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Rationalizing Investigations in Diagnosis of PCOS

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Abstract

Polycystic ovarian syndrome is most common endocrinopathy affecting women, with prevalence estimated between 8and 13%, depending on definition used and the population studied.It presents with several possible combinations of signs and symptoms and a wide range of phenotypes, which may include reproductive, endocrine and metabolic abnormalities. Despite of so many available guidelines, there still exists controversies in the diagnosis of PCOS particularly which androgen to measure and which method to use along with pertinent USG findings. This article aims to address various controversies andprovideconsensusin investigations during the diagnosis of polycystic ovary syndrome.

Keywords: PCOD, androgen measurements, ultrasound findings, metabolic abnormalities.

Introduction

Polycystic ovary syndrome is of heterogeneous nature, characterized by combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnosis.Addition of USG features has improved the detection of variation in the PCOS phenotype.It is a complex multigenic disorder with strong epigenetic and environmental influences on it. It is frequently associated with abdominal adiposity,obesity, insulin resistance, metabolic disorders and cardiovascular risk factors.Nationalinstitutes of Health (NIH) first proposed the diagnostic criteria for PCOS in 1990 followed by Rotterdam criteria in 2003,Androgen Excess Society in 2006,Global PCOS guideline in 2018.Despite of so many guidelines there are still several unresolved controversies in the diagnosis of PCOS.No one agreed upon the universal definition.But all definitions require that secondary causes to be excluded.Thecontroversiesinclude (a) methods of assessment and types of androgens to be measured to detect biochemical hyperandrogenism, (b) cut-off value for the diagnosis of clinical hyperandrogenism(c) ultrasound threshold of antral follicle count to diagnose polycystic ovaries(d)diagnosing PCOS in adolescence where there is no clear definition for 'irregular cycles'.So, for proper diagnosis and follow up of PCOS, judicious application ofwell-standardized diagnostic methodis essential.

Biochemical Hyperandrogenism

PCOS can be picked up by assessing biochemical hyperandrogenism,in those patients who don't have hirsutism or clear-cut evidence of clinical hyperandrogenism.More than three quarters of

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PCOS have increased circulating androgen levels.(1) But controversy arises on deciding which androgens to measure,which assays to use,defining the normal ranges for these androgens,costfactorfor the tests, easy access to high-quality tests, overlap of results between control and patients.

Which Androgen?

The commonlymeasured androgens in PCOS patients include total-testosterone (TT), Free testosterone (FT),Calculated bioavailable testosterone (BA-T), Calculated free testosterone (calculated FT) by the formula of Vermeulen and colleagues, Free androgen index [FAI=100 × (total testosterone/ sex hormone bindingglobulin[SHBG])],Androstenedione, Dehydroepiandrosterone sulphate (DHEAS).

While using TT as a diagnostic criterion,it identifies only 20–30% of PCOS women as hyperandrogenemia.FT can identify 50–60% of such women. But only 1–3% of testosterone is unbound to plasma proteins.Again,the levels of SHBG are reduced in women with PCOS,raising the concern that whether TT or FT is the most appropriate measure to diagnose PCOS.

There are certain limitations while measuring TT, value is variable depending on the sample collection time of the day.Again, many similar steroids present in the circulation tend to interfere with the assay. Reference range of TT is laboratory specific.Age and gender corrected reference values are lacking withno universally accepted testosterone calibrating standard.(2)

Mild to moderate elevation in androstenedione is seen in PCOS.But marked rise indicates adrenal pathology, mostly 21-OH deficient non-classic congenital adrenal hyperplasia (CAH).Here again the level of elevated 17 hydroxyprogesterone confirms the diagnosis. Sometimes ACTH stimulation test is required.

DHEAS may be increased (up-to 8 mcg/ml) in about 50% of anovulatory women with PCOS.DHEAS originates exclusively from adrenal gland, but the cause of adrenal hyperactivity is not known. DHEAS level measurementis not routinely recommended as it does not change the diagnosis or the management of hyperandrogenism.Higher level may be associated with steroidogenically active adrenal tumours where imaging is indicated.

Another important useful measure is FAI, which requires accurate measurementof testosterone and SHBG. It is the ratio of TT to SHBG multiplied by 100. So FAI can be biased by the inaccuracies in any one ofthetwo.There isacceptable correlation between FAI and FT. (2). It has been found that higher levels of FAI,FT and DHEA and reduced SHBG are effective measures for detection of PCOS (3).Again FAI $\geq$ 6,TT $\geq$ 2.4 nmol/L with certain USGfindings provide similar diagnostic accuracy. (4).

Other calculated testosterone indices like BA-T is also well corelated with TT,Androstenedione,DHEAS and non-biochemical parameters like hirsutism and ultrasound parameters of PCOS. (5)

ESHRE PCOS guideline group 2018(6) concluded that there is inadequate evidence to recommend which androgens to measure. They suggest that the best accuracy to detect biochemical hyperandrogenism is FT. The other hormones that could be tested are TT, DHEAS and androstenedione. But DHEAS and androstenedione on its own do not provide additional information regarding hyperandrogenemia in PCOS.

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Most ofthetime PCOS patients visiting to clinic are already onoral contraceptive pills. OCPs should be discontinued for at least 3months because of reduction in gonadotropin-dependent androgen production and increase in SHBG.

Which assay?

The Hormonal assays are used to measure androgen level for diagnosis of PCOS include - (a) liquid chromatography– tandem mass spectrometry (LC-MS/MS), (b) gas chromatographic mass spectrometry (GCMS), (c) radioimmunoassay (RIA), (d)chemiluminescence immunoassays (CLIA), (e) enzyme-linked immunosorbent assay (ELISA).

Many commercially available methods lack the sensitivity and specificity needed to accurately measure the very low androgen concentrations characteristic of women.(7)Till now consensus is thatfree testosterone is the most sensitive measure of hyperandrogenemia, provided the reliable methods such as equilibrium dialysis are used for its determination.Directradioimmunoassay analogue method must not be used (grossly inaccurate) for estimating free testosterone.(7,8)Valid alternative is the calculation of free testosterone concentrations from SHBG and TT.

Ideally, liquid chromatography/mass spectrometry (LC/MS) should be used for measurement ofTT (6). Some extraction or chromatography radio immunoassays and a few direct radio immunoassays might be reliable.Butall these assays are technically complex, expensive, timeconsuming.Thechance of exposure toradioactivitymight be there, which limit their broad use.

Currently used almost all immune-chemiluminescenceassays cannot accurately measure the low testosterone concentrations characteristic of women and children. Hopefully this situation will change in future.An updated direct immune-chemiluminescenceassay for TT was certified in 2016 by the Centers for Disease Control and Prevention (CDC) Laboratory/Manufacturer Hormone Standardization (HoSt) programme. The assay could detect testosterone levels of 8–1,000 ng/dl, which might be adequate for measuring levels of testosterone in women. (9)

Role of USG

Ultrasound features used to diagnose PCOS include-Antral follicle count (AFC),Follicular number per ovary (FNPO),Ovarian volume (Ov), Ovarian area (OA), Ovarian blood flow andratio of stroma to total ovarian size.AFC is considered to be a good measure to identify the severity of reproductive dysfunction in PCOS.Increased AFC was most significantly associated with increased androgens and LH:FSH ratio.(10) There are ample of evidences which suggest the associations among follicle populations with reproductive and metabolic features in women with PCOS.

Polycystic ovaries in PCOS can be confused with multifollicular ovaries, which may be physiological in puberty or pathological,in conditions such as hypothalamic anovulation, hyperprolactinaemia, central precocious puberty.In the early years of reproductive life (within 8years of menarche)multifollicularovaries are considered physiological.Sodiagnosing PCOS based on ultrasound criteria in adolescents is not appropriate.

Adams criteria,count of 10 or more follicles arranged peripherally around dense core stromawashavinggreatest sensitivity for defining PCOS(11),but 12 or more follicles offered a greater specificity.(12)Following a consensus opinion in 2003, the count changed to>12 follicles measuring 2–9 mm in diameter.

There is significant increase in FNPO with the advances in USG and better transducer frequencies (>8MHz). Previous threshold for FNPO was 12 or more (13). Eleven studies with 2961 participants looked at FNPO and concluded optimal sensitivity and specificity with>19 per ovary.

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For count of antralfollicle, the grid system method is most reproducible one (devised by Lujan and colleagues in 2010).This technique showed a sensitivity of 85% and specificity of 94% when 26 follicles per ovary was taken as a cut-off.

On transvaginalscan,FNPO of 20 or more in one or both the ovaries or OV>10mL without inclusion of dominant follicle or corpus luteum or any cysts (ESHRE PCOS guideline group 2018) taken as a cut-off for diagnosis of PCOS.With ultrasound machine of older technology,cut-off may decrease to 12. 3D scan with automaticvolume calculations of antral follicles (e.g. VOCAL™ and SonoAVC™)have better accuracy, reduced interobserver variation in follicle counts whencompared with manual 2D measurements.(14,15)Morestudies are necessary for recommendation of 3D scan.

Stromal hypertrophy and increased follicular countcorrelates to OV and OA. Women with PCOS have a higher ovarian size compared with normal women, matched for age and body weight. There are small changes in OV between the age of 20 and 39 years. So,an age-specific OV cut-off is not warranted.Rotterdam criteria have set a threshold of OV>10mL for diagnosis of PCOS. (16)When the image resolution does not allow an accurate AFC,OV measurement holds its place.

No cut-off values for blood flow have been proposed to differentiate PCOS from normal ovaries and no homogeneous data confirming the importance of measuring ovarian blood flow to diagnose PCOS till now.

Ovarian stroma is also taken as a diagnostic tool for polycystic ovaries.Stroma to total ovarian size ratio cut-off of 0.32, is associated with hyperandrogenemia(17).Stromal volume and ovarian size correlateswellbut adding stromal volume to clinical practice for diagnosis of PCOS does not provide extra value.

AMH

AMH is produced by preantral and antral follicles of ovary and significantly higher level of AMH is seenin women with PCOS compared with normal women(18,19).There is a direct correlation between AMH level and ultrasound parameters like FNPO and OV.(20)26 different studies looked at AUC-ROC of AMH for the diagnosis of PCOS (21 adult and 5 adolescents).The threshold of AMH ranges from 10-57pmol/L for adults (21)to 25-44pmol/L in adolescents.(22)There was a wide fluctuation in the levels of AMH. Variation in AMH assays,variation in the population and phenotype of women may be the possible explanation.Assay is an important issue in AMH estimation. Previously used assays likeDiagnostic Systems Laboratories (DSL) or Immunotech (IOT) assaysare not available anymore. Gen II kit assay which is used more recently need cautious interpretation. There are very little data on new automated assays (21).Again,International Federation of Clinical Chemistry does not provide a standard regarding assay methods.

In view of all these caveats, serum AMH value as a surrogate markerto diagnose PCOS is still not accepted.In future, AMH can be a potential marker for diagnosing PCOSprovided further research confirms its validation in vast population of different backgrounds.

Exclusion of Secondary Aetiologies

PCOS can be diagnosed by exclusion of certain other diseasesby clinical judgement (namely, Cushing syndrome) or byappropriateblood sampling. 17-hydroxyprogesterone for non-classic CAH, prolactin for hyperprolactinaemia, TSH for thyroid dysfunction and FSH for premature ovarian failure.The most important aetiology is to excludeadrenal and ovarian androgen-secreting tumours.

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Suspicion arises when the symptoms and signs of androgen excess start at any time other than the peripubertal period, accompanied by signs of virilization or defeminization and/or progress rapidly.

Imaging of the adrenal glands and ovaries must be performed immediately (23). Selective venous catheterization and sampling, laparoscopy and/or laparotomy might be needed in uncertain cases.The concentrations of androgen being less important in the diagnosis of these neoplasms.(23)

Assessment ofCardiometabolic Risk

PCOS is associated with insulin resistance, dyslipidemia, hypertension and increased risk for developing cardiovascular diseases.Euglycaemichyperinsulinaemic clamp for insulin resistance is reserved for research studies.(24)Standard oral glucose test with insulin may be analternativeoption.Butreliable immunoassays andthe establishment of a normative range for insulin is most important.

A complete lipid profile and oral glucose tolerance test are recommended at diagnosis and then at every 2 years in patients with obesity, or those at high risk of DM (such as advanced age, personal history of GDM or family history of type 2 DM) without obesity (25).

Non-classic cardiovascular risk factors such as increased levels of inflammatory mediators (26), advanced glycation end products(27),carotid intima–media thickness,endothelial dysfunction orsubclinical cardiac dysfunction might cluster inPCOS,butthe clinical utility of such determinants remains to be established. Currently, not enough evidence is available to justify their inclusion in the routine clinical workup of PCOS.

Routine screening for endometrial hyperplasia or cancer, obstructive sleep apnea(OSA) andnonalcoholicsteatohepatosis(NASH) are not recommended.

Conclusion

PCOS due to its undetermined phenotypic spectrum and varied etiology remain controversial till now, especially in the aspect of diagnostic criteria.Thereis insufficient evidence regarding the best method for measurement of androgens. Also, the methods which are used to measure are of insufficient precision.In the USG finding,FNPOhas been well researched. But no clear cut-off for the optimal OV is provided.There is also inadequate evidence for the use of other ultrasound parameters to diagnose PCOS.The use of AMH as a substitute for diagnosis of PCOS is hindered by the fact that current assays need improved standardisation. Despite so much advancement and research large scientific and clinical research is needed in this field for better accuracy of tests in diagnosis of PCOS.

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