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Assessment of anogenital distance as a diagnostic tool in polycystic ovary syndrome

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Key message

AGD_{AC} measurements might be a useful clinical tool in the diagnosis of PCOS, in particular improving the accuracy of phenotype 'O+POM'. AGD has embryological associations, so these findings are compatible with the fetal programming of PCOS. Gynaecologists may therefore be able to evaluate the individual likelihood of patients developing symptoms.

Abstract

Research question: Is anogenital distance (AGD) a useful clinical tool for predicting polycystic ovarian syndrome (PCOS) and its main National Institutes of Health (NIH) phenotypes?

Design: Case–control study conducted between September 2014 and May 2016 at the Department of Obstetrics and Gynecology of the University Clinical Hospital 'Virgen de la Arrixaca' in the Murcia region (south-eastern Spain). One hundred and twenty-six cases of PCOS and 159 controls without PCOS were included. AGD measurements were taken from the anterior clitoral surface to the upper verge of the anus (AGD_{AC}), and from the posterior fourchette to the upper verge of the anus (AGD_{AF}). Parametric and non-parametric tests and receiver operating characteristic (ROC) curves were used to assess associations between AGD and the presence of PCOS and its phenotypes.

Results: AGD_{AC}, but not AGD_{AF}, was associated with PCOS and all its phenotypes (*P*-values <0.001 to 0.048). The highest area under the curve (0.62; 95% confidence interval 0.55 to 0.71) was obtained for all PCOS with AGD_{AC} with a sensitivity and specificity of 50.0% and 73.0%, and positive and negative predictive value of 59.0% and 64.4%, respectively.

Conclusions: AGD_{AC} could moderately discriminate the presence of PCOS and may be a useful clinical tool.

Keywords
anogenital distance, biomarker, phenotypes, polycystic ovarian syndrome, prenatal hormonal milieu

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy affecting reproductive-aged women (Filippou and Homburg, 2017), ranging in prevalence from 4% to 21% depending on the diagnostic criteria (Lizneva *et al.*, 2016a). PCOS is responsible for most cases of anovulatory symptoms and hyperandrogenism in women (Azziz *et al.*, 2004). It is associated with a broad range of associated morbidities, including reproductive abnormalities, insulin resistance, increased risk of type 2 diabetes mellitus, coronary heart disease, atherogenic dyslipidaemia, cerebrovascular morbidity, anxiety and depression (Balen *et al.*, 1995; Deugarte *et al.*, 2005; Ferriman and Purdie, 1983; Jedel *et al.*, 2010; Krentz *et al.*, 2007; Legro *et al.*, 2001; Norman *et al.*, 2001; Wild *et al.*, 2000). There is a growing consensus from a clinical as well as from an epidemiologic perspective towards a phenotypic approach to PCOS. This approach characterizes PCOS into four phenotypes according to several key features (Azziz *et al.*, 2009; Lizneva *et al.*, 2016b; National Institutes of Health, 2012). The aetiopathogenesis of PCOS is complex and still unclear. The prevailing concept is that PCOS is the result of intrinsic ovarian characteristics that interact with one or more congenital or environmental factors, even during fetal life, to cause dysregulation of steroidogenesis (Ehrmann, 2005). Several observational studies have shown an association between fetal hormonal environment and subsequent development of PCOS (Cresswell *et al.*, 1997; Davies *et al.*, 2012; Ibáñez *et al.*, 1998; Melo *et al.*, 2010; Michelmore *et al.*, 2001; Pandolfi *et al.*, 2008).

Anogenital distance (AGD) is a sexually dimorphic attribute in placental mammals, almost twice as long in males as in females (Greenham and Greenham, 1977; Kurzrock *et al.*, 2000). Human studies have shown that AGD is an anthropometric biomarker of androgenic environment during the development of the reproductive system (Dean and Sharpe, 2013; Jain and Singal, 2013) and of prenatal exposure to endocrine disruptors (Bornehag *et al.*, 2014; Swan *et al.*, 2005). In reproductive-aged women, AGD has been related to female reproductive function (Barrett *et al.*, 2015; Mendiola *et al.*, 2012; Mira-Escolano *et al.*, 2014a, 2014b). AGD has also been proposed as a measure of reproductive toxicity. Exposure to antiandrogens results in shorter (more feminine) AGD in infant males (Barrett *et al.*, 2017; Swan *et al.*, 2015), whereas exposure to stressful events leads to a longer (more masculine) AGD in infant girls (Barrett *et al.*, 2013). Recently, Barret *et al.* (2017) have reported that prenatal exposure to bisphenol A (BPA), an endocrine disruptor, is associated with shorter AGD in the female fetus, as previously described in animal models (Boberg *et al.*, 2013; Christiansen *et al.*, 2014). Barret *et al.* (2018) have also reported that newborn daughters of women with PCOS had a longer AGD; strong associations between longer AGD and PCOS in Chinese (Wu *et al.*, 2017) and Mediterranean adult women have also been shown (Sánchez-Ferrer *et al.*, 2017).

However, it is thought that no study has previously assessed the predictive ability of AGD measurements to discriminate the presence of PCOS. This study

explored the usefulness of AGD measurements to assess the presence of PCOS and of its different phenotypes characterized following the recommendations of the National Institutes of Health (NIH) consensus panel (National Institutes of Health, 2012).

Materials and methods

Study population

A case-control study was conducted between September 2014 and May 2016 at the Department of Obstetrics and Gynecology of the University Clinical Hospital 'Virgen de la Arrixaca' in Murcia (south-east Spain). The study rationale and design have been previously described in detail (Sánchez-Ferrer *et al*., 2017). Patients between 18 and 40 years old were excluded from the study if they were pregnant or lactating, having oncological treatment, suffering from genitourinary prolapse, endocrine disorders (e.g. Cushing syndrome, congenital adrenal hyperplasia, androgen-secreting tumours, hyperprolactinaemia and hyper- and hypothyroidism) or taking any hormonal medication, including contraception, during the 3 months prior to the study. Cases (n = 126) were women attending the Gynecology Unit of the hospital, and included prevalent and incident cases. Cases were included only if a diagnosis could be established following the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004), including medical history with the Ferriman–Gallwey scale (Ferriman and Gallwey, 1961), transvaginal ultrasound (TVUS) and reproductive hormone levels (Sánchez-Ferrer *et al*., 2017). A diagnosis of PCOS required the presence of at least two of the following three criteria: (i) hyperandrogenism, either biochemical (total testosterone level ≥2.6 nmol/l) or clinical (mF-G score ≥12) (Afifi *et al*., 2017) with or without acne or androgenic alopecia; (ii) oligo- and/or anovulation (menstrual cycles >35 days or amenorrhoea >3 months); (iii) polycystic ovarian morphology (POM) on TVUS (≥12 follicles measuring 2–9 mm in diameter, mean of both ovaries) (Conway *et al*., 2014). Moreover, the following four phenotypic subtypes of PCOS were also assessed (National Institutes of Health, 2012) (**Table 1**): (A) hyperandrogenism + oligo/amenorrhoea (H+O), (B) hyperandrogenism + polycystic ovarian morphology (POM) (H+POM), (C) oligo/amenorrhoea + POM (O+POM), and (D) hyperandrogenism + oligo/amenorrhoea + POM (H+O+POM). Controls (n = 159) were women without PCOS (or other major gynaecological conditions, e.g. endometriosis) attending the gynaecological outpatient clinic for routine gynaecological examinations. Both cases and controls underwent the same procedures: anamnesis and questionnaires, physical examination with AGD measurements, TVUS and blood drawing for hormone levels. Written informed consents were obtained from all subjects. This study was approved by the Ethics Research Committee of the University of Murcia and the Clinical University Hospital (no. 770/2013, approved 3 October 2013).

Gynaecological history and physical examination

Cases and controls were interviewed in person by gynaecologists. Two gynaecologists using the same methodology performed all clinical evaluations. All participants filled out health questionnaires, gynaecological and obstetrical history, and underwent a physical and gynaecological examination including TVUS and blood draw at a scheduled clinical visit. Weight and height were

measured using a digital scale (Tanita SC 330-S, London, UK) and body mass index (BMI) was calculated. Uterine and ovarian morphology were evaluated with TVUS (Voluson E8® and 4–9 MHz transducer; General Electric Healthcare, USA).

Anogenital measurements

The AGD was measured with women in the lithotomy position with thighs at 45° to the examination table. Using a digital calliper (Stainless Steel Digital Calliper, VWR® International, LLC, West Chester, PA, USA), AGD_{AC} was measured from the anterior clitoral surface to the upper verge of the anus, and AGD_{AF} from the posterior fourchette to the upper verge of the anus as previously described (Mendiola *et al.*, 2016; Sánchez-Ferrer *et al.*, 2017). AGD_{AC} and AGD_{AF} presented normal distributions and were correlated (Pearson correlation [r] = 0.60, $P < 0.001$). Intra- and inter-examiner coefficients of variation were below 5% and intraclass correlation coefficients above 0.95 for both AGD measurements. Two gynaecologists unaware of the patient's status measured each distance three times, resulting in a total of six measurements for each woman. Average values of the six measurements were used in the analyses.

Statistical analyses

Descriptive statistics are shown using raw data. Continuous variables were summarized by arithmetic mean, standard deviation (SD) and median, and categorical variables given as number and percentage (%). Unpaired Student's t-tests and Mann–Whitney U-tests were used for comparison of continuous variables between cases and controls. The chi-squared test was used for categorical variables. Information for the following covariates was also obtained: age (years), BMI (kg/m^2), vaginal delivery (yes/no), episiotomy (yes/no) and parity (classified as 0, 1, 2+). In order to evaluate the discriminating ability of AGD to detect PCOS, receiver operating characteristic (ROC) curves were generated using maximum likelihood estimation to fit a binomial ROC curve to continuously distributed data. ROC curves were created comparing both AGD measurements (AGD_{AF} and AGD_{AC}) and presence of all PCOS and phenotypic subtypes (H+O; H+POM; O+POM; H+O+POM) versus controls. To calculate sensitivity, specificity, positive and negative predictive values (PV) and likelihood ratios (LR), AGD measurements were dichotomized by using optimal cut-off points based on maximum Youden index (J) value (Ruopp *et al.*, 2008). Age, BMI and episiotomy were taken into account in statistical models to get a better adjustment of the results. Age and BMI were included as potential confounders and episiotomy as a covariate that may influence AGD. It has been shown that AGD measures may differ among ethnic groups (Thankamony *et al.*, 2009), therefore a sensitivity analysis was performed excluding non-Caucasian women. All tests were two-tailed and the level of statistical significance was set at 0.05. Statistical analyses were performed with the statistical package IBM SPSS 19.0 (IBM Corporation, Armonk, New York, USA).

Results

Ninety-seven per cent of the participants were Caucasian. Overall, controls were older (mean [SD]: 30.7 years old [5.8] versus 27.4 [5.1]; $P < 0.001$) and presented more episiotomies (13.8% versus 5.6%; $P = 0.02$) than cases. Cases

presented higher BMI than controls (25.5 [5.9] versus 23.4 [4.5]; $P < 0.01$), but no significant differences were found for vaginal delivery or parity between both groups (**Table 2**). Cases showed significantly longer AGD_{AF} (27.8 mm [5.7] versus 26.5 mm [5.1]; $P < 0.001$) and AGD_{AC} than controls (80.5 mm [11.3] versus 76.0 mm [10.4]; $P = 0.048$). The diagnostic accuracy of AGD measurements in all PCOS and phenotypic subtypes (H+O; H+POM; O+POM; H+O+POM) are shown in **Table 3**. In all, the areas under the curve (AUC) were larger for AGD_{AC} compared with AGD_{AF}, showing therefore a better predictive value for AGD_{AC} measurements in discriminating presence of PCOS.

The highest AUC (0.62; 95% CI: 0.55 to 0.71) was obtained for all PCOS with the AGD_{AC} measurement (**Figure 1A**), with a sensitivity and specificity of 50.0% and 73.0%, positive and negative PV of 59.0% and 64.4%, and likelihood ratios of 1.82 and 0.70, respectively. The optimal cut-off of the predicted probability for this model was an AGD_{AC} of 82.0 mm. In the case of H+O ($n = 72$) versus controls (**Figure 1B**) the AUC of AGD_{AC} was 0.59 (95% CI: 0.51–0.67). For H+POM ($n = 88$) (**Figure 1C**) the AUC of AGD_{AC} was 0.59 (95% CI: 0.51–0.67); for O+POM ($n = 74$) (**Figure 1D**) the AUC of AGD_{AC} was 0.61 (95% CI: 0.54–0.69); and for the H+O+POM subgroup ($n = 54$) (**Figure 1E**) the AUC of AGD_{AC} was 0.59 (95% CI: 0.51–0.67). In the entire cohort (cases and controls), 36.5% (104 out of 285) presented an AGD_{AC} above the 82.0 mm cut-off. In particular, 52.0% of the cases (66 out of 126) were above that cut-off point. Lastly, similar results were obtained when excluding non-Caucasian women in a sensitivity analysis (data not shown).

Discussion

This is thought to be the first study to explore the accuracy of AGD, a biomarker of the intrauterine hormonal environment in the diagnosis of PCOS and its phenotypes. Women with PCOS showed significantly longer AGD than controls. AGD_{AC} was significantly larger in all phenotypes of PCOS compared with controls, while AGD_{AF} was significantly larger only in the O+POM phenotype. AGD_{AC} showed the best predictive value in discriminating the presence of PCOS and its phenotypic subtypes. The higher value of AUC was obtained including all PCOS, and for specific phenotypes, the largest AUC was for the O+POM phenotype.

Recently, Wu *et al.* (2017) and Sánchez-Ferrer *et al.* (2017) reported an association between the presence of PCOS and longer AGD in Chinese and Mediterranean women, respectively, supporting the aetiological hypothesis that PCOS could be associated with intrauterine exposure to androgens during fetal life (Callegari *et al.*, 1987). Our group has also reported a strong association between longer AGD and higher antral follicle count (Mendiola *et al.*, 2012) and higher testosterone levels in healthy women (Mira-Escolano *et al.*, 2014).

PCOS diagnosis continues to be a clinical challenge. Several studies have attempted to predict severity of PCOS based on clinical models (**Table 4**), many of which are based on anti-Müllerian hormone (AMH) levels. Although the role of AMH may replace antral follicle count or POM in the Rotterdam classification, currently there are technical difficulties in setting up consensual serum AMH levels and normal cut-off points (Dumont *et al.*, 2015). Regarding PCOS phenotypes, Georgopoulos *et al.* (2014) found an association between elevated serum androstenedione levels (>3.8 ng/ml) and the phenotypic severity of PCOS

(phenotypes H+O+POM and H+POM). However, a well-defined objective diagnostic marker for PCOS has yet to be established.

We propose a potential and additional new clinical tool to improve diagnosis of PCOS and phenotypic subtypes with moderate sensitivity and specificity based on an easy and inexpensive anthropometric measure. AGD is a reproducible and reliable measure within reach of any clinician. These measurements can be taken in a routine gynaecological examination and measurements can be accurate with a few sessions of training, but its use as a diagnostic criterion still needs to be elucidated with further studies. The majority of models for PCOS prediction (**Table 4**) include AMH, despite the negative correlation between age and AMH, whereas AGD remains stable throughout a woman's lifespan (Thankamony *et al.*, 2009). However, it has recently been shown that AGD might be positively related to age in pregnant women (Wainstock *et al.*, 2017), therefore this matter needs to be further investigated.

On the other hand, a phenotypic method for PCOS diagnosis has been shown to be a more convenient clinical approach, providing a simple diagnostic tool and avoiding the need to choose between different PCOS definitions (Lizneva *et al.*, 2016b). Furthermore, each PCOS phenotype has different comorbidities and long-term implications. In this way, AGD_{AC} could be particularly useful in the diagnosis of the O+POM phenotype. Up to one-quarter of unselected reproductive-aged women are diagnosed with POM (Azziz *et al.*, 2006), and POM is described in 75% of patients with PCOS (Azziz *et al.*, 2006). However, the false-positive rate is relatively high, as shown by the high rate of POM in the general population (Azziz *et al.*, 2006). In addition, there are technical limitations related to POM as it has a high explorer-dependent variability. In this context, AGD measurement could be useful in improving the accuracy of diagnosis of this phenotype of PCOS. As occurs with endometriosis (Sánchez-Ferrer *et al.*, 2017), AGD may not only be a good predictor of the presence of PCOS, but also a good predictor of worse prognostic cases, improving early diagnosis and treatment of these women.

This study has some limitations. Selection and measurement bias must be considered. Controls were patients attending the public hospital in the same period, and they stem from the same population from which cases emerged. Two different gynaecologists, who were uninformed of the patient's status and were not involved in the diagnosis, performed AGD measures. Misclassification may have occurred but, if present, it would contribute to underestimating the true magnitude of the association. With a case-control design, the possibility cannot be ruled out that elevated androgens during childhood or adulthood, as well as other effects of factors during critical periods of development (e.g. hormonal medication or fetal exposure to stressful life events), resulted in longer AGD in women with PCOS. There is no clear explanation for the difference between AGD_{AC} and AGD_{AF}. It has been argued that AGD_{AF} measurements tend to be more difficult to reproduce due to uncertainty about the appropriate landmarks (Sathyanarayana *et al.*, 2015; Swan *et al.*, 2015). Nonetheless, a few studies have shown stronger associations for AGD_{AF} with ovarian follicle number in females (Mendiola *et al.*, 2012) and for the long (equivalent) measurement (anopenile distance) in male newborns (Sathyanarayana *et al.*, 2015; Swan *et al.*, 2005).

In conclusion, AGD_{AC} measurements might be a useful clinical tool in the diagnosis of PCOS, in particular improving the accuracy of diagnosing phenotype O+POM. As AGD has important clinical and embryological associations, these findings are compatible with the fetal programming of PCOS as recently reported by Barrett *et al.* (2018). Therefore, gynaecologists may be able to evaluate the individual likelihood of patients developing symptoms. The findings of this study have additional public health implications in the prevention of prenatal exposure to hormonal substances and endocrine disruptors. It opens up a real possibility for strategies to prevent PCOS. More research is needed to confirm these findings in other populations.

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Declaration

The authors report no financial or commercial conflicts of interest.

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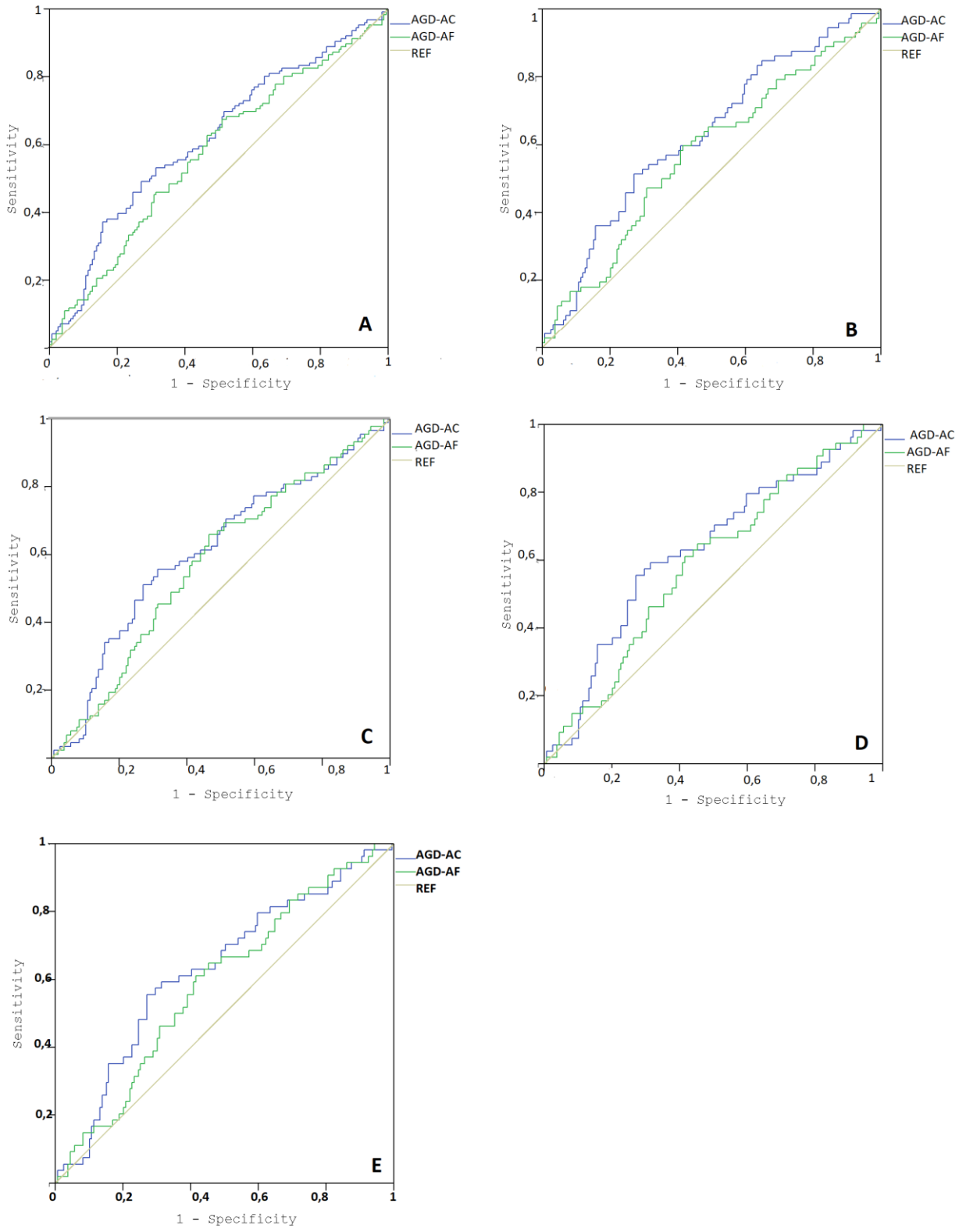

Fig. 1. Receiver operating characteristics (ROC) curves for anogenital distance (AGD) and presence of all PCOS and phenotypic subtypes of PCOS. These analyses examine the ability of AGD to predict PCOS and phenotypic subtypes. Blue and green solid lines represent AGD_{AF} and AGD_{AC}, respectively. (A) Presence of all PCOS (*n* = 126) versus controls (*n* = 159); (B) hyperandrogenism +

oligo/amenorrhoea (H+O) (*n* = 72) versus controls (*n* = 159); (C) hyperandrogenism + polycystic ovarian morphology (POM) (H+POM) (*n* = 88) versus controls (*n* = 159); (D) oligo/amenorrhoea + POM (O+POM) (*n* = 74) versus controls (*n* = 159); (E) hyperandrogenism + oligo/amenorrhoea + POM (H+O+POM) (*n* = 54) versus controls (*n* = 159).

Table 1. Diagnostic criteria for polycystic ovarian syndrome and proposed phenotypes.

	NIH, 1990	ESHRE/ASRM, 2003	AE-PCOS, 2006	NIH extension of ESHRE/ASRM, 2012
Criteria	H	H	H	H
	O	O	O and/or POM	O
		POM		POM
Requirements	Two of two criteria	Two of three criteria	Two of two criteria	Two of three criteria. Phenotypes:
				A: H + O
				B: H + POM
				C: O + POM
				D: H + O + POM

H = hyperandrogenism; O = oligo/anovulation; POM = polycystic ovarian morphology.

Table 2. Comparison of characteristics in controls, all PCOS cases and phenotypic subtypes of PCOS (hyperandrogenism + oligo/amenorrhoea [H+O]; hyperandrogenism + polycystic ovarian morphology [POM] [H+POM]; oligo/amenorrhoea + POM [O+POM]; hyperandrogenism + oligo/amenorrhoea + POM [H+O+POM]).

Variables	Controls (*n* = 159)	All PCOS (*n* = 126)	*P*-value[a]	H+O (*n* = 72)	*P*-value[a]	H+POM (*n* = 88)	*P*-value[a]	O+POM (*n* = 74)	*P*-value[a]	H+O+POM (*n* = 54)	*P*-value[a]
Age	30.7 (5.8); 32.0	27.4 (5.1); 28.0	<0.001	26.9 (5.3); 27.0	<0.001	26.7 (4.8); 27.0	<0.001	27.0 (4.9); 27.0	<0.001	26.3 (4.8); 26.5	<0.001
BMI (kg/m^2)	23.4 (4.5); 22.3	25.5 (5.9); 24.1	<0.01	26.1 (6.4); 24.9	<0.01	24.8 (5.5); 23.6	<0.01	25.8 (5.6); 25.0	<0.01	25.5 (5.8); 24.5	<0.01
Vaginal delivery	32 (20.1)	23 (18.3)	NS	10 (13.9)	NS	13 (14.8)	NS	12 (16.2)	NS	6 (11.1)	NS

Episiotomy	22 (13.8)	7 (5.6)	0.02	2 (2.8)	<0.01	4 (4.5)	0.02	3 (4.1)	0.02	1 (1.9)	<0.01
Parity											
0	111 (69.8)	88 (69.8)	NS	51 (70.8)	NS	66 (75.0)	NS	53 (71.6)	NS	41 (75.9)	NS
1	18 (11.3)	8 (6.3)		4 (5.6)		4 (4.5)		4 (5.4)		2 (3.7)	
2+	30 (18.9)	30 (23.8)		17 (23.6)		18 (20.5)		17 (23.0)		11 (20.4)	

Values are expressed as mean (SD); median or *n* (%).[a]Student's t-test or Mann–Whitney U-tests or chi-squared test, compared with control participants.

Table 3. Diagnostic accuracy of anogenital distance (AGD) measurements in all PCOS cases and phenotypic subtypes of PCOS (hyperandrogenism + oligo/amenorrhoea [H+O] hyperandrogenism + polycystic ovarian morphology [POM] [H+POM]; oligo/amenorrhoea + POM [O+POM]; hyperandrogenism + oligo/amenorrhoea + POM [H+O+POM]).

Variables	Controls (*n* = 159)		Cases		*P*-value[a]	AUC (95% CI)	Diagnostic accuracy						
	Mean	SD	Mean	SD			Cut-off[b]	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Positive LR	Negative LR
All PCOS (*n* = 126)													
AGD_{AF} (mm)	26.5	5.1	27.8	5.7	<0.001	0.57 (0.49–0.65)	>25.6	67.0	49.0	51.0	66.0	1.32	0.66
AGD_{AC} (mm)	76.0	10.4	80.5	11.3	0.048	0.62 (0.55–0.71)	>82.0	50.0	73.0	59.0	64.4	1.82	0.70
H+O (*n* = 72)													
AGD_{AF} (mm)	26.5	5.1	27.8	5.8	NS	0.55 (0.47–0.63)	>27.3	60.0	56.0	32.0	80.0	1.35	0.71
AGD_{AC} (mm)	76.0	10.5	80.7	10.6	0.002	0.59 (0.51–0.67)	>82.0	51.4	67.8	35.6	80.0	1.60	0.72
H+POM (*n* = 88)													
AGD_{AF}	26.	5.1	27.	4.9	NS	0.56	>26.	66.0	52.2	39.	76.	1.38	0.65

(mm)	5		6			(0.48 – 0.65)	2			5	4		
AGD_{AC} (mm)	76.0	10.6	79.6	10.4	<0.01	0.59 (0.51 – 0.67)	>80.0	56.0	66.1	43.8	75.9	1.64	0.67
O+POM (*n* = 74)													
AGD_{AF} (mm)	26.5	5.1	28.2	5.3	0.02	0.58 (0.51 – 0.64)	>23.5	86.5	28.3	30.3	85.3	1.21	0.48
AGD_{AC} (mm)	76.0	10.5	81.1	11.5	<0.001	0.61 (0.54 – 0.69)	>82.0	55.7	66.1	43.8	76.0	1.64	0.67
H+O+POM (*n* = 54)													
AGD_{AF} (mm)	26.5	5.1	28.0	4.9	NS	0.56 (0.48 – 0.66)	>27.2	65.0	52.3	25.2	85.7	1.36	0.67
AGD_{AC} (mm)	76.0	10.6	80.3	10.2	0.008	0.59 (0.51 – 0.67)	>81.2	57.4	66.5	29.8	86.3	1.71	0.64

Age, BMI and episiotomy were taken into account in statistical models to get a better adjustment of the results.
AUC = area under the receiver operating characteristic curve; AGD_{AF} = anogenital distance from the upper verge of the anus to the posterior fourchette; AGD_{AC} = anogenital distance from the upper verge of the anus to the anterior clitoral surface; BMI = body mass index; CI = confidence interval; LR = likelihood ratio; NPV = negative predictive value; NS = not statistically significant; PCOS = polycystic ovary syndrome; PPV = positive predictive value; SD = standard deviation.
[a]Student's t-test or Mann–Whitney U-test compared with control participants.
[b]Dichotomized based on Youden index (J) (maximum potential effectiveness for sensitivity and specificity) (Ruopp *et al.*, 2008).

Table 4 – Studies to predict severity of PCOS based on clinical models.

Study	Study type	Population type (no. of participants)	Phenotypic differentiation of PCOS	Main results	AUC	Sensitivity	Specificity	AMH threshold (ng/ml)
Detti *et al.* (2015)	Cross-sectional	374 (PCOS)	No	FAI as predictor of glucose intolerance	0.606	49%	69%	N/A
				FAI as predictor of hyperinsulinemia	0.404	55%	66%	N/A
				FAI as predictor	0.731	78%	59%	N/A

				of dyslipidaemia				
				Ovarian volume as predictor of SBP	0.596	39%	45%	N/A
				Ovarian volume as predictor of DBP	0.623	83%	78%	N/A
				AFC as predictor of luteal phase defect	0.663	74%	55%	N/A
Deepika *et al.* (2013)	Case-control	571 (259 PCOS, 315 controls)	No	ACE I/D gene polymorphism as molecular marker for susceptibility to PCOS and onset of clinical symptoms	N/A	N/A	N/A	N/A
Georgopoulos *et al.* (2014)	Cross-sectional	1276 (PCOS)	Yes	Elevated serum androstenedione is associated with more severe PCOS phenotypes	N/A	N/A	N/A	N/A
Eilertsen *et al.* (2012)	Cross-sectional	262 (PCOS)	No	AMH as replacement for POM	0.992	94.6%	97.1%	2.8
Sahmay *et al.* (2013)	Case-control	570 (419 PCOS, 151 controls)	No	AMH as a new diagnostic marker for PCOS	0.916	80%	89.8%	3.94
Sahmay *et al.* (2014)	Cross-sectional	606 (PCOS)	No	AMH + Rotterdam criteria	0.92	83%	100%	3.8

				AMH + NIH criteria	0.86	83%	89%	3.8
				AMH + AES criteria	0.87	82%	93.5%	3.8
Kim *et al.* (2017)	Case-control	89 (46 PCOS, 43 controls)	No	AMH as biomarker of PCOS in obese adolescent girls	0.788	67%	81%	6.26

ACE I/D = angiotensin-converting enzyme insertion/deletion; AES = Androgen Excess Society; AFC = antral follicle count; AMH = anti-Müllerian hormone; AUC = area under the curve; DBP = diastolic blood pressure; FAI = free androgen index; N/A = not applicable; NIH = National Institutes of Health; PCOS = polycystic ovary syndrome; POM = polycystic ovarian morphology; SBP = systolic blood pressure.