The additional value of ultrasound markers in the diagnosis of polycystic ovary syndrome

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Objective: To study the value of current definitions for follicle number per ovary and ovarian volume in the diagnosis of polycystic ovary syndrome (PCOS).
Design: Cross-sectional study.
Setting: University tertiary care center.
Patient(s): Women diagnosed with PCOS after standardized screening were eligible for inclusion in the PCOS group. Women without PCOS who underwent the same screening, had regular menstrual cycles, normal hormonal values, and no other endocrine pathology were eligible for inclusion.
Intervention(s): Not applicable.
Main Outcome Measure(s): Follicle number per ovary and ovarian volume in women with PCOS, stratified by age. Linear regression models to investigate the influence of body mass index (BMI) on follicle number per ovary and ovarian volume. Differences in follicle number per ovary and ovarian volume between the PCOS phenotypes and the additional value of ovarian volume compared with follicle number per ovary.
Result(s): A total of 2,492 women (16–50 years) with PCOS and 152 women without PCOS were included. Most women with PCOS up to age of 35 exhibit a follicle number per ovary $\geq$20 (87.8%–100%) (using an ultrasound transducer $\geq$8 MHz) or $\geq$12 (95.1%–98.6%) (using a transducer <8 MHz), followed by a decline in follicle number per ovary >35 years. Median ovarian volume was below the 10 mL cutoff in every age group, for both ultrasound transducers. Follicle number per ovary and ovarian volume were higher in women with PCOS compared with women without PCOS in every age category. In our cohort, 13/2,297 women with PCOS (0.6%) would not have received the diagnosis if ovarian volume was not considered a marker for polycystic ovarian morphology. For both ultrasound transducers, women with phenotype A (ovulatory dysfunction + hyperandrogenism + polycystic ovarian morphology) exhibited the highest follicle number per ovary and ovarian volume, followed by phenotype D (ovulatory dysfunction + polycystic ovarian morphology), then phenotype C (hyperandrogenism + polycystic ovarian morphology), and then phenotype B (ovulatory dysfunction + hyperandrogenism). No clinically significant correlation between BMI and follicle number per ovary or ovarian volume was observed.
Conclusion(s): Criteria to define follicle number per ovary should be established per age category, as follicle number per ovary decreases with age. Ovarian volume shows a less clear decline with age and has a lower discriminative power, and therefore could be excluded from the diagnostic criteria. Follicle number per ovary does not need to be stratified by BMI. (Fertil Steril® 2024;■: ■–■. ©2024 by American Society for Reproductive Medicine.)
Key Words: PCOS, ultrasonography, follicle count, ovarian volume

Polycystic ovary syndrome (PCOS) is the most prevalent endocrinopathy in reproductive-aged women, affecting approximately 13% of the population (1, 2). Currently, PCOS is advised to be diagnosed according to the algorithm from the 2023 PCOS Guideline, which requires the presence of at least two of the following: clinical/biochemical hyperandrogenism; ovulatory dysfunction; and polycystic ovaries on ultrasound or elevated antimüllerian hormone (AMH) levels, after other causes of these features are excluded (1). Although the 2023 PCOS Guideline introduced AMH as a diagnostic marker, it acknowledges the lack of a consensus on specific cut-off values to define polycystic ovarian

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morphology (PCOM). Consequently, the use of AMH for detecting PCOM is not yet common in clinical practice and ultrasound remains the modality of choice for defining PCOM.

The ultrasound-based definition of PCOM supported by the 2023 PCOS Guideline largely aligns with that of the 2018 PCOS Guideline. Polycystic ovarian morphology is preferentially defined as a follicle number per ovary of ≥20 in at least one ovary when using a TVUS transducer with a frequency bandwidth including 8 MHz (1). A threshold of follicle number per ovary ≥12 in at least one ovary could be applied when using a transducer <8 MHz (3, 4). In instances where image quality or the age of the equipment precludes a reliable follicle count, an ovarian volume of ≥10 mL in at least one ovary could be used to define PCOM. These definitions of PCOM are based on consideration of a small number of studies (n = 15 for follicle number per ovary and n = 17 for ovarian volume), wherein the majority of studies used the older National Institutes of Health (NIH) criteria, instead of the current Rotterdam criteria, to define PCOS cases (5). Differences in PCOS criteria, age, and body mass index (BMI) across study populations have made it difficult to compare studies. Furthermore, the majority of the studies on ovarian ultrasound are based on small sample sizes. The 2023 PCOS Guideline concluded that the quality of evidence for the proposed evidence-based recommendations for the ultrasound diagnosis of PCOS is low. A recently published study, which included the meta-analysis conducted to inform the 2023 PCOS Guideline, recommends investigating ovarian ultrasound markers in a large population and to stratify for age (6). Additionally, it advises to investigate whether ovarian markers differ across the BMI spectrum. This is particularly relevant as limited information exists, even among women without PCOS, regarding the impact of BMI on measures such as total follicle count (7). Given the aforementioned uncertainties and recommendations, our aim was to evaluate the additional value of the ultrasound markers (follicle number per ovary and ovarian volume) in the diagnosis of PCOS and the associations with age and BMI.

MATERIALS AND METHODS

Study design

The study design was a retrospective cross-sectional study in which we used data from patients who underwent a standardized endocrine screening, between January 1, 2001 and March 1, 2023, at the Division of Reproductive Endocrinology and Infertility of the Erasmus University Medical Center in Rotterdam, the Netherlands. The Medical Ethical Review Board of the Erasmus University Medical Center approved retrospective studies within this patient population (MEC-2020-0534).

Study population

Women diagnosed with PCOS after the aforementioned endocrine screening were eligible for inclusion in this study if they met the Rotterdam 2003 consensus criteria and since 2018 the International PCOS Guideline criteria (3, 8). This included the presence of at least two of the following: ovulatory dysfunction (menstrual irregularities, including oligo- or amenorrhea); PCOM; and clinical and/or biochemical hyperandrogenism. The application of these criteria results in four phenotypes: phenotype A (ovulatory dysfunction + hyperandrogenism + PCOM), phenotype B (ovulatory dysfunction + hyperandrogenism), phenotype C (hyperandrogenism + PCOM), and phenotype D (ovulatory dysfunction + PCOM). Ovulatory dysfunction was defined as oligomenorrhea (menstrual cycle <21 days, >35 days, or <8 cycles per year) or amenorrhea (absence of vaginal bleeding exceeds >182 days) (3, 8). PCOM was defined by ≥12 follicles per ovary using <8 MHz transducer or ≥20 follicles per ovary using ≥8 MHz transducer and/or ovarian volume >10 mL in at least one ovary (3). All ultrasound examinations were preferably performed using a transvaginal transducer. When women declined this procedure, an abdominal ultrasound was conducted. Clinical hyperandrogenism was defined as a modified Ferriman–Gallwey score ≥5 (3, 9). Biochemical hyperandrogenism was defined as a Free Androgen Index >4.5 (measured by radioimmunoassay) and >2.9 (measured by liquid-chromatography tandem mass spectrometry) (10). Women without PCOS underwent the same standardized screening, had regular menstrual cycles, normal hormonal values, and no other endocrine pathologies. Women without PCOS had undergone screening as part of their participation in research as healthy controls or were those that had suspicion of an endocrinological issue that was not confirmed.

Participant selection

A total of 4,119 women were diagnosed with PCOS after endocrine screening of 4,476 patients (Supplemental Fig. 1, available online). Women were excluded for the following reasons: <15 years (n = 2), >50 years (n = 3), presence of cysts (n = 965), use of the oral contraceptive pill (OCP) ≤3 months before screening (n = 252), confounding medical factors (including chemotherapy, radiotherapy in that area, and stem cell transplantation) (n = 31), missing data (n = 329), and analyzed by transabdominal ultrasound (TAUS) instead of transvaginal ultrasound (TVUS) (n = 45). As a result, data from 2,492 women with PCOS were available for analysis. In total 357 women had regular menstrual cycles and normal hormonal values. We excluded women based on missing data (n = 30), confounding factors in medical history (n = 36), presence of cysts (n = 90), and use of OCP (n = 48). As a result, 152 women without PCOS were included in the study.

Standardized screening

Detailed information about the standardized endocrine screening has been published previously (11). In short, women with (suspicion of) ovulatory dysfunction or endocrine disorders (including PCOS) underwent this endocrine screening at the Division of Reproductive Endocrinology in the Erasmus Medical Center. The screening process included assessment of the menstrual cycle, measurement of anthropometrics, evaluation of hirsutism, ultrasound, and blood withdrawal, all conducted on the same day. Data from this screening has

been collected over the years and was used for this study. The screening took place randomly throughout menstrual cycle for women with PCOS and on cycle days 3, 4, or 5 for women without PCOS. Ultrasound was performed to assess follicle number per ovary and ovarian volume. Before May 5, 2019, the Philips EnVisor machine was used with a transvaginal probe (C8-4V) operating at a frequency of 4 to 8 MHz, and a trans abdominal probe (C5-2) with a frequency range of 2–5 MHz. Since May 5, 2019, the Affiniti70 has been used with a transvaginal probe (C10-3V) operating at a frequency of 3–10 MHz, and a trans abdominal probe (C5-1) with a frequency range of 1–5 MHz. Follicle number per ovary was assessed in real time by counting the number of follicles with sizes between 2 and 10 mm throughout the entire ovary. Ovarian volume was assessed in real time by measuring three dimensions of the ovary and calculated using the standard simplified formula of an ellipsoid (0.523 × length × width × thickness) (12, 13). The presence of cysts was noted (including endometriomas, dermoid cysts, etc.), and follicles larger than 10 mm were classified as physiological cysts, as both affect ovarian volume. We compared women with PCOS who underwent TAUS (n = 30) to women with PCOS who underwent TVUS (n = 30), all analyzed with the use of an ultrasound probe <8 MHz, and matched by age within a range of 2 years (Supplemental Table 1, available online). Mean ovarian volume, left ovarian volume, and right ovarian volume were similar in both groups. Mean follicle number per ovary, left follicle number per ovary and right follicle number per ovary were significantly lower in the TAUS group. Therefore, for all other analyses, we excluded women who were assessed by TAUS (n = 45, Supplemental Fig. 1).

Statistical analysis

Mann-Whitney *U* test was performed to compare continuous variables and χ^2 test to compare categorical variables between two groups. Linear regression analyses were performed for age categories defined in 5-year increments to investigate the association between BMI and follicle number per ovary or ovarian volume. We applied Bonferroni–Holm correction to adjust for multiple testing. The correction was performed separately for all six variables. The *P* values were ranked from smallest to largest, and each *P* value was multiplied by its rank (up to six). The significance threshold for the adjusted *P* values was set at .05. The Kruskal-Wallis test was used to compare variables between the different PCOS phenotype groups. To adjust for age, linear regression analyses were performed. When data was not normally distributed it was transformed before regression analysis. Statistical significance was defined as a probability value of <.05.

RESULTS

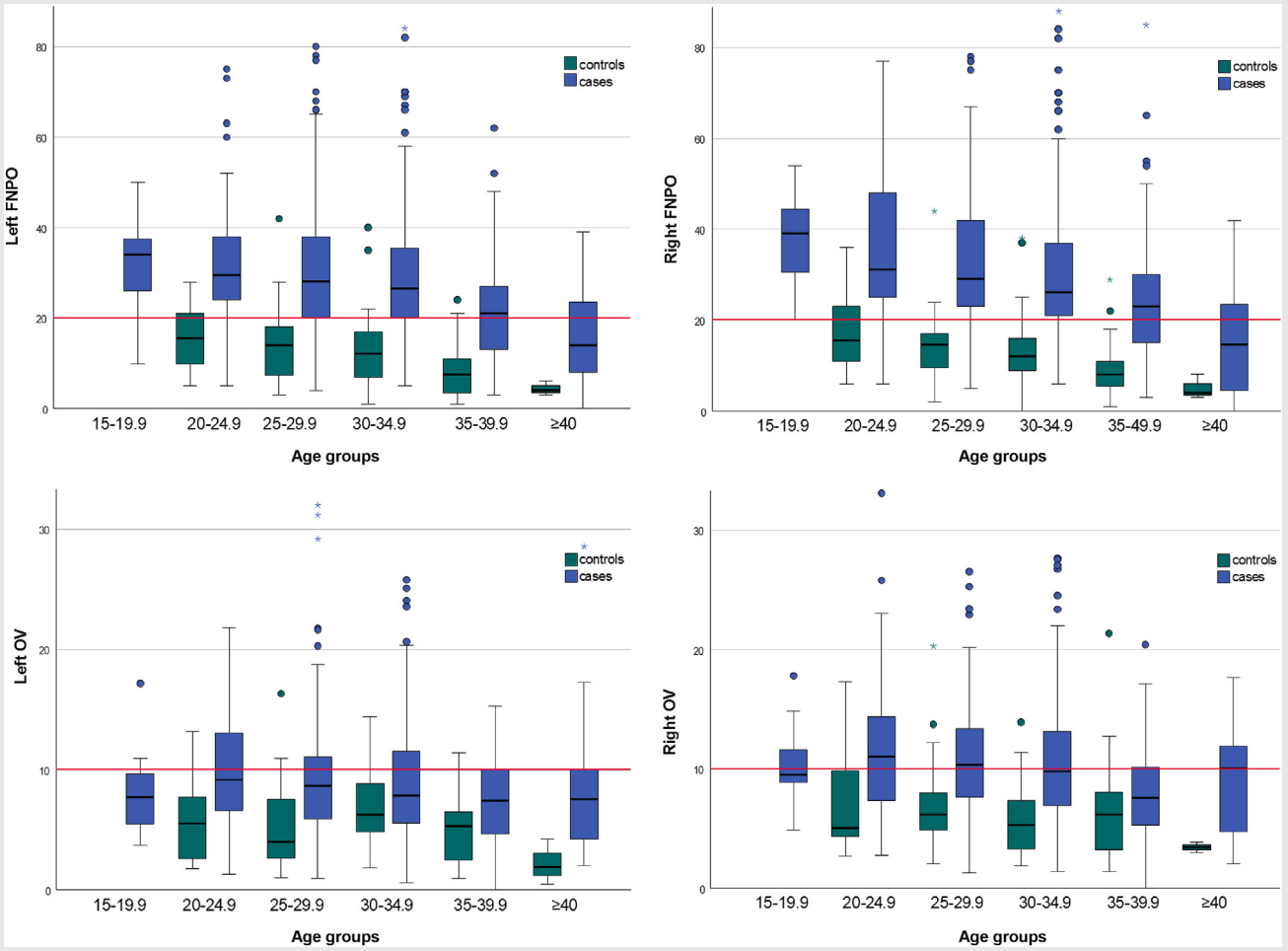
Ovarian markers stratified by age

Age, BMI, and ovarian markers are shown for the total group of PCOS divided by the two different ultrasound probes and stratified by age groups (Supplemental Table 2). Follicle number per ovary was highest in the age group 16.0–19.9 years for both ultrasound transducers in the PCOS group (using ultrasound ≥8 MHz: median of 35.5 follicles [interquartile range {IQR}, 27.5–43.0]) and decreased with age in all subsequent age groups. Ovarian volume was highest in the age group 20.0–24.9 years for both ultrasound transducers (using ultrasound >8 MHz: median of 9.6 mL [IQR, 7.7–15.0]) compared with the age group of 16.0–19.9 years (median of 8.3 mL [IQR, 7.7–10.1]), and compared with the older age groups. For all age groups, except ≥40 years, the medians of follicle number per ovary were higher than the cutoff values for PCOM according to the PCOS Guidelines (Fig. 1 and Supplemental Table 2). There is a significant decline in follicle number per ovary across all ages (*P*<.001) in both women with and without PCOS, with a notable drop occurring specifically from the age of 35 onward, independent of the ultrasound transducer used. In the cohort in which women underwent an ultrasound with a transducer frequency ≥8 MHz, the percentage of women with PCOM due to follicle number per ovary ≥20 (in one or both ovaries) was 100% (16.0–19.9 years), 97.4% (20.0–24.9 years), 93.2% (25.0–29.9 years), 87.8% (30.0–34.9 years), 72.% (35.0–39.9 years), and 50.0% (≥40 years). In all age groups, the medians of ovarian volume were below the recommended cutoff value of 10 mL (Fig. 1 and Supplemental Table 2). There is a gradual decline in mean ovarian volume by age in the PCOS group (*P*<.001), and it is not clearly indicated at which age this decrease becomes prominent (Fig. 1 and Supplemental Table 2). In the ≥8 MHz cohort, it seems to start from the age category of 25.0–29.9 years. In the ≥8 MHz cohort, the percentage of women with PCOM due to ovarian volume >10 mL (of one or both ovaries) was 53.3% (16.0–19.9 years), 67.9% (20.0–24.9 years), 62.9% (25.0–29.9 years), 56.1% (30.0–34.9 years), 45.6% (35.0–39.9 years), and 55.0% (≥40 years). Women without PCOS did not show a significant decline in ovarian volume by age (Fig. 1). The boxplots showed more overlap in ovarian volume than in follicle number per ovary among women with and without PCOS (Fig. 1).

Associations of ovarian markers with BMI

To investigate whether BMI influences follicle number per ovary or ovarian volume we performed regression analyses in different age categories (Supplemental Table 3). In the cohort in which an ultrasound <8 MHz was used, BMI was positively associated with mean follicle number per ovary (β, 0.209, *P*=.004) and left follicle number per ovary (β, 0.762; *P*=.002) in the group 16.0–19.9 years, and with left ovarian volume (β, 0.097; *P*=.004) in the group 20–24.9 years, after correction for multiple testing. In the group 30.0–34.9 years, BMI was positively associated with mean ovarian volume (β, 0.112; *P*<.001) and with left ovarian volume (β, 0.134; *P*<.001), after correction for multiple testing. In the cohort in which ultrasound >8 MHz was used, BMI was negatively associated in the age group 20–24.9 years with left ovarian volume (β, –0.215; *P*=.004), after correction for multiple testing. In all other age groups, BMI was not significantly associated with any of the ovarian markers.

FIGURE 1



Boxplots of left follicle number per ovary, right follicle number per ovary, left ovarian volume, and right ovarian volume per age category of the cohort of women who underwent ultrasound using a probe ≥8 MHz. Number of included women in the group: 16–19.9 years (PCOS = 15, controls = 0), 20–24.9 years (PCOS = 78, controls = 18), 25–29.9 years (PCOS = 205, controls = 28), 30–34.9 years (PCOS = 180, controls = 29), 35–39.9 years (PCOS = 79, controls = 24), and ≥40 years (PCOS = 20, controls = 3). *Dots* mean outliers including the formula: IQR × 1.5. The *stars* mean far outliers, including the formula: IQR × 3.0. FNPO = follicle number per ovary; IQR = interquartile range; OV = ovarian volume; PCOS = polycystic ovary syndrome.

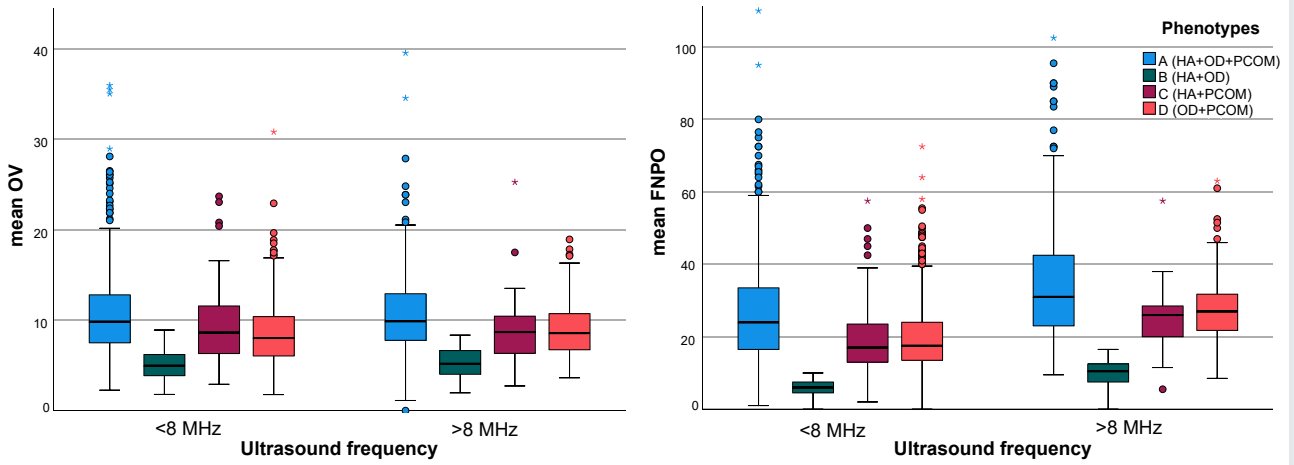
van der Ham. Ovarian ultrasound markers for polycystic ovary syndrome diagnosis. Fertil Steril 2024.

PCOS phenotypes

Ovarian markers were contrasted among the four PCOS phenotypes in women aged 20.0–39.9 years. We excluded 350 women (age <20 years or ≥40 years) due to evolving PCOS features during this life stage and 60 due to missing data, leaving 1,772 women measured with an ultrasound probe <8 MHz. In total, 1,015 women had phenotype A, 40 with phenotype B, 59 with phenotype C, and 801 with phenotype D (Supplemental Table 4). In total, 525 women were assessed using an ultrasound probe >8 MHz, of which 320 women had phenotype A, 33 women phenotype B, 29 women phenotype C, and 143 women phenotype D. For both ultrasound frequencies, women with phenotype A exhibited the highest follicle number per ovary and ovarian volume, followed by phenotype D, then phenotype C, and then phenotype B (all *P* values <.001 after adjusting for age) (Fig. 2). In the cohort with the high-frequency probe (>8 MHz), the percentage of women with follicle number per ovary ≥20 (in one or both ovaries) in phenotype A was 97.2%, phenotype C was 93.1%, and phenotype D was 95.1%.

Mean ovarian volume was the highest in phenotype A in both ultrasound groups. In no phenotype group did the median ovarian volume reach or exceed the cutoff value of 10 mL. To investigate how many women with PCOS received the diagnosis in our cohort based on different ultrasound markers, we assessed the different criteria for PCOM stratified by the four phenotypes (Supplemental Table 5). In a small percentage of cases (1.2% for phenotype A, 3.4% for phenotype C, and 1.2% for phenotype D), PCOM diagnosis was solely based on ovarian volume. If ovarian volume was not a marker for PCOM, women with phenotypes A and B would still receive the diagnosis of PCOS. In total, 13 women (0.6% of

FIGURE 2



Boxplots of mean follicle number per ovary and mean ovarian volume of the four PCOS phenotypes, divided by ultrasound frequency probe <8 MHz and ≥8 MHz. *Dots* mean outliers including the formula: IQR × 1.5. The *stars* mean far outliers, including the formula: IQR × 3.0. Phenotype A: ovulatory dysfunction + hyperandrogenism + polycystic ovarian morphology, phenotype B: ovulatory dysfunction + hyperandrogenism, phenotype C: hyperandrogenism + polycystic ovarian morphology, and phenotype D: ovulatory dysfunction + polycystic ovarian morphology. FNPO = follicle number per ovary; IQR = interquartile range; OV = ovarian volume; PCOS = polycystic ovary syndrome.

van der Ham. Ovarian ultrasound markers for polycystic ovary syndrome diagnosis. Fertil Steril 2024.

all women in this cohort) would not have received the diagnosis of PCOS if ovarian volume had not been considered as a marker for PCOM.

DISCUSSION

The 2003 Rotterdam Consensus defined PCOM as ≥12 follicles (2–9 mm) and/or ovarian volume >10 mL in at least one ovary using transvaginal ultrasonography (8). The 2018 International PCOS Guideline introduced a cutoff ≥20 follicles using a preferred high-frequency transducer (≥8 MHz), but criteria for ovarian volume was never revised (3). Despite changes in PCOM criteria and advancements in ultrasound technology, these cutoffs have remained largely uncontested with few studies attesting to their external validity. The 2023 PCOS Guideline emphasizes revising thresholds regularly with advancing ultrasound technology, preferably in large cohorts, and determining the impact of age and BMI on PCOM criteria (1).

In line with these recommendations, we assessed follicle number per ovary and ovarian volume in a large well-phenotyped cohort and investigated the influence of age and BMI on these ovarian ultrasound markers. Our findings showed that follicle number per ovary decreased with age and ovarian volume showed a less clear decline. Therefore, cutoff values for follicle number per ovary should be defined per age category. We found no clinically relevant correlation between BMI and follicle number per ovary or ovarian volume. Furthermore, the median of ovarian volume in this large PCOS population was lower in every age group than the cutoff of 10 mL that is currently advised.

Our results suggest that the optimal cutoff for ovarian volume is lower than the recommended threshold. To explain this difference, it is important to consider that almost all studies included in the evidence synthesis of the PCOS Guideline enrolled women diagnosed with PCOS according to the original NIH criteria (1, 6, 14). Some of these studies suggest that follicle number per ovary ≥20 and ovarian volume >10 mL are appropriate thresholds. However, women with PCOS who fulfill the NIH criteria represent the extreme PCOS phenotypes, including the most common "full-blown" phenotype A. We showed that non-NIH phenotypes (also known as phenotypes C and D) exhibit lower values of ovarian volume and follicle number per ovary compared with phenotype A. This finding aligns with other research (15, 16). Le et al. (16) also reported a lower ovarian volume than 10 mL, in 119 PCOS patients diagnosed according to the Rotterdam 2003 criteria. It is important to note that the sample sizes for phenotypes B and C in our study were relatively small, which necessitates a cautious interpretation of these findings.

Given the introduction of the diagnostic algorithm in the new 2023 International Guideline for PCOS, a threshold for PCOM is no longer necessary for women who experience both oligo-anovulation and hyperandrogenism. This algorithm specifies that an ultrasound is only required if either hyperandrogenism or ovulatory dysfunction is present in isolation. This implies that ultrasound is only necessary to diagnose the previously termed phenotypes C and D. Therefore, it is crucial to focus specifically on these women. Our results demonstrate that for these women, a lower cutoff for ovarian volume may be needed to identify these PCOS phenotypes. In addition, we also showed that only 0.6% (13/2,297) of the

patients in our cohort would not receive the diagnosis of PCOS if ovarian volume was not included in the criteria. These findings allow us to speculate that ovarian volume could even be omitted for the detection of PCOM in the diagnosis of PCOS. The relatively poorer diagnostic accuracy associated with ovarian volume further supports this notion (6). However, we were unable to evaluate the additional value of ovarian volume measured with TAUS, as there was no data available in this cohort to compare ovarian volume measurements between TAUS and TVUS within the same subjects.

Previous studies also reported a relatively higher follicle number per ovary and ovarian volume in the right ovary compared with the left one (16, 17). Two possible explanations have been suggested to explain this difference in ovarian size. First, the decreased space in the left lower pelvic cavity resulting from the S-shaped curve and descent of the sigmoid colon could limit ovarian growth and expansion. Second, there is a known difference in venous drainage between the right and left ovaries (18). However, the meta-analysis from Pea et al. (6) did not investigate any potential for left or right specific cutoffs. Our results demonstrate that with the high-frequency transducer ovarian volume, but not follicle number per ovary, differed between the left and the right ovary. Based on these findings, different cutoff values for follicle number per ovary between the left and right ovary are not necessary, and the added value of ovarian volume is already questioned here.

Antimüllerian Hormone has been proposed as a substitute for PCOM (19). However, there are many factors influencing AMH levels that prevent the establishment of a universal cutoff, such as different AMH assays, age, and BMI (5, 20). As such, ultrasound remains the most used method to assess PCOM in patients with PCOS. Previous studies have shown an inverse association between BMI and AMH levels in patients with PCOS (21), although this relationship remains uncertain. Our results showed no clinically significant correlation between BMI and follicle number per ovary or ovarian volume. By contrast, a clear relationship exists between age and follicle number per ovary and our study emphasizes the importance of age-specific cutoffs, particularly in the latter stages of reproductive life.

One of the strengths of our study was the inclusion of a large, well-phenotyped cohort of women with PCOS. A lot of clinical data was available, which gave us the opportunity to use reliable information to answer the research questions. Consequently, we were able to exclude women with ovarian cysts or those using OCP, as OCP usage is known to influence ovarian follicle counts (22). Using this real-world clinical data allowed us to investigate the results of two different types of ultrasound machines and transducers (high and low frequency). A potential limitation of using this data is the variation in ultrasound operators, which could introduce variability in the results. However, this variation mirrors real-world clinical settings where multiple sonographers and sonologists conduct ultrasound examinations and diagnose PCOS across different regions globally. Another limitation of this study is that, since our clinical data primarily originated from the Department of Reproductive Endocrinology, the number of women without PCOS that could be included was limited, resulting in small sample sizes per age category. Nevertheless, the data from controls were highly reliable due to their uniform endocrine screening and the exclusion of PCOS. It is noteworthy that the control group may include women with PCOM. There is an ongoing debate about whether they should be excluded. To our knowledge, PCOM alone is not considered a clinical condition associated with adverse health outcomes. We choose not to exclude women in the control group with solely PCOM, despite their potential to reduce the discriminatory power of the ovarian markers.

In conclusion, our data suggest that ovarian volume has no discriminative power in addition to follicle number per ovary to detect PCOM as a criterion for PCOS. The cutoff for follicle number per ovary should ideally be stratified by age, but there is no necessity to stratify by BMI.

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CRediT Authorship Contribution Statement

Kim van der Ham: Writing – original draft, Methodology, Formal analysis, Conceptualization, Data curation, Visualization. **Federica Barbagallo:** Writing – review & editing. **Emiliya van Schilfgaarde:** Writing – original draft, Formal analysis. **Marla E. Lujan:** Writing – review & editing, Methodology. **Joop S.E. Laven:** Writing – review & editing, Supervision. **Yvonne V. Louwers:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Declaration of Interests

K.v.d.H. has nothing to disclose. F.B. has nothing to disclose. E.v.S. has nothing to disclose. M.E.L. has nothing to disclose. J.S.E.L. reports grants from Ansh Labs, Ferring, Roche Diagnostics, Merck, outside the submitted work; personal fees from Ferring, Titus Healthcare, Gedeon Richter, Ansh Labs, Roche Diagnostics; honoraria and travel expenses from Ferring and Roche Diagnostics; data safety monitoring board for LOCI Trail UK; an unpaid Board Member and President of the Androgen Excess and PCOS Society; and a member of the American Society for Reproductive Medicine Research Integrity Committee, outside the submitted work. Y.V.L. reports honoraria fees from Ferring and Merck; travel support from Ferring; and funding from Synergy Erasmus Medical Center grant, outside the submitted work.

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