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Original Research

AMH as part of the diagnostic PCOS workup in large epidemiological studies

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Abstract

Objectives: Previous studies have shown good correlation between polycystic ovarian morphology (PCOM) and serum anti-Müllerian hormone (AMH) levels. We evaluated the utility of AMH as a surrogate for PCOM as a part of the polycystic ovary syndrome (PCOS) diagnosis by describing how the use of different AMH cut-off values would change the prevalence of PCOS.

Methods: A general population-based birth cohort study. Anti-Müllerian hormone concentrations were measured from serum samples taken at age 31 years ($n = 2917$) using the electrochemiluminescence immunoassay (Elecsys). Anti-Müllerian hormone data were combined with data on oligo/amenorrhoea and hyperandrogenism to identify women with PCOS.

Results: The addition of AMH as a surrogate marker for PCOM increased the number of women fulfilling at least two PCOS features in accordance with the Rotterdam criteria. The prevalence of PCOS was 5.9% when using the AMH cut-off based on the 97.5% quartile (10.35 ng/mL) and 13.6% when using the recently proposed cut-off of 3.2 ng/mL. When using the latter cut-off value, the distribution of PCOS phenotypes A, B, C, and D was 23.9%, 4.7%, 36.6%, and 34.8%, respectively. Compared with the controls, all PCOS groups with different AMH concentration cut-offs showed significantly elevated testosterone (T), free androgen index (FAI), luteinizing hormone (LH), LH/follicle-stimulating hormone (FSH) ratio, body mass index (BMI), waist circumference, and homoeostatic model assessment of insulin resistance (HOMA-IR) values, as well as significantly decreased sex hormone-binding globulin (SHBG) values.

Conclusions: Anti-Müllerian hormone could be useful surrogate for PCOM in large data sets, where transvaginal ultrasound is not feasible, to aid the capturing of women with typical PCOS characteristics. Anti-Müllerian hormone measurement from archived samples enables retrospective PCOS diagnosis when combined with oligo/amenorrhoea or hyperandrogenism.

Keywords: polycystic ovary syndrome, PCOS, anti-Müllerian hormone, polycystic ovarian morphology, PCOM

Significance

In large epidemiological data sets, where transvaginal ultrasound is not feasible, AMH is a useful tool in PCOS case and phenotype identification when combining the measurement with oligo/amenorrhoea and hyperandrogenism.

Introduction

The diagnosis of polycystic ovary syndrome (PCOS), the most common endocrine disorder in women, has been under debate for several decades. To date, the 2018 international evidence-based guideline for the assessment and management of adult PCOS recommends using modified criteria based on the 2003 Rotterdam consensus.[1] According to the criteria, the presence of two out of three features: oligo/amenorrhoea (OA), clinical or biochemical hyperandrogenism (HA) and polycystic ovarian morphology (PCOM), after the exclusion of other causes, is sufficient to establish the diagnosis. The Rotterdam criteria produce four different phenotypes (A: HA + OA + PCOM, B: HA + OA, C: HA + PCOM, and D: OA + PCOM) that seem to present with different hormonal and metabolic profiles.[2]

In epidemiological studies, which are crucially needed for the understanding of the long-term consequences of PCOS, it is often not feasible to perform transvaginal ultrasound (TVUS) assessment of the ovaries, when preferably thousands of study subjects should be investigated. This limits the use of

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Rotterdam criteria and prevents more detailed phenotyping of PCOS cases. The latter would be clinically important as previous studies have shown that the most severe PCOS phenotype in terms of reproductive and metabolic outcomes is the phenotype A that include all three features of the syndrome.[3] Considering also that the ultrasonogram performance regarding ovarian anatomy has been shown to vary among different investigators,[4,5] a simple objective measurement replacing ultrasonography in PCOM diagnostics would be welcomed.

Anti-Müllerian hormone (AMH), a glycoprotein part of the transforming growth factor-B superfamily produced by ovarian granulosa cells, could provide a surrogate tool for PCOM. Indeed, AMH has previously been shown to correlate well with the ovarian antral follicle count (AFC) and is suggested to be involved in PCOS pathogenesis through its local and systemic effects.[6-9] Anti-Müllerian hormone has also been suggested as a surrogate for PCOM and part of PCOS diagnosis, as the serum levels are 2- to 3-fold higher in women with PCOS, even when aging or pregnancy is considered.[10-12] During the development of AMH assays, several different thresholds have been suggested for PCOM detection,[13-15] but with the new automated analysers, the sensitivity and specificity of the measurement have improved, and availability or price is rarely an issue anymore. A recent study using the fully automated Elecsys AMH assay reported that an AMH cut-off of 3.2 ng/mL as a surrogate for PCOM resulted in a sensitivity of 88.6% and specificity of 80.3% in women aged 23-35 years.[13]

The aim of the present study was to evaluate how the addition of AMH measurement as a surrogate for PCOM would change the prevalence of PCOS, defined as the presence of OA and HA, as well as the metabolic and hormonal profiles of PCOS groups in a large general population-based study setting. We hypothesized that the combination of AMH, OA, and HA information could be used as a tool to capture PCOS cases in large epidemiological data sets and in the general population.

Materials and methods

Study design and setting

This study is based on the prospective general population-based Northern Finland Birth Cohort 1966 (NFBC1966). In 1966, 12,231 children (5889 female) were born in the two northernmost provinces of Finland (covering 48% of Finnish territory) and included in the cohort. Originally, the study was set to evaluate early-life factors on long-term health and work ability. Since the beginning, the cohort population has been followed at four different time points: 1, 14, 31, and 46 years of age (set by the cohort centre). Comprehensive questionnaires on female health and clinical examinations with biological data collection have been performed at ages 31 and 46 years; thus, the present study builds on these data collection points.

The detailed cohort description and follow-up protocol have been published previously.[16,17] Briefly, in 1997 (the 31-year follow-up), postal questionnaires regarding health, behaviour, work, and social background were sent to all individuals still alive and with known addresses ($n = 5608$ women), and 4523 (81%) of them responded. The postal questionnaire included wide range of questions about female health, such as "Have you ever been pregnant?". In addition, those living in the Northern Finland area or in the Helsinki metropolitan area ($n = 4074$ women) were invited to a clinical examination, in which 3127 (77%) women participated. In 2012 (the 46-year follow-up), postal questionnaires and an invitation to the clinical examination were sent to all individuals still alive and with a known address ($n = 5123$ women). Of them, 3706 (72%) women responded to the questionnaires, and 3280 women (64%) participated in the clinical examination. The flow chart of the NFBC1966 study is illustrated in Figure 1.

Study population

The postal questionnaire at age 31 included two questions on PCOS symptoms: OA ("Is your menstrual cycle often [more than twice a year] longer than 35 days?") and hirsutism ("Do you have bothersome, excessive body hair growth?"). In total, 463 (10.5%) women reported only OA, 471 (10.6%) women reported only hirsutism, and 153 (3.5%) women reported both hirsutism and OA, whereas 3339 (75.4%) women reported having neither of the symptoms.

In addition, at age 46, the postal questionnaire included the question "Have you ever been diagnosed as having polycystic

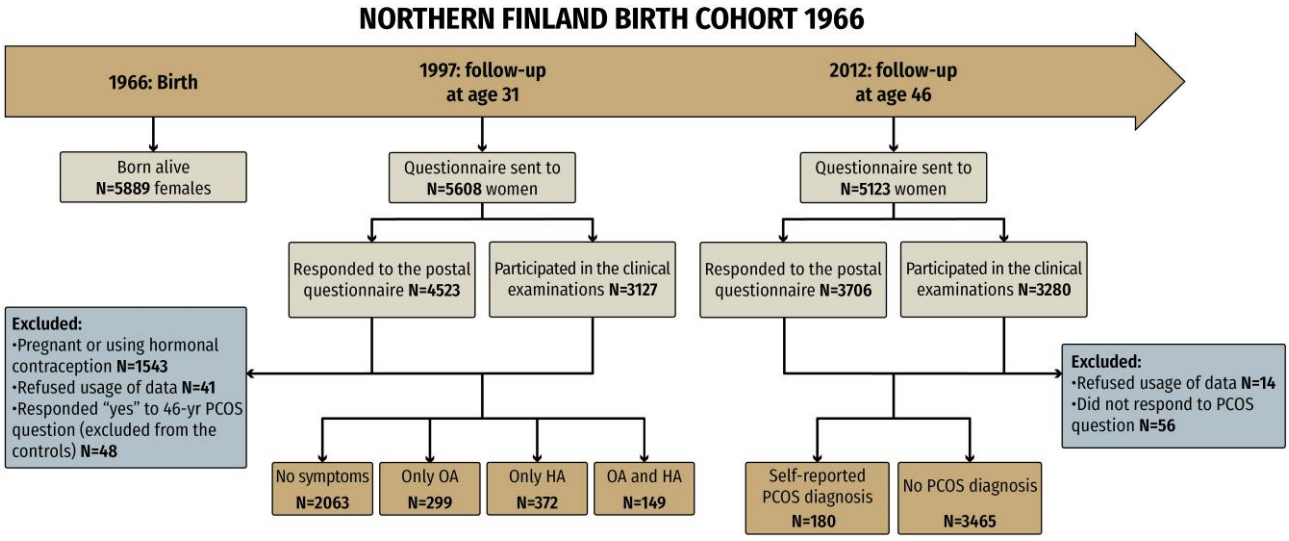

Figure 1. The flowchart of the Northern Finland Birth Cohort 1966 study.
OA, oligo/amenorrhoea; HA, hyperandrogenism (biochemical or clinical hyperandrogenism); PCOS, polycystic ovary syndrome.

ovaries and/or polycystic ovary syndrome (PCOS)?". The women who responded "yes" were excluded from the control group at age 31 (Figure 1). Women who were using hormonal contraceptives ($n = 1275$) or who were pregnant ($n = 268$) at age 31 were excluded from the data analysis, as during pregnancy or use of hormonal contraceptives, it is not possible to reliable asses menstrual cycle or HA. After exclusions, the respective numbers for women with both hirsutism and OA, OA only, hirsutism only, and no PCOS symptoms at age 31 were respectively 124 (4.3%), 323 (11.2%), 318 (11.0%), and 2114 (73.4%).

The ability of these two questions to identify women with PCOS has been previously validated, and we have shown that the women present with typical changes in hormone and metabolic profile, ovarian morphology, and psychological derangements.[18-22] Ovarian morphology was assessed in a small subgroup of the study participants in 1997, and considering that the resolution of ultrasound devices in 1997 does not meet the current standards, we did not aim to investigate the correlation between AMH and AFC in the present study, as several previous studies have already shown good correlations between AMH and AFC.

Clinical examinations

In the clinical examinations at ages 31 and 46 years, weight, height, and waist circumference were measured by trained professionals. Weight (kg) was measured using a regularly calibrated digital scale, and height (cm) was measured twice using a standard and calibrated stadiometer. Body mass index (BMI) was calculated (kg/m^2) using the average of the two height measurements and weight. If the study subjects had not participated in the clinical examination, weight and height information was supplemented from the postal questionnaire to minimize missing data. Waist circumference was measured at the level midway between the lowest rib margin and the iliac crest. Blood samples were drawn after overnight fasting in the morning.

Laboratory methods

The serum levels of testosterone (T), sex hormone-binding globulin (SHBG), and insulin, as well as plasma glucose levels, were analysed in NordLab Oulu, a testing laboratory (T113) accredited by the Finnish Accreditation Service (FINAS) (EN ISO 15189), as previously described.[21] The serum T levels were assayed using liquid chromatography–mass spectrometry equipment (Agilent Technologies, Wilmington, DE, USA) and SHBG by fluoroimmunoassay (Wallac Inc. Ltd., Turku, Finland). The free androgen index (FAI) was calculated using the formula FAI = 100 × T (nmol/L)/SHBG(nmol/L) to detect women with biochemical HA at age 31. Based on our laboratory's reference ranges (97.5% cut-off), T above 2.3 nmol/L and FAI above 5.6 were used to define biochemical HA. Fasting plasma glucose and fasting serum insulin values were used to calculate the homoeostatic model assessment of insulin resistance (HOMA-IR) index with the following formula: fasting glucose (mmol/L) × fasting insulin (mIU/L)/22.5.

Anti-Müllerian hormone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) levels were analysed in 2020 from serum samples drawn in 1997 (the 31-year follow-up) and stored at −20°C since then. Due to low number of −80°C samples available, the −20°C samples were used for the study. Serum AMH, LH, and FSH concentrations were measured using the automated Elecsys electrochemiluminescence immunoassay on a cobas e 411 analyser, according to the manufacturer's instructions (Roche Diagnostics, Germany). For a quality check, as the serum samples had not been thawed or opened before the analysis day, they were first visually inspected and then further tested to be of good quality: We compared AMH levels of 11 individuals who had serum samples stored both at −80°C and −20°C and found that the mean values did not differ and that AMH, LH, and FSH levels of the samples stored at −80°C or −20°C were highly correlated (Pearson correlation 0.980 for AMH, 0.984 for LH, and 0.985 for FSH). The mean AMH level of the serum samples stored at −80°C was 5.7% lower than that of the samples stored at −20°C, possibly due to minimal evaporation over the storage period. More specific details are provided in the Supplemental material. The assay limits of detection and quantitation were 0.01 and 0.03 ng/mL for AMH. The limits for detection were 0.100 mIU/mL for LH and 0.100 mUI/mL for FSH. The intraassay and interassay coefficients of variance were 1.0%-1.8% and 2.9%-4.4% for AMH, 0.8%-1.8% and 1.9%-5.2% for LH, and 1.4%-2.0% and 2.9%-5.3% for FSH. Limits above the measuring ranges were 23 ng/mL for AMH and 200 mUI/mL for LH and FSH.

Statistical methods

We first analysed the prevalence of PCOS according to the NIH criteria that applied during the time of 31-year follow-up study and then investigated how the addition of AMH information would change the prevalence of PCOS. The addition of AMH serves as a surrogate for PCOM and thus would produce a prevalence defined according to the Rotterdam criteria.

The data were analysed using IBM SPSS Statistics version 27 (IBM Corporation, Armonk, NY, USA). Error bar plots were created using GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA). A P-value < .05 was considered statistically significant, and due to the nature of the analyses and specified questions, there was no need to correct for multiple testing. Normally distributed variables are presented as means with standard deviations and skewed data as medians with (25th and 75th percentiles). A two-sided t-test or Mann–Whitney U-test was used to test the differences between group characteristics.

Ethical approval

The study followed the principles of the Declaration of Helsinki. The Ethics Committee of the Northern Ostrobothnia Hospital District approved the research (decision number 94/2011). All participants took part on a voluntary basis and signed informed consent forms.

Results

Prevalence of PCOS according to NIH criteria

Women who had both OA and clinical or biochemical HA at age 31 fulfilled the NIH criteria for PCOS. The prevalence of PCOS according to the NIH criteria was 5.2% ($n = 149$) at age 31. The group of women with PCOS according to the NIH criteria had significantly higher T, FAI, LH, LH/FSH ratio, BMI, waist circumference, and HOMA-IR values, as well as significantly lower SHBG than the control women (Table 1). In these analyses, we defined the controls as women

Table 1. Hormonal and metabolic characteristics at age 31 in differently defined PCOS groups.

At age 31	Controls	OA + HA	OA + HA + AMH[a] 10.35 ng/mL	OA + HA + AMH[a] 5.0 ng/mL	OA + HA + AMH[a] 3.2 ng/mL	OA + HA + AMH[a] 3.2 ng/mL / srPCOS
T (nmol/L)	0.88 (0.69; 1.14) ($n = 746$)	1.46 (1.21; 2.19)* ($n = 101$)	1.44 (1.19; 2.04)* ($n = 123$)	1.35 (1.08; 1.83)* ($n = 230$)	1.29 (0.98; 1.71)* ($n = 337$)	1.25 (0.94; 1.69)* ($n = 409$)
SHBG (nmol/L)	46.6 (35.0; 61.5) ($n = 763$)	30.9 (22.0; 48.1)* ($n = 103$)	34.0 (23.0; 48.2)* ($n = 125$)	39.4 (26.5; 53.7)* ($n = 237$)	39.5 (26.4; 55.5)* ($n = 349$)	40.1 (27.3; 56.0)* ($n = 402$)
FAI	1.9 (1.4; 2.6) ($n = 736$)	5.1 (3.1; 7.2)* ($n = 100$)	4.5 (3.0; 6.6)* ($n = 122$)	3.7 (2.4; 5.9)* ($n = 229$)	3.4 (2.1; 5.5)* ($n = 336$)	3.2 (1.9; 5.2)* ($n = 387$)
LH (IU/L)	6.4 (4.3; 8.9) ($n = 740$)	10.0 (6.7; 16.9)* ($n = 99$)	10.7 (6.9; 16.9)* ($n = 121$)	9.7 (6.5; 14.9)* ($n = 233$)	8.8 (5.9; 13.6)* ($n = 345$)	8.5 (5.3; 12.9)* ($n = 414$)
FSH (IU/L)	5.8 (4.1; 7.8) ($n = 740$)	5.9 (3.5; 7.5) ($n = 99$)	6.1 (4.0; 7.7) ($n = 121$)	6.0 (4.0; 7.4) ($n = 233$)	6.0 (4.1; 7.6) ($n = 345$)	5.8 (3.8; 7.5) ($n = 414$)
LH/FSH—ratio	1.13 (0.77; 1.75) ($n = 727$)	1.94 (1.39; 2.99)* ($n = 97$)	1.92 (1.42; 2.83)* ($n = 119$)	1.76 (1.21; 2.58)* ($n = 229$)	1.64 (1.09; 2.49)* ($n = 339$)	1.55 (1.04; 2.40)* ($n = 414$)
BMI (kg/m^2)	22.7 (20.8; 25.4) ($n = 1474$)	25.3 (22.3; 30.1)* ($n = 142$)	24.8 (22.1; 29.5) ($n = 166$)	24.1 (21.7; 28.1)* ($n = 273$)	24.1 (21.7; 27.9)* ($n = 383$)	24.0 (21.7; 27.7)* ($n = 486$)
Waist (cm)	76.0 (70.5; 84.0) ($n = 773$)	82.8 (74.8; 94.4)* ($n = 102$)	82.0 (73.0; 93.5)* ($n = 123$)	79.0 (71.3; 91.0)* ($n = 233$)	79.0 (71.0; 90.1)* ($n = 341$)	79.0 (71.5; 89.0)* ($n = 407$)
HOMA-IR	1.52 (1.23; 1.99) ($n = 753$)	1.97 (1.50; 3.25)* ($n = 100$)	1.84 (1.45; 2.80)* ($n = 122$)	1.75 (1.36; 2.40)* ($n = 231$)	1.70 (1.30; 2.25)* ($n = 338$)	1.70 (1.31; 2.24)* ($n = 411$)
Has been pregnant %	77.6% ($n = 1159/1493$)	67.8% ($n = 99/146$)§	70.8% ($n = 119/168$)§	74.5% ($n = 207/278$)	72.2% ($n = 280/288$)§	73.3% ($n = 356/486$)§

The number of study subjects in each analysis is shown in the parenthesis, as the number of cases in each analysis varied due to randomly missing data. The results are reported median with (25; 75) percentiles. The difference between groups was tested by the Student's *t*-test or Mann–Whitney *U*-test, when appropriate. In this table, we have reported the descriptives for controls that were defines as the following: no OA, no HA, and AMH < 3.2 ng/mL. However, the PCOS groups in which different AMH cut-offs were used as a surrogate for PCOM were compared to controls using corresponding AMH cut-off as a surrogate for normal ovarian morphology. The descriptives of these control groups are shown in the Supplemental material.
Abbreviations: AMH, anti-Müllerian hormone; BMI, body mass index; FAI, free androgen index; FSH, follicle-stimulating hormone; HA, hirsutism or biochemical hyperandrogenaemia at age 31; HOMA-IR, homoeostatic model assessment of insulin resistance; LH, luteinizing hormone; OA, oligo/amenorrhoea at age 31; SHBG, sex-hormone binding globulin; srPCOS, self-reported PCOS diagnosis at age 46; T, testosterone; Waist, waist circumference.
[a]Presence of two of the three features (OA, HA, or AMH as a surrogate for polycystic ovarian morphology) fulfilled the PCOS definition according to the Rotterdam criteria.
*$P < .001$ compared with controls.
§$P < .05$ compared with controls.

who did not have OA, hirsutism or biochemical HA at age 31 and did not report being diagnosed with PCOS by the age of 46 ($n = 2063$).

Use of different AMH cut-offs as a surrogate for PCOM in the diagnostic evaluation of PCOS according to the Rotterdam criteria

We then tested how many women would fulfil the Rotterdam criteria for PCOS after applying the different AMH cut-off values as a surrogate marker for PCOM (Figure 2). First, we tested the cut-offs based on the 97.5% (10.35 ng/mL) and 95% percentiles (8.10 ng/mL), as often done when defining the laboratory cut-offs, and then AMH cut-offs of 3.2 ng/mL based on the previous study by Dietz de Loos et al.[13] and 5.0 ng/mL based on the previous study by Bell et al.[15] and supported by our data (Figure S3). In general, the addition of AMH as a surrogate marker for PCOM increased the number of women classified as having PCOS. The prevalence of PCOS increased from 5.2% to 5.9% when using the highest AMH cut-off (97.5% percentile) and up to 13.6% when using the AMH 3.2 ng/mL as a cut-off, respectively. Moreover, when also including women who had reported a history of PCOS by age 46, the prevalence of PCOS further increased to 16.9%.

When using AMH 3.2 ng/mL cut-off as a surrogate for PCOM, up to 40.1% (120 of 299) of women originally having isolated OA and 33% (126 of 372) of women originally having isolated HA fulfilled the PCOS criteria.

When restricting the analysis to those women with PCOS who had all available data as regards of OA, HA, and AMH >3.2 ng/mL as a surrogate for PCOM, the prevalence of PCOS phenotypes A, B, C, and D were 23.9% ($n = 81$), 4.7% ($n = 16$), 36.6% ($n = 124$), and 34.8% ($n = 118$), respectively.

Hormonal and metabolic profiles of differently defined PCOS populations

All differently defined PCOS populations showed typical hormonal and metabolic traits of PCOS and differed significantly from the controls. In these analyses, each PCOS group was compared to controls who did not have OA and HA and had the corresponding AMH-cut-off value to define normal ovarian morphology (Table S2). Tables 1 and 2 illustrate how hormonal and metabolic profiles change when using different AMH cut-offs as markers for PCOM. The PCOS populations defined by NIH or Rotterdam criteria with the highest cut-off for AMH (>10.35 ng/mL) showed the highest values of T, FAI, LH/FSH ratio, HOMA-IR, waist circumference, and BMI, as well as the lowest SHBG levels. Even women with the broadest definition of PCOS in this study, ie, at least two of the following features at age 31: OA, HA, lowest AMH cut-off >3.2 ng/mL, or self-reported PCOS diagnosis by age 46, showed typical PCOS characteristics when compared with non-PCOS controls (Table 1). Of note, the prevalence of women who had been pregnant before the 31-year follow-up was higher among controls than in women with differently defined PCOS (Table 1).

Women with all PCOS features (OA, HA, and AMH >3.2 ng/mL), ie, with phenotype A, showed the worst

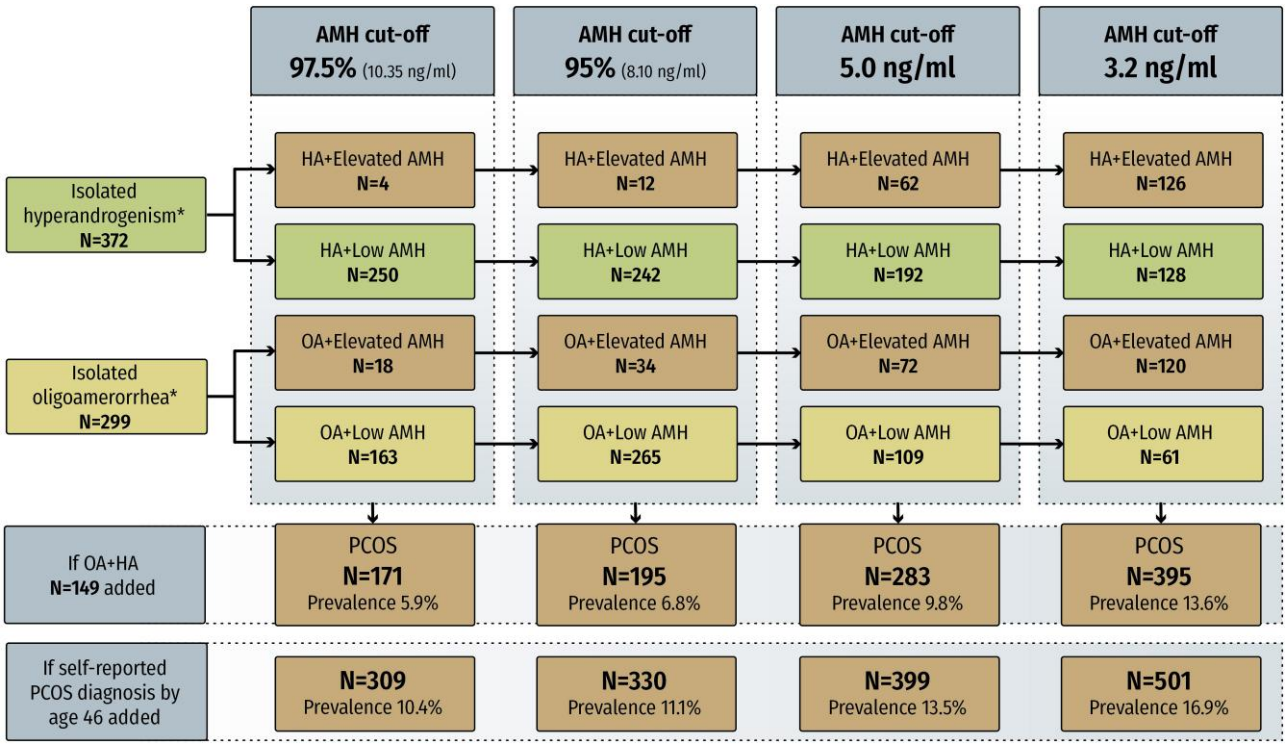

Figure 2. The flowchart of prevalence for different PCOS groups.
*From both the isolated HA and isolated OA groups, AMH information was not available for 118 individuals, as they had not participated in the blood sample collection. AMH, anti-Müllerian hormone; OA, oligo/amenorrhoea; HA, hyperandrogenism (biochemical or clinical hyperandrogenism); PCOS, polycystic ovary syndrome.

Table 2. Hormonal and metabolic characteristics at age 31 in control women and in women with PCOS phenotypes A, C, and D.

At age 31	Controls	PCOS phenotype A (23.9%)	PCOS phenotype C (36.6%)	PCOS phenotype D (34.8%)
Testosterone (nmol/L)	0.88 (0.69; 1.14) ($n = 744$)	1.54 (1.24; 2.33)* ($n = 78$)	1.30 (0.94; 1.80)* ($n = 121$)	1.11 (0.89; 1.43)* ($n = 112$)
SHBG (nmol/L)	46.5 (35.0; 61.4) ($n = 760$)	30.9 (21.35; 45.33)* ($n = 81$)	41.0 (27.7; 60.0) ($n = 124$)	41.6 (30.6; 53.2)§ ($n = 118$)
FAI	1.9 (1.4; 2.6) ($n = 734$)	5.45 (3.22; 7.45)* ($n = 78$)	3.4 (1.9; 5.8)* ($n = 121$)	2.7 (1.7; 3.8)* ($n = 112$)
LH (IU/L)	6.4 (4.3; 9.0) ($n = 737$)	12.39 (7.17; 17.44)* ($n = 81$)	8.1 (5.1; 13.3)* ($n = 124$)	8.7 (6.2; 11.9)* ($n = 118$)
FSH (IU/L)	5.8 (4.1; 7.8) ($n = 737$)	5.93 (3.57; 7.66) ($n = 81$)	5.9 (4.1; 7.6) ($n = 124$)	6.1 (4.7; 7.6) ($n = 118$)
LH/FSH—ratio	1.13 (0.77; 1.76) ($n = 737$)	2.12 (1.55; 3.36)* ($n = 81$)	1.62 (1.00; 2.41)* ($n = 124$)	1.37 (1.03; 2.24)* ($n = 118$)
BMI (kg/m^2)	22.8 (20.8; 25.4) ($n = 1493$)	25.7 (22.3; 31.7)* ($n = 80$)	23.8 (21.1; 27.8)* ($n = 124$)	23.4 (21.2; 26.2) ($n = 117$)
Waist (cm)	76.0 (70.5; 84.0) ($n = 784$)	82.5 (72.8; 93.8)* ($n = 81$)	78.0 (70.5; 88.0)§ ($n = 123$)	77.0 (70.0; 85.8) ($n = 116$)
HOMA-IR	1.52 (1.23; 1.98) ($n = 765$)	1.94 (1.49; 3.12)* ($n = 80$)	1.71 (1.36; 2.22)§ ($n = 122$)	1.49 (1.17; 1.94) ($n = 116$)

PCOS phenotype A: oligo/amenorrhoea, hyperandrogenism, and AMH cut-off of 3.2 ng/mL as a surrogate for PCOM. PCOS phenotype C: hyperandrogenism and AMH cut-off of 3.2 ng/mL as a surrogate for PCOM. PCOS phenotype D: oligo/amenorrhoea and AMH cut-off 3.2 ng/mL as a surrogate for PCOM. Prevalence of each PCOS phenotype is shown in parenthesis after the name of phenotype. The number of the study subjects in each analysis is shown in the parenthesis, as the number of cases in each analysis varied die to randomly missing data. The results are reported median with (25; 75) percentiles. The difference between groups was tested by the Student's *t*-test or Mann–Whitney *U*-test, when appropriate. Controls were defined as the following; no OA, no HA, and AMH <3.2 ng/mL, and no PCOS by age 46.
Abbreviations: BMI, body mass index; FAI, free androgen index; FSH, follicle- stimulating hormone; HOMA-IR, homoeostatic model assessment of insulin resistance; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; SHBG, sex-hormone binding globulin; Waist, waist circumference.
*$P < .001$ compared with controls.
§$P < .05$ compared with controls.

hormonal and metabolic profiles (Table 2). Moreover, women with the nonhyperandrogenic PCOS phenotype with the lowest AMH cut-off of 3.2 ng/mL showed milder hormonal and metabolic changes but still significantly differed from the controls regarding T, SHBG, FAI, LH, and LH/FSH ratio values (Table 2). Not surprisingly, all the differently defined PCOS populations showed significantly higher AMH levels compared with the controls (Figure S4). For example, the group of women with PCOS defined based on the presence of at least two of the following: OA, HA, or AMH >3.2 ng/m had a median AMH of 5.51 (4.16; 7.81) ng/mL, whereas the median AMH of control women was 1.93 (1.36; 2.56) ng/mL.

Discussion

The main finding of this large, general population-based cohort study is that the use of different AMH cut-offs, including the lowest cut-off of 3.2 ng/mL, could be considered a surrogate for PCOM, instead of a TVUS finding, when evaluating large epidemiological datasets. Indeed, the use of AMH as one of PCOS characteristics identified a group of women with typical hormonal and metabolic features of PCOS, in line with previous studies.[13,23-25]

To date, AMH has not yet been recommended as a surrogate marker for PCOM, most importantly due to the

diagnostic inaccuracies of AMH assays and the lack of decision whether age-related cut-offs should be applied.[5] However, the performance of AMH assays has markedly improved with the introduction of the fully automated assays.[26-28] A recent study using the fully automated Elecsys AMH assay reported that an AMH cut-off of 3.2 ng/mL as a surrogate for PCOM resulted in a sensitivity of 88.5% and specificity of 80.3% in women aged 23-35 years.[13] In the present study, we were able to validate this cut-off further as a surrogate marker for PCOM in a population-based study setting. Women classified as PCOS using this cut-off and at least one other PCOS symptom exhibited typical hormonal and metabolic traits of PCOS. To our knowledge, many other studies examining the AMH cut-off for PCOM have used older generation AMH assays, such as the manual AMH Gen II assay of Beckman Coulter, which gives higher AMH values than the automated assays,[29] thus limiting the comparability of studies.

The use of AMH as a surrogate for PCOM had a significant impact on the prevalence of PCOS, as the prevalence of PCOS increased from 5.2% when applying the NIH criteria to 13.6%, when applying Rotterdam criteria and AMH 3.2 ng/mL cut-off as a surrogate of PCOM. These findings are in line with the findings of previous studies,[30,31] as the inclusion of PCOM has been shown to double the prevalence of PCOS.[32] Moreover, when using the AMH cut-off of 3.2 ng/mL, PCOS phenotype C was the most common phenotype in this study population (36.6%), in line with previous findings.[33] By contrast, the prevalence of phenotype B (4.7%) was low, although this is in line with some previous studies.[3] In addition, phenotype D showed higher prevalence (34.8%) than generally seen in nonselected study populations, which may reflect the used AMH cut-off value of 3.2 ng/mL. It must also be noted that the phenotype analysis reflects the distribution of different phenotypes at age 31. The exclusion of women using hormonal contraceptives may also lead to exclusion of the most severe cases, shifting the phenotype distribution from A towards D. Moreover, there might be some ethnic or geographic differences in the distribution of PCOS phenotypes, as the two previously published studies from Europe have found similar phenotype distribution as in the present study; in a study from another Nordic country (Denmark), the prevalence of PCOS phenotype B was also 4.7% and the most common phenotype was C,[24] and in a study from Turkey, the prevalence of phenotype B was 5.1% and the most common phenotype was C,[34] in line with our findings.

We also further investigated the metabolic and hormonal profiles of the PCOS groups that were formed by using different AMH cut-offs as a surrogate for PCOM. Not surprisingly, the group that was formed using the highest AMH cut-off value (10.35 ng/mL) showed a significant shift towards unfavourable T, LH/FSH ratio, and BMI values. This is in line with a previous finding of AMH levels reflecting the severity of the PCOS phenotype.[35,36] In addition, the groups with PCOS phenotypes C (HA + PCOM) and D (OA + PCOM) showed typical PCOS traits when the AMH cut-off of 3.2 ng/mL was applied. When the diagnostic criteria of any disease are widened or new diagnostic tools are adapted, the general concern is the possibility of overdiagnosis or misdiagnosis. However, the comparable prevalence of PCOS in the present and previous studies using Rotterdam criteria, as well as the typical metabolic and hormonal PCOS phenotype of all groups, supports the use of AMH as a surrogate for PCOM.

Strengths and limitations

The major strengths of this study are the prospective, general population-based design and high participation rate. These strengths minimize the possibility of selection bias and provide an estimate that applies to the general population. We studied AMH only at age 31, which could be considered both a strength and a limitation. As we studied individuals of similar ages, the variation in AMH levels across the study sample was not affected by age. On the other hand, as age is known to have a major impact on AMH levels, it is important to further investigate whether the use of age specific AMH cut-offs is needed to improve the diagnostic accuracy of AMH.[14,37] Indeed, the study by Mulders et al.[38] found that the yearly decline in AMH levels was 15% in normo-ovulatory controls compared to 8% in women with normogonadotrophic anovulation (including PCOS). The work by Dietz de Loos et al.,[13] however, suggested applying the same cut-off for women between ages 25 and 45 with acceptable accuracy, although the sensitivity and specificity were better in women aged 25-35 years than those in women aged 36-45 years. The lack of ovarian ultrasonography data and PCOM morphology assessments as well as self-reported hirsutism also adds to these limitations. However, due to limited resources and the timing of data collection in 1997, it would not have been possible to perform TVUS examinations for all women with modern equipment. Furthermore, the serum samples for AMH, LH, and FSH measurements were stored in −20°C since 1997; however, the quality of samples was validated before the analysis (Supplemental material). Even though the use of self-reported hirsutism could be considered a limitation, it is important to note that even in clinical practice, the clinical evaluation of hirsutism, using Ferriman–Gallwey scoring, can be challenging; due to widely applied hair-removal methods, the clinical HA evaluation often relies on self-reported information, similarly to menstrual history. Moreover, the question regarding hirsutism did not specify affected body areas and thus might overestimate the prevalence of hirsutism compared to modified Ferriman–Gallwey scoring, which does not account excess hair growth in upper and lower extremities. Androgen measurement with the golden standard LCM-MS method can be included as a strength, although by excluding women under combined contraceptives, we may have excluded most hyperandrogenic women. By evaluating T and FAI levels as a biomarker for hyperandrogenaemia, we followed the PCOS guideline[1] but also acknowledge that this method might have missed same cases who have only elevated androstenedione or DHEA levels.

Conclusions

New automated AMH assays that perform well in distinguishing PCOM have now become widely available. We found that the use of previously validated AMH threshold of 3.2 ng/mL as a surrogate marker for PCOM in addition to OA and HA resulted in a group of women with typical hormonal and metabolic traits of PCOS and resulted in a prevalence of PCOS that is in line with previous general population-based studies. This approach serves large data sets, as it enables a more accurate evaluation of the prevalence of PCOS as well as the phenotyping of PCOS.

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Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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