Diagnosis of Polycystic Ovary Syndrome

Which Criteria to Use and When?

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KEYWORDS

• Polycystic ovary syndrome • PCOS • Hyperandrogenism • Diagnostic criteria
• Disease classification

KEY POINTS

- Current diagnostic criteria for polycystic ovary syndrome (PCOS) are based on expert opinion, the lowest level of evidence. There has never been a formal consensus process to determine criteria for the diagnosis of PCOS.
- Individual diagnostic criteria have limitations that may result in misclassification of National Institutes of Health (NIH) and non-NIH Rotterdam PCOS phenotypes.
- Although current criteria have identified 2 major groups (NIH and non-NIH Rotterdam) that have different metabolic risks, recent genetic analyses have suggested that these criteria do not identify biologically distinct PCOS subtypes.
- Diagnosis of PCOS, therefore, should depend on management goals. The assessment of polycystic ovarian morphology is not needed to manage the endocrine and metabolic features of PCOS but is critical for reproductive endocrinologists managing infertility associated with PCOS.
- Agnostic data mining approaches have identified PCOS subtypes (metabolic and reproductive) that appear genetically distinct, potentially providing a biologically meaningful way to establish criteria for the diagnosis of PCOS.

WHAT IS POLYCYSTIC OVARY SYNDROME ?

PCOS is a highly heritable complex genetic disorder.[1] It is the most common disorder of reproductive-aged women, affecting up to 15% of this population worldwide, depending on the diagnostic criteria applied.[2,3] PCOS is a leading cause of anovulatory infertility, obesity, and type 2 diabetes mellitus.[3,4] It is a syndrome, a collection of signs and features, of unknown etiology. Despite its high prevalence and major

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morbidities, affected women remain remarkably underserved.[1] Diagnosis frequently is delayed[5] and physicians often are poorly informed about PCOS.[6]

PCOS is characterized by enhanced luteinizing hormone (LH) relative to follicle-stimulating hormone release, increased LH-dependent ovarian testosterone (T) production, frequent adrenal androgen excess, profound insulin resistance, dysglycemia, and obesity.[1,3] Antimüllerian hormone levels are increased in PCOS, and recent rodent[7] and human[8] studies suggest antimüllerian hormone plays a direct role in PCOS pathogenesis.

Polycystic ovarian morphology (PCOM) is characterized by an excessive number of antral follicles, which may be the result of accelerated follicle growth and/or prolonged survival of small follicles.[9,10] Additional hallmarks of PCOM are ovarian stromal hypertrophy, theca cell hyperplasia, and ovarian cortical thickening.[11] Theca cells in women with polycystic ovaries secrete more androgens, basally and in response to LH and insulin.[12] Abnormalities of both theca and granulosa cells may contribute to the arrest of follicular development seen in PCOS.[12–14]

DIAGNOSTIC CRITERIA

National Institutes of Health Criteria

Stein and Leventhal[15] generally are credited with the original description in 1935 of what has come to be known as PCOS, although there are clear reports of the disorder dating back to Hippocrates in the fifth century BCE.[16] It was not until 1990, however, that there was a formal effort to develop standard diagnostic criteria as part of a meeting of experts in medical and reproductive endocrinology sponsored by the NIH.[17] The participants were asked to vote on the potential diagnostic features of PCOS; features receiving the most votes, clinical and/or biochemical evidence of hyperandrogenism (HA) and ovulatory dysfunction (OD) with the exclusion of secondary causes, became known as the NIH criteria.[17] These criteria did not include PCOM because, even at that time, it was recognized that PCOM was present in 20% to 30% of women with regular menses and no hyperandrogenic symptoms.[18] The prevalence of PCOS has been constant, at 5% to 8%, using the NIH criteria.[19]

Rotterdam Criteria

In 2003, the European Society of Human Reproduction and Embryology and the American Society for Reproductive Medicine (ASRM) sponsored another meeting of experts in Rotterdam, Netherlands, during which PCOM was added as a diagnostic criterion.[20,21] The Rotterdam criteria required 2 of the 3 features for the diagnosis of PCOS: (1) OD, (2) HA, and (3) PCOM. The intent of the Rotterdam criteria was to encompass the NIH criteria as well as to broaden the definition of PCOS. The result was 2 new phenotypes, HA + PCOM and OD + PCOM (**Fig. 1**). The prevalence of PCOS using the Rotterdam criteria is as high as approximately 15% of reproductive-aged women.[22,23]

Androgen Excess Society Criteria

In 2006, the Androgen Excess Society (now known as the Androgen Excess and PCOS Society [AE-PCOS]) convened another task force of experts.[24] This group recommended that HA should be a requirement for the diagnosis of PCOS, which limited the diagnosis of PCOS to 3 of the 4 Rotterdam phenotypes: HA + OD + PCOM, HA + OD, and HA + PCOM.[24,25] The AE-PCOS criteria have never been widely adopted.

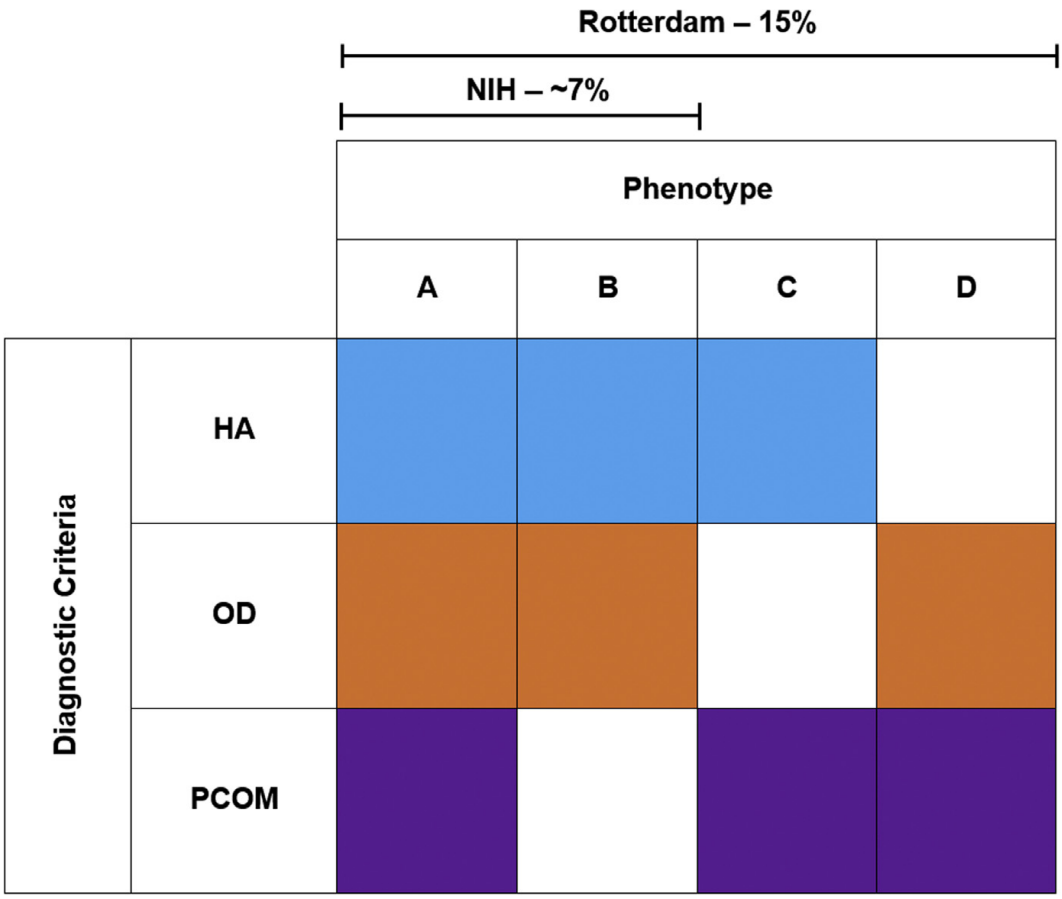

Fig. 1. Comparing components of different diagnostic criteria. NIH PCOS must include both HA + OD, ± PCOM. There are no endocrine or metabolic differences between phenotypes A and B; there is no need to assess PCOM in NIH PCOS. Rotterdam criteria require at least 2 of the 3 criteria to be present, adding 2 new phenotypes HA + PCOM and OD + PCOM. The prevalence of PCOS increases from approximately 7% to 15% when including the 2 non-NIH Rotterdam phenotypes.

National Institutes of Health–Sponsored Evidence-Based Methodology Workshop on Polycystic Ovary Syndrome

In 2012, the NIH sponsored an evidence-based methodology workshop on PCOS to review the current state of the science in the field.[26] This meeting differed from the previous PCOS meetings in that it followed a formal consensus process. Although it was not an official NIH Consensus Development Conference (https://consensus.nih.gov/), the meeting followed the same court model, where the evidence is presented to a panel that functions as a jury. The panel consisted of individuals who are experts in their fields (gynecology, diabetes and metabolism, cardiology, and primary care) but are not engaged in PCOS research, allowing independent assessment of the scientific evidence by an unbiased panel. The panel's final report noted that "the name 'PCOS' was a distraction and an impediment to progress," and that the emphasis on PCOM created confusion because it was neither necessary nor sufficient for the diagnosis of PCOS.[26] They recommended using the Rotterdam criteria with precise specification of phenotype (see **Fig. 1**) and proposed a comprehensive research agenda that included assessment of the epidemiology and long-term health outcomes of the PCOS phenotypes.

Evidence-Based Guidelines

Consensus conferences since have been replaced by evidence-based guidelines for the diagnosis and management of PCOS, such as those published in 2013 by the Endocrine Society[27] and the 2018 international evidence-based guidelines.[28–30] The quality of the evidence on which these guidelines are based, however, is predominantly low due to a paucity of randomized clinical trials (RCTs). Unfortunately, the key research initiatives recommended by the 2012 Evidence-based Methodology Workshop on Polycystic Ovary Syndrome to critically assess the utility of the PCOS diagnostic criteria as well as the optimal therapies and long-term health outcomes have not been undertaken, in large part due to underfunding of the field.[31] Of the 34 recommendations in the Endocrine Society Clinical Practice Guideline,[27] the evidence supporting 24 of these was rated as "low" or "very low." There were almost 175 recommendations in the international guideline,[28–30] of which only 31 were ranked as evidence based; the remainder were clinical consensus recommendations or clinical practice points.

LIMITATIONS OF THE INDIVIDUAL DIAGNOSTIC CRITERIA

Hyperandrogenism

Clinical hyperandrogenism

Signs of HA in women include hirsutism, acne, and alopecia. Male pattern terminal hair growth is a fairly reliable indicator of androgen action.[32] The development of hirsutism is determined by number of pilosebaceous units and their sensitivity to androgen action, which genetically are determined and vary by race and ethnicity.[32,33] Although increased male pattern terminal hair growth assessed by ethnicity-specific Ferriman-Gallwey scores is pathognomonic for HA, as many as 50% of HA women do not have clinically significant terminal hair growth.[34] Furthermore, women often remove unwanted hair using mechanical methods. Acne and alopecia can reflect HA but are not reliable enough to use as surrogate markers for androgen excess.[35,36]

Biochemical hyperandrogenism

Hyperandrogenemia is characterized by elevated circulating endogenous androgen levels. T circulates specifically bound to sex hormone–binding globulin (SHBG) and loosely associated with albumin; only approximately 1% of circulating T is free T (FT).[37] Both FT and albumin-associated T are biologically available[37] and are referred to collectively as non-SHBG bound T (NSB-T). FT is elevated in approximately 70% of women with PCOS.[25] NSB-T, however, provides a better index of T that is bioavailable.[38]

The main limitation of biochemical HA as a diagnostic criterion is that it can be difficult to detect at the lower circulating levels present in women and children.[39] Measuring T is challenging because steroids structurally are similar, and antibodies used in immunoassays can cross-react with other steroids.[39] The gold standard for measuring total T (TT) is liquid chromatography followed by tandem mass spectrometry (LC/MS-MS)[39]; this is the method recommended by the Endocrine Society[39] and the Centers for Disease Control and Prevention.[40] Unfortunately, some clinical laboratories still use inaccurate TT assays, such as electrochemiluminescence immunoassays, performed directly on serum or plasma (direct assays).[39] These methods lack sensitivity and specificity and should not be used,[39] especially in women and children.

The gold standard for measuring FT is equilibrium dialysis; however, it is expensive, cumbersome, and subject to variability if not correctly performed.[39] Direct FT assays are unreliable and should not be used.[39] Therefore, guidelines[25,39] recommend

calculating FT and NSB-T based on the binding affinity of T to SHBG and albumin utilizing the law of mass action.[41] It is possible to order calculated FT and NSB-T as part of T profiles offered by clinical laboratories. It also is easy to perform these calculations using measured TT and SHBG values (e.g. http://www.issam.ch/freetesto.htm); albumin levels can be assumed to be normal.[41] The accuracy of these calculations depend on the quality of the TT assay.[39] Unreliable TT assays may lead to a failure to detect biochemical HA and result in misclassification of NIH PCOS as the non-NIH Rotterdam OD + PCOM phenotype (**Table 1**).

Assessment of HA should include measurement of dehydroepiandrosterone sulfate (DHEAS) because a substantial minority of women with HA have isolated DHEAS elevations.[25] DHEAS also is a marker of adrenal androgen-secreting neoplasms. Measurement of androstenedione does not increase the detection of HA in most cases.[42] Recently, 11-oxygenated C19 steroids, such as 11β-hydroxyandrostenedione, have been recognized as an important pool of circulating adrenal androgens,[43] including in women with PCOS.[44] Nevertheless, there is no current evidence to suggest that these steroids should be included in the clinical assessment of HA.[45]

Ovulatory Dysfunction

Among women with PCOS who report regular menstrual cycles, 20% to 30% have been found to have anovulatory cycles with biochemical assessment of ovulation.[24,25] Oligomenorrhea (defined as <6–8 menstrual cycles per year) is virtually pathognomonic for anovulation, but more frequent cycles do not necessarily indicate ovulation. A recent study of greater than 600,000 menstrual cycles revealed that there is more variation within normal menstrual cycles than previously recognized,[46] suggesting that it may be difficult to determine ovulatory status based solely on self-report of 21-day to 35-day cycles. To increase detection of anovulatory cycles, the ASRM recommends that women with other signs of PCOS who report regular menstrual cycles undergo ovulatory monitoring using basal body temperatures or measurement of a serum progesterone level in the luteal phase.[47] Lack of confirmation of ovulation can lead to misclassification of NIH PCOS as the ovulatory non-NIH Rotterdam HA + PCOM phenotype (see **Table 1**).

Polycystic Ovarian Morphology

Despite the intrinsic abnormalities seen in PCO,[9–11] the presence of PCOM is neither necessary nor sufficient for diagnosis of PCOS.[18,48] There is no evidence that the presence of PCOM has any implications with regard to the endocrine or metabolic features of PCOS.[27,49] There also is no evidence to support the addition of assessment of PCOM to the diagnosis of NIH PCOS. The international evidence-based guidelines state that ultrasound is not needed for the diagnosis of PCOS in women with HA + OD.[28–30] Similarly, the Endocrine Society guideline for the management of hirsutism states that demonstrating PCOM to diagnose ovulatory PCOS (HA + PCOM) is unlikely to affect management of hirsutism.[50] Furthermore, PCOM is so common in adolescents that ultrasound is not recommended for the diagnosis of PCOS in this age range.[28–30,42,51]

Ovarian ultrasound is a critical component of assisted reproductive technology (ART), because assessment of antral follicle count predicts ovarian responses to medications[52] as well as risk of ovarian hyperstimulation syndrome.[53] All women undergoing infertility evaluation and treatment routinely have pelvic ultrasounds at baseline and throughout their treatment to monitor follicular growth. The detection of PCOM is dependent on the sensitivity of the ultrasound equipment, the skill of the operator, the approach (vaginal vs abdominal), and the weight of the patient.[28–30] Furthermore,

Table 1
Misclassification of Rotterdam polycystic ovarian syndrome phenotypes

	Hyperandrogenism	Ovulatory Dysfunction	Polycystic Ovarian Morphology	Interpretation
Each criteria appropriately assessed	√	√	√	NIH phenotype
HA not detected due to use of incorrect TT or FT assay	X	√	√	Non-NIH Rotterdam phenotype
Anovulation not detected due to reported regular menses	√	X	√	Non-NIH Rotterdam phenotype
Ovarian morphology not detected due to technology, operator, or patient factors	√	√	X	NIH phenotype

ovarian ultrasound examinations performed by clinical radiologists frequently do not use the Rotterdam criteria to interpret their findings. Therefore, it is challenging for medical endocrinologists to obtain an accurate ovarian ultrasound assessment of PCOM. In summary, assessment of PCOM is not needed for the endocrine management of PCOS or hirsutism; access to accurate ovarian sonography is limited outside of reproductive endocrinology.

Polycystic Ovary Syndrome Phenotypes

Application of the Rotterdam criteria results in 4 PCOS phenotypes: (A) HA + OD + PCOM, (B) HA + OD, (C3) HA + PCOM, and (D) OD + PCOM (see **Fig. 1**). Phenotypes A and B also are known as NIH PCOS; phenotypes C and D also are known as non-NIH Rotterdam PCOS. There is no evidence, however, that there are endocrine or metabolic differences between HA + OD and HA + OD + PCOM.[27,49] Thus, there is no rationale for stratifying HA + OD cases by the presence of absence of PCOM. Stratification by the presence or absence of hirsutism has been proposed in the Androgen Excess Society guidelines,[24] adding 12 potential phenotypes. Hirsutism is a distinct biologic process related to androgen action and there are insufficient data to support using it to further subgroup PCOS.[50]

TOWARD A BIOLOGICAL BASIS FOR POLYCYSTIC OVARY SYNDROME CLASSIFICATION

Current Polycystic Ovary Syndrome Classification

Michael Crichton stated, "the work of science has nothing whatever to do with consensus. Consensus is the business of politics. Science, on the contrary, requires only one investigator who happens to be right…consensus is evoked only in situations where the science is not solid enough."[54] Despite labeled as consensus criteria, all the PCOS diagnostic criteria are based on expert opinion—the lowest level of evidence.[55] As better scientific evidence accumulates, consensus no longer is necessary. Unfortunately, the newer so-called evidence-based PCOS guidelines[27–30] still are largely based on expert opinion because of the paucity of high-quality RCTs addressing most features of PCOS.[31]

Validation of Diagnostic Criteria

Diagnostic criteria should identify discrete biologically meaningful subgroups that are stratified by associated disease risk, biomarker profiles, responses to treatment, and/or genetic architecture. The consistent difference among the PCOS phenotypes is the severity of insulin resistance. It has been well established that the NIH PCOS phenotype is at higher risk for insulin resistance and associated metabolic abnormalities compared with non-NIH Rotterdam PCOS phenotypes.[3,56,57] Ovulatory PCOS (HA + PCOM) tend to have lower body mass index (BMI) than the NIH phenotype[58] and have either mild or no metabolic abnormalities.[59]

Although current criteria have identified 2 major subgroups (NIH vs non-NIH Rotterdam) with differing metabolic risk, a recent well-powered meta-analysis of PCOS genome-wide association studies (GWAS) has suggested that current criteria do not identify genetically distinct subgroups.[60] GWAS permit the agnostic interrogation of the entire genome for regions that are associated with a given trait or disease and provide insight into causal pathways.[61] GWAS in PCOS case-control cohorts of Han Chinese and European ancestry have implicated pathways regulating gonadotropin secretion and action, androgen biosynthesis, insulin resistance, and ovarian aging in the pathogenesis of PCOS.[1,62,63] The largest genetic analysis of PCOS to date—a GWAS meta-analysis that contained greater than 10,000 PCOS cases and 100,000

controls—compared NIH, non-NIH Rotterdam, and self-reported PCOS and found no significant differences in genetic architecture for 13 of 14 identified loci.[60] There was 1 locus that was associated significantly more strongly with NIH PCOS than with non-NIH Rotterdam and self-reported PCOS,[60] which suggests that this locus is involved in pathways regulating insulin action because NIH PCOS are more insulin resistant than the other PCOS phenotypes. This meta-analysis suggests that, overall, the genetic architecture of NIH PCOS, non-NIH Rotterdam PCOS and self-reported PCOS is similar.

Objective Approaches to Polycystic Ovary Syndrome Classification

Cluster analysis is a well-established mathematical approach to aggregate traits into clusters of similar data. Cluster analysis increasingly is applied to large biomedical datasets to identify subtypes of disorders, such as type 2 diabetes mellitus.[64] The authors performed unsupervised hierarchical cluster analysis in women of European ancestry with PCOS diagnosed by NIH criteria using quantitative anthropometric, reproductive, and metabolic traits and identified 2 distinct clusters, designated *reproductive* and *metabolic* (**Fig. 2**).[65] The reproductive subtype had higher LH and SHBG levels with relatively low BMI and insulin levels, whereas the metabolic subtype had higher BMI, glucose, and insulin levels with lower SHBG and LH levels.[65] The authors replicated these clusters in an independent cohort of European ancestry NIH PCOS cases. GWAS was performed limiting the cases to the reproductive or metabolic subtypes. Five novel susceptibility loci were discovered, 4 associated with the reproductive subtype and 1 associated with the metabolic subtype.[65] The authors' findings suggest that these subtypes are biologically relevant because they appear to have distinct genetic architectures. This study presents an objective, unbiased mathematical approach to PCOS classification and validates this approach by demonstrating that the reproductive and metabolic subtypes thus identified are associated with novel and distinct gene regions. These PCOS subtypes need to be replicated in PCOS diagnosed by Rotterdam criteria as well as in PCOS cohorts of non-European ancestry.

Which Polycystic Ovary Syndrome Diagnostic Criteria to Use When

The NIH criteria of HA + OD are sufficient to detect women at high risk of insulin resistance and associated metabolic abnormalities.[3] The presence of PCOM has no impact on the diagnosis or endocrine management of NIH PCOS. In contrast, ovulatory women with HA are at low risk for insulin resistance and associated metabolic abnormalities, whether or not they have PCOM.[3,56] Therefore, medical endocrinologists should focus on determining whether an HA patient is ovulatory. Usually, OD is not a subtle finding as it is accompanied by oligomenorrhea (<6–8 menses per year). Because a substantial minority of women reporting regular menstrual cycles may be anovulatory, however, it is prudent to confirm ovulation in this subgroup of HA patients.[47] Glucose tolerance and lipid levels should be evaluated in NIH PCOS.[3,27,66]

Symptom-based management for hirsutism, or for acne and alopecia with biochemically documented HA, can be undertaken in ovulatory women without additional metabolic screening.[3] As highlighted in the Endocrine Society guideline for the management of hirsutism, establishing a diagnosis of Rotterdam PCOS by assessing ovarian morphology does not affect management.[50] There is no evidence, however, to support the use of the Rotterdam criteria for the diagnosis of PCOS, that is, the addition of PCOM, for the management of endocrine or metabolic features of PCOS. In contrast, treatment of anovulatory infertility associated with PCOS using ART requires monitoring of ovarian morphology. Assessment of HA is important for establishing a diagnosis of NIH PCOS because these patients require monitoring for

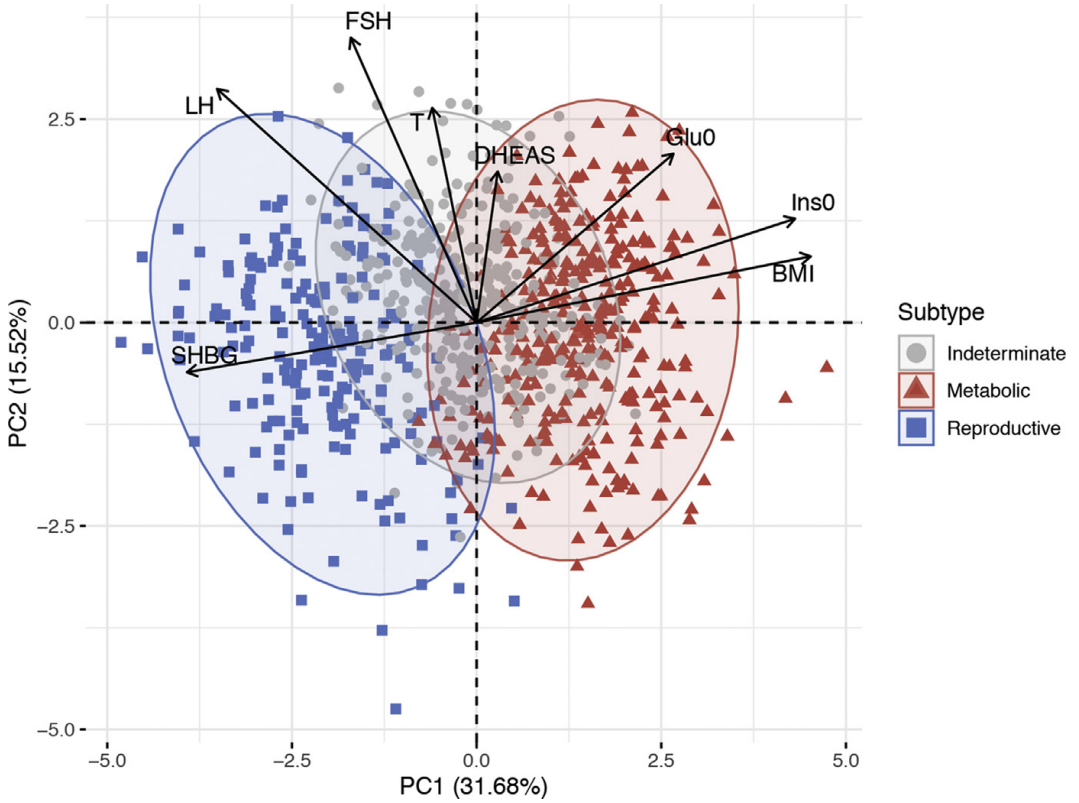

Fig. 2. Principal component analysis plot of quantitative traits for a genotyped PCOS clustering cohort showing a biologically driven method of PCOS classification. Women with PCOS were clustered into distinct groups—metabolic, reproductive, and indeterminate—based on BMI, fasting insulin, fasting glucose, DHEAS, T, follicle-stimulating hormone, LH, SHBG, and genotype data. The relative magnitude and direction of trait correlations with the principal components are shown with black arrows. Glu0, fasting glucose; Ins0, fasting insulin; PC, principal component. (*From* Dapas M, Lin FTJ, Nadkarni GN, et al. Distinct subtypes of polycystic ovary syndrome with novel genetic associations: An unsupervised, phenotypic clustering analysis. PLoS Med. 2020;17(6):e1003132; with permission.)

associated metabolic abnormalities. The presence or absence of HA, however, does not affect short-term reproductive management.

SUMMARY

The diagnostic criteria for PCOS are based on expert opinion and do not identify genetically distinct subgroups of women. Furthermore, each individual diagnostic criterion has limitations that may lead to misclassification between PCOS phenotypes. The endocrine management of PCOS does not require assessment of ovarian morphology. Unsupervised cluster analysis of clinical and biochemical traits has shown promise in identifying genetically distinct metabolic and reproductive PCOS subtypes. Such objective approaches enable the transition from PCOS classification based on expert opinion, which is subjective, to classification based on demonstrable biologic differences.

CLINICS CARE POINTS

- Hirsutism is sufficient to diagnose HA but not all HA women are hirsute.
- Acne and alopecia are not reliable markers of HA.

- LC/MS-MS assay is needed for accurate TT measurement. FT and NSB-T should be calculated based on TT and SHBG.
- Women presenting with HA who report regular menses should undergo testing to confirm ovulation.
- Ovarian morphology is not needed for the endocrine management of PCOS.

DISCLOSURE

The authors have nothing to disclose.

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