Hormone profiling, including anti-Müllerian hormone (AMH), for the diagnosis of polycystic ovary syndrome (PCOS) and characterization of PCOS phenotypes

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Abstract

Objectives

To evaluate serum AMH levels in polycystic ovary syndrome (PCOS) and in its different phenotypes in relation to clinical, endocrine and metabolic parameters using a new automated VIDAS® method and to compare it with the Gen II method.

Study design

Multi-centre study including 319 PCOS women and 109 healthy controls.

Results

Serum AMH levels measured using VIDAS® were significantly higher in PCOS women than controls ($p<0.001$), and they correlated with those measured using the AMH Gen II method. An AMH cut-off value of 42.1pmol/L distinguished PCOS women from controls with 67% sensitivity and 83% specificity. The PCOS women with three Rotterdam criteria or hyperandrogenism displayed significantly higher AMH levels compared with those with two Rotterdam criteria or normoandrogenism. In PCOS, AMH levels correlated positively with luteinizing hormone (LH), androgen and sex hormone-binding globulin (SHBG) levels and negatively with BMI, abdominal obesity, follicle-stimulating hormone (FSH), fasting glucose and insulin, and insulin resistance.

Conclusions

AMH evaluated using the VIDAS® method distinguished PCOS patients from healthy controls relatively well, especially in those with more severe phenotypes. Further studies are needed to establish whether AMH measurements can distinguish PCOS patients with different metabolic risk factors.

54 **Keywords:** Polycystic ovary syndrome, Anti-Müllerian hormone, Hyperandrogenism,
55 Phenotype of PCOS, Metabolic risks

Introduction

Polycystic ovary syndrome (PCOS) is characterized by oligoamenorrhoea (OA), hyperandrogenism (HA) and polycystic ovary morphology (PCOM) on ultrasound (1,2). The diagnosis of the syndrome requires the presence of at least two of the three aforementioned criteria (3, 4).

AMH is a member of the transforming growth factor-beta superfamily produced by the ovarian granulosa cells (5). The main physiological roles of AMH in the ovary are the prevention of primordial follicles recruitment and the modulation of FSH action in early follicular development (6,7). Serum AMH levels are correlated with the ovarian antral follicle count (AFC) in women with and without PCOS (8,9). As AMH levels are strongly correlated with both biochemical HA and AFC, studies have suggested that AMH levels could be used as a surrogate tool of PCOM in the diagnosis of PCOS (10,11). However, AMH assays lack an international standard, and concentrations and cut-off values are method dependent.

The presence of relatively high AMH levels in the peripheral circulation suggests that circulating AMH may have also a function outside the reproductive system. Low AMH levels could be associated with cardiovascular disease and metabolic disorders (12) whereas elevated AMH levels seem to be related to PCOS severity (13,14,15,16,17,18).

In a population study of Nordic Caucasian women, our first objective was to evaluate serum AMH levels and their diagnostic value in PCOS using the VIDAS® (bioMérieux SA, Marcy-l'Etoile, France) kit. Our second aim was to examine the correlation of serum AMH levels measured with this kit with those obtained using the AMH Gen II enzyme-linked immunosorbent assay (ELISA) (Beckman Coulter, Inc., CA, USA). A formal comparison between VIDAS® and Gen II methods has not been published before, but both methods have been recently compared with Elecsys® (Roche Diagnostics) (19,20).

In addition, in PCOS patients, we investigated serum levels of AMH in different phenotypes of the syndrome, as well as the association of AMH levels with AFC, and with hormonal and metabolic parameters.

Materials and methods

Subjects

The PCOS (*n* = 319) group had been originally recruited to a randomized controlled study investigating the efficacy of metformin in the treatment of anovulatory infertility (21). The inclusion and the exclusion criteria have been reported earlier (21).

Hyperandrogenism (HA) was defined as clinical, defined as a Ferriman–Gallwey score>7 or biochemical, defined as a testosterone level ≥+2SD (i.e. ≥2.3nmol/L). The PCOS patients were divided further into four phenotypes according to the Rotterdam diagnosis criteria: A:PCOM+HA+OA (*n* = 106), B:HA+OA (*n* = 18), C:HA+PCOM (*n* = 12) and D:OA+PCOM (*n* = 124) (3).

The control subjects consisted of 96 healthy Caucasian women (18–39 years; BMI:19-35kg/m^2) recruited from the community by advertisements in local newspapers (22,23). All were non-smokers and none of them used any hormonal contraception or other hormonal preparations, had regular menstrual cycles, and none had hirsutism/hyperandrogenaemia or were using any medications.

After an overnight fast, serum samples were obtained in the follicular phase 1–7 days after spontaneous menstruation (oligomenorrhoeic PCOS patients and controls) or at a convenient time (amenorrhoeic PCOS women) during 2004–2009 and were immediately frozen at -20°C.

Laboratory and clinical measurements

AMH concentrations and serum levels of LH, FSH and estradiol (E_2) were measured in the stored samples using the VIDAS® automated enzyme immunoassay with fluorescent detection (bioMérieux). The measuring range for AMH was 0.14–64.3pmol/L (0.02–9.00ng/mL), and the intra-assay coefficient of variation was 5.15%. For concentrations over 64.3pmol/L (9ng/mL), a dilution procedure was used. A detailed description of this new automated method has been published recently (21).

In women with PCOS, the determinations of AMH had been also performed earlier with the AMH Gen II enzyme linked immunosorbent assay (Beckman Coulter).

Waist and hip circumference, serum levels of glucose, insulin, testosterone and SHBG, and calculation of the free androgen index (FAI), homeostasis model assessment-estimated insulin resistance index (HOMA-IR) and the areas under the curve for incremental insulin and glucose were measured as reported earlier (21).

Statistical analyses

An independent sample Student's *t*-test was used for continuous variables if their distributions were not skewed. Correlations between variables were analysed by Spearman's correlation test. A receiver operating characteristic (ROC) curve analysis was used to determine the best cut-off point for AMH to distinguish PCOS women from controls. Intraclass correlation coefficient and its 95% confidence interval between AMH values measured with VIDAS® and Gen II method was calculated based on mean-rating (k=3), consistency, 2-way mixed-effects model. Statistical analyses were performed using IBM SPSS Statistics 20.0 (SPSS, Inc., IBM Corp, New York, USA.). A p-value <0.05 was considered statistically significant.

Results

Anthropometric, metabolic and hormonal parameters of the women with PCOS and the controls are presented in Table 1.

Serum AMH concentrations in the PCOS patients and controls

The serum levels of AMH were significantly higher in the PCOS women than in controls (66.1±47.4pmol/L vs. 30.7±17.4pmol/L, $p<0.001$, Figure 1a), and the levels correlated significantly with AFCs (r=0.58, $p<0.001$).

The sensitivity and specificity of the serum concentration of AMH in distinguishing PCOS women from controls were evaluated using cut-off values according to the ROC curve. The best combined sensitivity (67%) and specificity (83%) was obtained using an AMH cut-off value of 42.1pmol/L with the VIDAS® kit (Figure 2a).

Comparison of serum AMH concentrations between the VIDAS® and Gen II ELISA methods

In the PCOS group, the mean AMH serum level was 66.1±47.4pmol/L with the VIDAS® and 58.9±33.9pmol/L with the AMH Gen II method. Intraclass correlation coefficient value (0.927 (95% confidence interval 0.909 – 0.941)) indicated an excellent level of reliability.

Serum AMH concentrations according to PCOS phenotypes

PCOS women with the phenotype A had significantly higher serum AMH and testosterone levels as compared with those of PCOS women who fulfilled only two of the Rotterdam criteria (phenotypes B/C/D) (Table 1, Figure 1b). In addition, PCOS women with phenotype A (91.7±61.9pmol/L) had significantly higher serum AMH levels than those with phenotype B (43.6±17.4pmol/L, $p<0.001$) or D (61.0±33.3pmol/L, $p<0.001$). An AMH cut-off value of

49.0pmol/L showed sensitivity of 79% and specificity of 92% in distinguishing PCOS women with the phenotype A from controls (Figure 2b).

Serum levels of AMH and E_2 and the BMI values were significantly higher in hyperandrogenic (A/B/C) PCOS phenotypes as compared with the normoandrogenic (D) phenotype (Table 1, Figure 1c). An AMH cut-off value of 49.0pmol/L displayed a sensitivity of 71% and a specificity of 92% in distinguishing hyperandrogenic PCOS women from controls (Figure 2c) and a cut-off value of 42.4pmol/L had a sensitivity of 66% and specificity of 83% in distinguishing phenotype D (normoandrogenic phenotype) from controls.

Serum AMH concentrations and hormonal and metabolic parameters

In the PCOS group, there was a statistically significant positive correlation between AMH and AFC (r=0.58,p<0.001), SHBG (r=0.18,p=0.002), testosterone (r=0.49,p<0.001), FAI (r=0.20,p<0.001) and LH (r=0.32,p<0.001) and a statistically significant negative correlation between AMH and BMI (r=-0.26,p<0.001), waist circumference (r=-0.23,p<0.001), waist-hip-ratio (r=-0.13,p=0.028), fasting glucose (r=-0.12,p=0.039), fasting insulin (r=-0.27,p<0.001), AUC insulin (r=-0.14,p=0.017), HOMA-IR (r=-0.26,p<0.001) and FSH (r=-0.13,p=0.026).

The serum AMH levels of normal weight PCOS women (BMI<25kg/m^2) were significantly higher than those of overweight PCOS women (BMI>25kg/m^2: 75.7±54.1pmol/L vs. 58.1±39.0pmol/L, p=0.001) or obese PCOS women (BMI >30kg/m^2: 52.1±35.0pmol/L, p<0.001).

Discussion

In this study, we report higher AMH levels for PCOS women compared to controls and several AMH cut-off values for PCOS phenotypes to distinguish them from controls. Moreover, in women with PCOS, serum AMH levels correlated positively with SHBG, androgen and LH levels and negatively with obesity, fasting glucose and insulin, and insulin resistance.

The results obtained in the present study using the VIDAS® method are in line with those of previous studies, showing higher mean serum AMH levels in women with PCOS than in controls and a significant correlation between AMH and AFC (16,24,25,26,27,28,29). Importantly, the differences in serum AMH values between PCOS women and controls were significant even though the women in the control group were slightly younger than the women with PCOS. The best cut-off value (42.1pmol/L) to distinguish PCOS patients from controls was similar to that reported in some studies (30,31) but lower than that found in some other studies (28,29), with 67% sensitivity and 83% specificity. Importantly, serum AMH levels determined by the VIDAS® method were strongly and positively correlated with those measured earlier using the AMH Gen II method. Some of the samples in the present study had been frozen for a mean time of ten years before measurements with VIDAS assay, pointing to good reliability of the method and stability of the samples.

In PCOS women there was a positive correlation between serum levels of AMH and those of testosterone, LH and FAI, in line with the results of previous studies (27,29,32,33,34,35,36,37) and pointing to an interaction between AMH, LH and androgen secretion. Such an interaction may contribute to the pathogenesis of PCOS (38), as demonstrated by a previous study, which reported increased gonadotropin-releasing hormone (GnRH)-dependent pulsatility and LH surges through GnRH- neurone AMH receptor

activation in mice (39). In the present study, hyperandrogenic PCOS phenotypes displayed significantly higher AMH levels as compared with those in normoandrogenic phenotypes. Moreover, the phenotype A (full-blown syndrome) expressed higher levels than the phenotypes including only two Rotterdam criteria. Again, these findings are in agreement with that of previous studies, which showed that women with more severe PCOS manifestations exhibited elevated serum AMH levels (13,14,15,16,17,18). Of note, AMH was able to distinguish hyperandrogenic and phenotype A PCOS patients from controls with the best sensitivity and specificity. Whether hyperandrogenism itself induces enhanced AMH production remains unresolved and could not be clarified in the present study design.

Women with PCOS are known to present with an altered metabolic profile, characterized by abdominal obesity, insulin resistance, metabolic syndrome and an elevated risk of type 2-diabetes (40,41,42). In the present study, in accordance with the findings of some (37,43) but not all (38,44) studies, we found negative correlations between serum AMH levels, BMI and several metabolic risk factors. Moreover, in the PCOS group, AMH levels were significantly higher in women of normal weight as compared with those of overweight women (BMI>25kg/m^2), and the difference was even greater when we compared the normal weight and obese group (BMI>30kg/m^2). Interestingly, low AMH levels have been associated with an elevated risk of metabolic syndrome in PCOS (45) and with an increased risk of cardiovascular disease in non-PCOS women (12). The negative correlations between AMH and metabolic parameters could be driven by obesity, as the significance disappeared in a multivariate regression analysis including BMI (37,43). On the other hand, in another study, the hyperandrogenic phenotypes of PCOS with the highest AMH concentrations display the most unfavourable metabolic profile (46). Likewise, in the present study, the group with full-blown PCOS had higher serum levels of AMH and testosterone, and greater FAI values as compared with the group fulfilling only two Rotterdam criteria. However, there were no

differences in anthropometric or metabolic parameters between these groups. Further studies are therefore needed to clarify the nature of the complex relationship between AMH levels, hyperandrogenism and metabolic risk factors in humans.

Strengths and limitations

The strengths of this study are the homogenous study population, which included only Nordic Caucasian women and the well-defined patient and control groups. As for limitations of the study, the control group and some of the PCOS phenotypic subgroups included a relatively low number of participants. In addition, as we did not measure AMH levels in the control group using the AMH Gen II, we were not able to compare the sensitivity and specificity of the two methods to distinguish PCOS from controls. Furthermore, the study population was not population based but consisted of women who had visited an infertility clinic. These women probably had more severe PCOS. Given, that the control group did not include women with any PCOS symptoms, namely isolated hyperandrogenism or oligoamenorrhea, this could result into higher differences in the serum levels of AMH between the two study groups. Last, the serum samples had been stored for 6–11 years and had gone through at least one previous freeze-thaw cycle. This may have affected the reliability of some laboratory determinations.

Conclusion

In conclusion, AMH concentrations measured with the VIDAS® method correlated well with those measured using the AMH Gen II method and were able to distinguish women with PCOS from healthy controls with 67% sensitivity and 83% specificity. Moreover, serum AMH correlated positively with hyperandrogenism and negatively with unfavourable metabolic factors, underlining the close relation of AMH with pivotal pathogenic factors of PCOS. Further studies are needed to clarify the nature of the complex relationship between

AMH levels and the risk of metabolic disorders and to establish whether AMH levels may serve as a useful tool to distinguish PCOS patients with different metabolic risk factors.

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Disclosure statement

Laure Morin-Papunen has received speaker's fees for delivering lectures to bioMérieux personnel. The other authors have no conflict of interest to declare.

Figure legends

Figure 1: Box-plots show serum AMH concentrations in all PCOS women and controls (A), in PCOS patients with three Rotterdam criteria and two Rotterdam criteria (B) and in the hyperandrogenic and normoandrogenic phenotypes of PCOS (C). *P*-values according to the independent sample t-test. AMH serum levels are measured by the bioMerieux VIDAS® method.

Figure 2: Receiver operating characteristic-curves show the best cut-off values of serum AMH levels between PCOS and controls (A), phenotype A of PCOS and controls (B) and hyperandrogenic PCOS and controls (C). Cut-off points with the best-combined sensitivity and specificity are shown in the Figure. AMH serum levels are measured by the bioMerieux VIDAS® method.
Abbreviations: PCOS, polycystic ovary syndrome; AMH, Anti-Müllerian hormone;

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Table 1. Anthropometric, metabolic and hormonal parameters in all PCOS patients and in different PCOS subgroups. *P*-values according to the independent sample t-test.

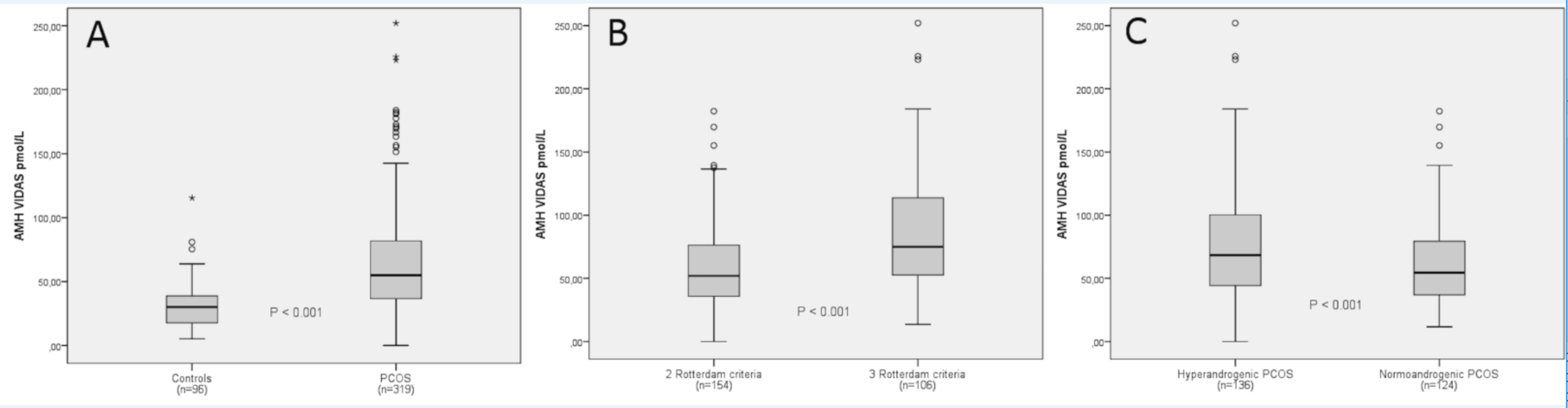
[1] Comparisons between all PCOS women and controls.

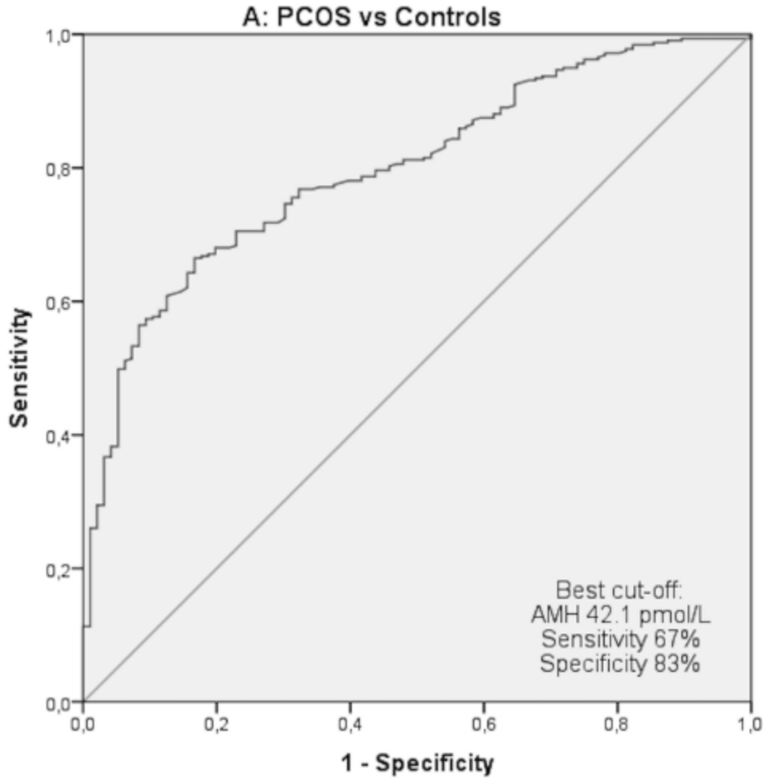
[2] Comparisons between PCOS three-criteria and PCOS two-criteria.

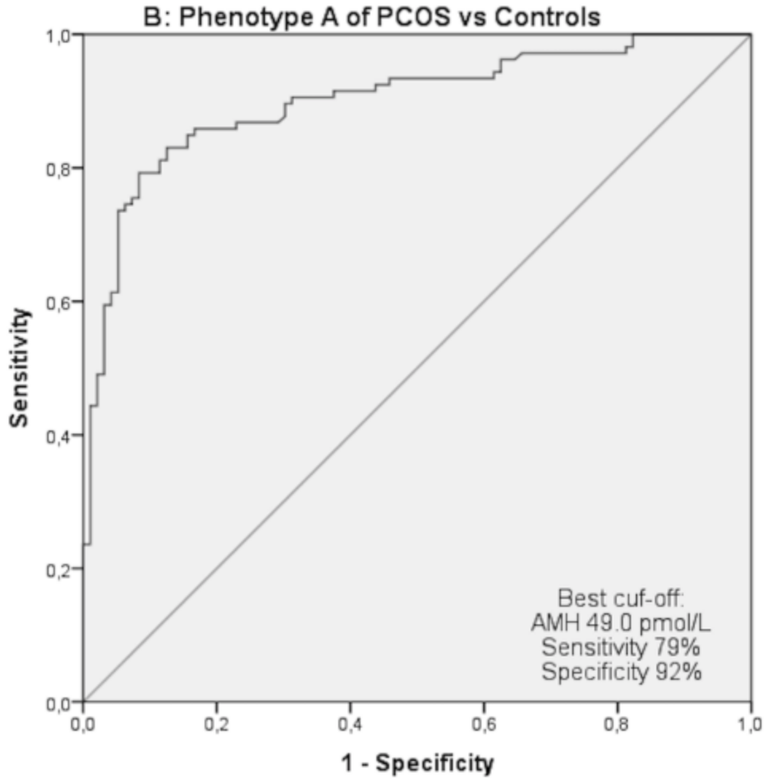
[3] Comparisons between hyperandrogenic PCOS group and normoandrogenic PCOS group.

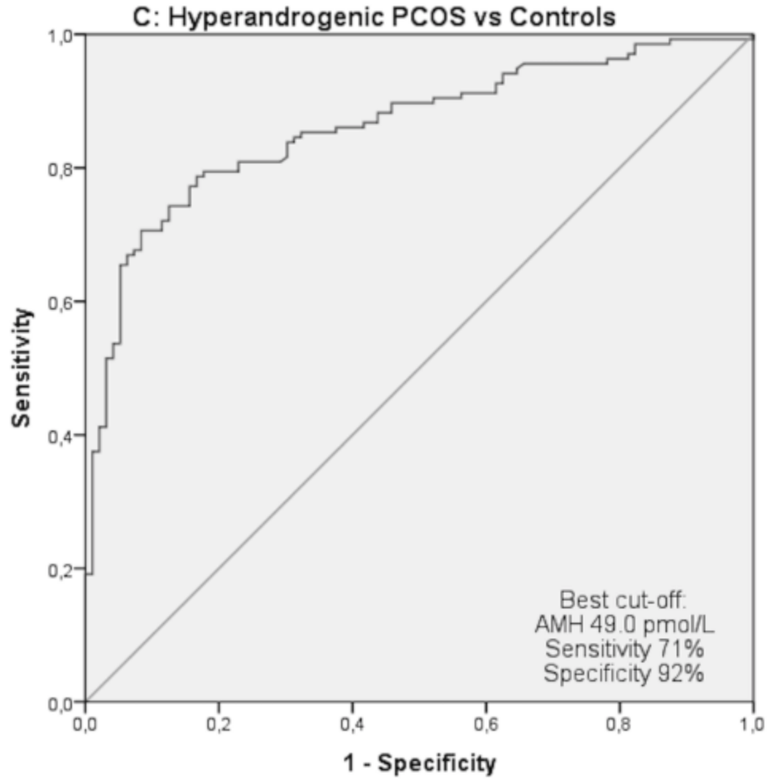
Abbreviations: BMI, Body mass index; AFC, Antral follicle count; AUC, Area under curve; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; FSH, Follicle-stimulating hormone; LH, Luteinizing hormone; E_2, Estradiol; T, Testosterone; SHBG, Sex hormone-binding globulin; FAI, Free androgen index; AMH, Anti-Müllerian hormone.

	All PCOS women (n=319)	Controls (n=96)	*p*-value[1]	PCOS 3-criteria (n=106)	PCOS 2-criteria (n=154)	*p*-value[2]	Hyperandrogenic PCOS (n=136)	Normoandrogenic PCOS (n=124)	*p*-value[3]
Age (yr)	28.1 ± 4.3	26.0 ± 5.2	<0.001	28.3 ± 3.7	28.1 ± 4.3	ns (0.7)	28.2 ± 3.9	28.1 ± 4.3	ns (0.8)
BMI (kg/m^2)	27.3 ± 6.3	22.8 ± 3.6	<0.001	27.5 ± 6.2	26.9 ± 6.5	ns (0.4)	28.0 ± 6.5	26.2 ± 6.2	0.024
Waist (cm)	85.0 ± 15.0			85.8 ± 15.3	84.1 ± 15.1	ns (0.4)	86.6 ± 15.8	82.7 ± 14.3	0.042
Waist-hip-ratio	0.8 ± 0.1			0.8 ± 0.1	0.8 ± 0.1	ns (0.3)	0.8 ± 0.1	0.8 ± 0.1	ns (0.1)
AFC	23.4 ± 6.7			26.5 ± 7.3	23.4 ± 5.1	<0.001	25.1 ± 7.4	24.2 ± 4.8	ns (0.3)
Fasting glucose (mmol/L)	5.1 ± 0.5			5.1 ± 0.4	5.1 ± 0.4	ns (0.5)	5.1 ± 0.4	5.0 ± 0.5	ns (0.5)
Fasting insulin (mU/L)	11.2 ± 11.5			10.4 ± 7.9	11.9 ± 13.5	ns (0.3)	11.5 ± 10.6	11.0 ± 12.6	ns (0.7)
AUC_{gluc}	767.4 ± 167.5			784.9 ± 180.4	757.8 ± 160.6	ns (0.2)	785.4 ± 173.2	750.6 ± 163.4	ns (0.1)
AUC_{ins}	8412.7 ± 6937.2			9243.9± 7602.9	8116.6 ± 6755.4	ns (0.2)	9179.3 ± 7409.0	7924.9 ± 6766.6	ns (0.2)
HOMA-IR	2.6 ± 2.8			2.4 ± 1.9	2.8 ± 3.3	ns (0.3)	2.6 ± 2.5	2.6 ± 3.2	ns (0.9)
FSH (mIU/mL)	6.2 ± 2.1	5.7 ± 2.1	ns (0.05)	6.1 ± 1.9	6.3 ± 2.0	ns (0.5)	6.1 ± 1.9	6.3 ± 1.9	ns (0.4)
LH (mIU/mL)	6.9 ± 4.8	3.3 ± 1.6	<0.001	7.6 ± 3.9	6.7 ± 5.1	ns (0.1)	7.5 ± 4.7	6.6 ± 4.6	ns (0.1)
E_2 (pmol/L)	268.5 ± 207.9	155.9 ± 74.4	<0.001	296.3 ± 193.1	268.7 ± 234.1	ns (0.3)	309.3 ± 238.8	248.2 ± 189.7	0.024
T (nmol/L)	1.6 ± 0.7			2.0 ± 0.7	1.4 ± 0.5	<0.001	2.0 ± 0.7	1.3 ± 0.4	<0.001
SHBG (nmol/L)	50.9 ± 27.7			51.8 ± 26.7	51.6 ± 29.9	ns (0.9)	51.5 ± 26.9	51.8 ± 30.4	ns (0.9)
FAI	3.8 ± 2.5			4.8 ± 2.7	3.5 ± 2.3	<0.001	4.7 ± 2.6	3.4 ± 2.3	<0.001
AMH VIDAS (pmol/L)	66.1± 47.4	30.7 ± 17.4	<0.001	91.7 ± 61.9	58.6 ± 32.5	<0.001	82.3 ± 58.8	61.0 ± 33.3	<0.001


A
AMH VIDAS pmol/L
250,00
200,00
150,00
100,00
50,00
,00
P < 0.001
Controls
(n=96)
PCOS
(n=319)
B
AMH VIDAS pmol/L
250,00
200,00
150,00
100,00
50,00
,00
P < 0.001
2 Rotterdam criteria
(n=154)
3 Rotterdam criteria
(n=106)
C
AMH VIDAS pmol/L
250,00
200,00
150,00
100,00
50,00
,00
P < 0.001
Hyperandrogenic PCOS
(n=136)
Normoandrogenic PCOS
(n=124)


A: PCOS vs Controls
Sensitivity
1 - Specificity
0,0
0,2
0,4
0,6
0,8
1,0
Best cut-off:
AMH 42.1 pmol/L
Sensitivity 67%
Specificity 83%


B: Phenotype A of PCOS vs Controls
Sensitivity
1 - Specificity
0,0
0,2
0,4
0,6
0,8
1,0
Best cuf-off:
AMH 49.0 pmol/L
Sensitivity 79%
Specificity 92%


C: Hyperandrogenic PCOS vs Controls
Sensitivity
1 - Specificity
0,0
0,2
0,4
0,6
0,8
1,0
Best cut-off:
AMH 49.0 pmol/L
Sensitivity 71%
Specificity 92%