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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

Introduction

- PCOS, the most common endocrine disorder in women, has been under debate for several decades.
- 2018 international evidence- based guideline for the assessment and management of adult PCOS recommends using modified criteria based on the 2003 Rotterdam consensus.

Introduction

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,

D: OA + PCOM

that seem to present with different hormonal and metabolic profiles.

Introduction

- In epidemiological studies it is often not feasible to perform TVUS assessment of the ovaries, when preferably thousands of study subjects should be investigated.
- This limits the use of Rotterdam criteria and prevents more detailed phenotyping of PCOS cases.
- simple objective measurement replacing ultrasonography in PCOM diagnostics would be welcomed.

Introduction

- 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.
- 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.
- 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.

Introduction

- 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.
- 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
- 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

- 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.
- **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.

- 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.
- In addition, 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.

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 ovaries and/or polycystic ovary syndrome (PCOS)?". The women who responded "yes" were **excluded from the control group at age 31 .**

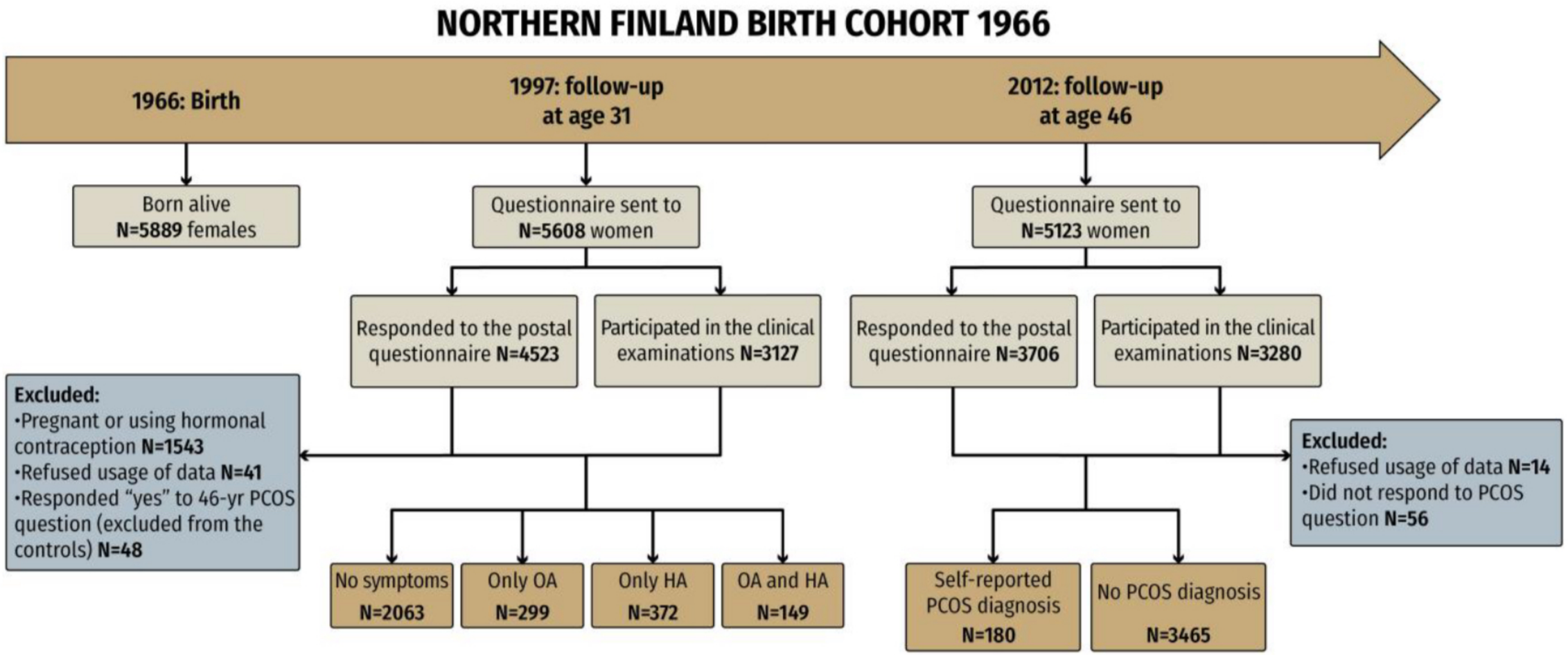

Figure 1. The flowchart of the Northern Finland Birth Cohort 1966 study.
OA, oligo/amenorrhoea; HA, hyperandrogenism (biochemical or clinical hyperandrogenism); PCOS, polycystic ovary syndrome.

Clinical examinations

- In the clinical examinations at ages 31 and 46 years, weight, height, and waist circumference were measured
- 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,
- The serum T levels were assayed using liquid chromatography–mass spectrometry equipment
- 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, LH, FSH levels were analysed in 2020 from serum samples drawn in 1997 (the 31-year follow-up) and stored at −20°C since then.

- For a quality check, as the serum samples had not been thawed or opened before the analysis day, they were first visually inspected and
- 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
- Serum AMH, LH, and FSH concentrations were measured using the automated Elecsys electrochemiluminescence immunoassay
- 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.
- 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 according to the Rotterdam criteria.
- The data were analysed using IBM SPSS Statistics version 27
- A P-value < .05 was considered statistically significant,
- 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.

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
- In these analyses, we defined the **controls** as **women 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 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. and 5.0 ng/mL based on the previous study by Bell et al.
- 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.
- when also including women who had reported a history of PCOS by age 46, the prevalence of PCOS further increased to **16.9%**.

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

- 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 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.

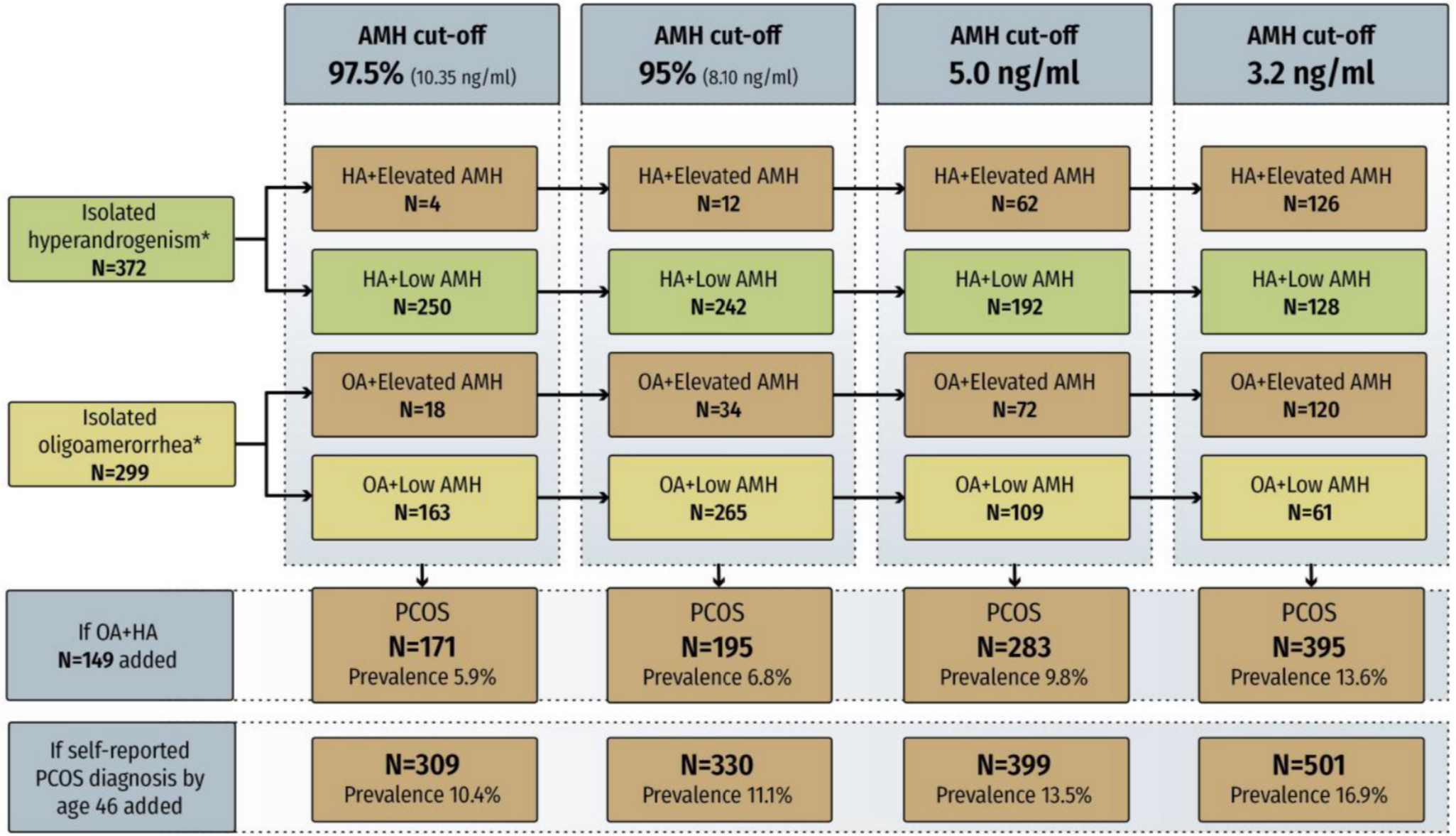

Figure 2. The flowchart of prevalence for different PCOS groups.
*From both the isolated HA and isolated OA groups, AMH information was not available for 118 individuals, as they had not participated in the blood sample collection. AMH, anti-Müllerian hormone; OA, oligo/amenorrhoea; HA, hyperandrogenism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

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**
- The PCOS populations defined by NIH or Rotterdam criteria with the highest cut-off for AMH (**>10.35 ng/m**L) 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/m**L, or self-reported PCOS diagnosis by age 46, showed **typical PCOS characteristic**s 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).

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.

Hormonal and metabolic profiles of differently defined PCOS populations

- Women with all PCOS features (OA, HA, and AMH >3.2 ng/mL), ie, with **phenotype A,** showed the **worst** hormonal and metabolic profiles (Table 2).
- women with the **nonhyperandrogenic** PCOS phenotype with the 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
- Not surprisingly, all the **differently defined** PCOS populations showed significantly **higher AMH levels compared with the controls.**

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.

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.
- 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.

- 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.
- 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.
- **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.**

- 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 as the **inclusion of PCOM has been shown to double the prevalence of PCOS.**
- 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.
- prevalence of phenotype B (4.7%) was low, although this is in line with some previous studies

- 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 **contraceptive** may also lead to exclusion of the most severe cases, shifting the **phenotype distribution from A towards D.**

- 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.**
- 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

- 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
- The lack of ovarian ultrasonography data and PCOM morphology assessments as well as self-reported hirsutism also adds to these limitations.

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** enables a more **accurate evaluation** of the **prevalence** of PCOS as well as the **phenotyping** of PCOS.