for AL-105i, 0–164 pmol/L (2 points calibration curve) for Cobas e, and 0–171 pmol/L (6 points calibration curve) for Access Dxi analyzer. All assays, except Gen II and Cobas e, use rhAMH as a calibrator dissolved in either protein-rich buffer (AL-105i, Access Dxi) or human serum-based matrix (EIA AMH/MIS). Gen II and Cobas-e assays use bovine AMH as a calibrator solubilized in animal serum. Assays were run simultaneously (two ELISA on the same day and the third ELISA and the automated assays on the next day) from November 2014 to January 2015. Manual immunoassays were performed in duplicate by the same operator. Each sample that generated an AMH