Polycystic ovary syndrome (PCOS), the main cause of androgen excess in women of reproductive age, is a multifaceted, dynamic and clinically heterogenic disorder. Rotterdam 2003 ESHRE/ASRM definition criteria were recently reinforced at the NIH Consensus Meeting 2012. Concomitant identification of the clinical phenotypes of the syndrome is mandatory in medical care and clinical studies, as these are strongly related to reproductive, cardiovascular and metabolic outcomes. Documentation of polycystic ovarian morphology (PCOM) is challenging, with the AE/PCOS Task Force 2014 suggesting a threshold of ≥25 follicles/ovary in 18-35 years old women when using high-frequency transducers. Elevated levels of total testosterone and/or free testosterone and/or low sex hormone-binding globulin (SHBG) stand for androgen excess in women, as stated by the ESE Position Statement 2014. Despite evidence of increased metabolic and cardiovascular risk, increased prevalence of cardiovascular events linked to PCOS status per se is still insufficient documented, mainly because of the clinical heterogeneity of studies populations and lack of prospective data. First-line therapy in the medical management of PCOS is metformin, at least 1.5 g/d, in all patients with documented insulin resistance and hyperinsulinemia. According to Endocrine Society Guidelines 2013, other insulin-sensitizers (e.g. thiazolidinediones) raise safety concerns on the long-term, whereas statins need further evaluation to demonstrate their benefits in the treatment of PCOS, however, are indicated in dyslipidemic patients. Anti-androgens and combined oral contraceptives (COC) are targeting androgen excess, particularly in non-insulin resistant patients, with an overall benefit to risk ratio in PCOS favoring benefits.

**Key words:** polycystic ovary syndrome, androgens, testosterone, insulin resistance, endothelial dysfunction, cardiovascular risk.

**INTRODUCTION**

Polycystic ovary syndrome (PCOS) clusters, variably, a complex group of symptoms and signs including androgen excess, chronic oligo/anoovulation, insulin resistance and dysglycemia, and obesity and further associates increased risk of non-alcoholic steatohepatitis (NASH), sleep apnea syndrome, vascular calcifications, mental health disorders and endometrial neoplasia. Etiopathogenetically, PCOS cannot be explained by a unifying theory, intervention of both genetic and environmental factors being hypothesized. It is a dynamic clinical condition, in the sense that dominating reproductive symptoms in young-aged patients switch to a cardio-metabolic and neoplastic high-risk clinical pattern in peri- and postmenopausal women (1).

PCOS definition has been a constant matter of controversy. While the presence of androgen excess is mandatory with National Institutes of Health (NIH) 1990 and Androgen Excess/Polycystic Ovary Syndrome (AE/PCOS) 2006 diagnosis criteria, the broader inclusionary diagnostic criteria of Rotterdam European Society for Human Reproduction (ESHRE)/American Society of Reproductive Medicine (ASRM) 2003 accept the combination of anovulation and polycystic ovarian morphology (PCOM) as diagnosis criteria of PCOS. Use of Rotterdam criteria in the definition of PCOS was recently reinforced at the NIH Consensus Meeting 2012 (2), while excluding of other causes of androgen excess and specifically mentioning the PCOS clinical phenotype represent prerequisites for the diagnosis.

**PCOM or PCOS?**

Ultrastructure studies of the PCOS ovary showed years ago that increased theca cells thickness is a PCOS trait, being, in addition, the main source of androgen excess. Abnormally high LH results in theca cells hyperplasia and augmented androgen biosynthesis but androgen excess is also non-LH-mediated, via insulin resistance and hyperinsulinemia, which lower sex hormone-binding globulin (SHBG) liver synthesis and insulin-like growth factor binding protein (IGFBP)-1.
liver and ovarian production, respectively, increasing free androgens on one hand and enhancing IGF-1 bioavailability on the other hand. Up-regulation of ovarian IGF-1 receptor by insulin allows stimulated sex steroid synthesis and increases aromatase activity at the PCOS ovary level thus increasing androgen production (Fig. 1). Moreover, an intrinsic abnormality of the PCOS ovary is suggested by the observation that PCOS ovary theca cells cultured in vitro persistently secrete significantly higher amounts of androgens compared to theca cells cultures derived from unaffected ovarian tissue (3). The intrinsic abnormality of theca cells could have a genetic basis as suggested by a very recent study that shows that PCOS theca cells overexpress an alternatively spliced form of the DENND1A gene (DENND1A.v2), which encodes a protein (connecden1) associated with clathrin-coated pits that secrete androgens. Connecden1 may stimulate CYP17A1- and CYP11A1-dependent steroidogenesis in theca cells (4).

To the clinician, an ovarian volume ≥10 cm³ or a 2-9 mm diameter follicle number per ovary (FNPO) of at least 12 certifies the polycystic ovarian morphology. However, these criteria seem to be obsolete given the ability of high-frequency (>8MHz) ultrasound transvaginal transducers to visualize follicles below 2 mm diameter; in a recent task force report from the AE/PCOS Society setting the threshold for FNPO at ≥25 follicles in women aged 18-35 years was recommended (5).

The anti-müllerian hormone (AMH) is a product of granulosa cells that reflects the greater number of ovarian follicles. AMH represents another indicator of polycystic ovarian morphology and elevated AMH levels are found in PCOS patients, to the highest degree in PCOS patients with oligo/anovulation, particularly the amenorrhoeic PCOS phenotype; to a lower degree, serum AMH is elevated in healthy women with PCOM (6-9). When comparing the performance of transvaginal ultrasonography against the AMH level in the diagnosis of PCOS, in women with either oligo-anovulation or hyperandrogenism as a sole manifestation, a FNPO ≥...
PCOS evaluation and management

20 and/or serum AMH >5ng/ml define PCOS whereas positive ultrasound or AMH criteria in the absence of dysovulation or androgen excess rather suggests PCOM and not PCOS (10).

Polycystic ovarian morphology is not specific to PCOS; it can be present in up to 25-30% of cycling, non-hyperandrogenic women and has been also reported in various clinical conditions (Table 1). On the other hand, the PCOM pattern fades with age, particularly after menopause (1).

Table 1. Main clinical conditions associating polycystic ovarian morphology (PCOM)

<table>
<thead>
<tr>
<th>Clinical condition</th>
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<tr>
<td>- Eugonadal women (25-30%)</td>
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<td>- Polycystic ovary syndrome</td>
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<tr>
<td>- Functional hypothalamic amenorrhea</td>
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<tr>
<td>- Hyperprolactinemia</td>
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<tr>
<td>- Hypothyroidism</td>
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<tr>
<td>- Type 1 Diabetes mellitus</td>
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<tr>
<td>- Congenital adrenal hyperplasia</td>
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<tr>
<td>- Androgen-secreting tumors</td>
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<tr>
<td>- Cushing’s syndrome</td>
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<td>- Severe Insulinresistance</td>
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Assessment of Androgen Excess in PCOS

The poor accuracy and sensitivity of traditional immunoassays to assess testosterone in the low range has been advocated as the cause of total testosterone as a poor hyperandrogenism marker in women. Total testosterone levels may be elevated in PCOS but there is important overlap between PCOS and healthy women. High-accuracy, gold standard techniques measurements of steroid hormones are mass spectrometry (MS)-based. Gas chromatography (GC)-MS is very accurate for steroids measurements but needs complex sample processing and long assay run times; liquid chromatography (LC)-MS/MS implies simple pre-assay sample preparation for precise measurement, with the drawback that this is a relatively expensive options in comparison to traditional immunoassays.

In a population of healthy women, diagnostic agreement between LC-MS/MS and radioimmunoassay in the measurement of circulating androgens was evaluated and it was shown that, of all androgens, total testosterone determined by immunoassay exhibited the highest predictive value (11). Therefore, one can say that measurement of total or salivary testosterone using a high-quality double antibody radioimmunoassay with variation coefficients below 10% appears to be a reasonable option for clinical routine in the investigation of androgen excess in women.

When comparing the free androgen index (FAI) with total testosterone and androstendione, in a series of women who addressed a reproductive medicine clinic, Barth et al. (12) clearly demonstrated for FAI the highest area-under-the-curve (AUC) in the diagnosis of PCOS. Compared to total testosterone, calculated levels of free testosterone, in the form of either FAI or Vermeulen’s formula represent a more sensitive marker of ovarian androgen excess in women.

Sex hormone-binding globulin is a valuable parameter that tests low in PCOS; it not only allows calculation of free testosterone concentration but also provides a surrogate marker for insulin resistance. Based on testosterone and SHBG measurements, the European Society of Endocrinology (ESE) Special Interest Group recommends a paradigm for identifying hyperandrogenism in women (9).

About 60-80% of PCOS cases present with elevated testosterone levels whereas increased serum dehydroepiandrosterone (DHEA)-sulphate or androstendione, each, may represent the sole androgen hormones abnormality in about 10% of patients. Simultaneous measurement of multiple hormone compounds by LC-MS/MS gives us a picture on the so-called steroid profiling in PCOS and reveals concomitant elevation of testosterone, androstendione, 17-hydroxyprogesterone and 3b-hydroxydehydrogenase, the enzyme converting DHEA into androstendione (11). Basal and corticotrophin-stimulated 17-hydroxyprogesterone and 11-deoxicortisol is recommended to 21-hydroxylase or 11-beta hydroxylase deficiency, respectively, when congenital adrenal hyperplasia is suspected.

Cardio-Metabolic Risk and Cardiovascular Disease in PCOS

Based on Rotterdam definition criteria, four clinical phenotypes are described in PCOS, that are differently related to cardio-metabolic and reproductive PCOS outcomes. Insulin resistance and highest cardiovascular risk seem to characterize the classic PCOS phenotype, associating hyperandrogenism (HA), anovulation (AO) and PCOM, and to a lesser extent the hyperandrogenic PCOS phenotypes, HA+AO and HA+PCOM. Metabolically, the non-hyperandrogenic phenotype of PCOS (AO+PCOM) is similar to non-PCOS women. Infertility is most severe in the anovulatory phenotype (AO+PCOM), with additive effects of elevated androgen levels and insulin resistance.

The prevalence of obesity is difficult to be established due to lack of representative population-based data but it is estimated around 49% (9). Obesity,
particularly the abdominal phenotype, may precede PCOS development; it is presumed that obese PCOS associate more severe metabolic and reproductive phenotypes (1).

PCOS patients are at increased cardiovascular risk, irrespective of age or body mass index. Impaired flow-mediated vasodilation of the brachial artery (FMD) is a constant finding in PCOS. In a case-control evaluation, we were able to find three-fold lower values in both lean and obese young PCOS women and, in agreement to that, about three-fold higher endothelin-1 concentrations were noted in the study (13, 14). In a meta-analysis of observational studies, low FMD was confirmed in PCOS (15). While FMD marks changes of the function of the vascular endothelium, the intima-media thickness of the carotid artery (CIMT) gives information of the endothelium structure. Several observational studies show increased CIMT in PCOS and recent meta-analyses favor increased CIMT in PCOS (16) underpinning subclinical cardiovascular disease.

Increased prevalence of aortic and/or coronary artery calcifications has been reported in PCOS by relatively few observational studies, even in relatively young, premenopausal subjects (17). Very recently, a prospective cohort study indicates PCOS status and fasting glucose as significant risk factors for coronary artery calcifications (18). We have shown that osteoprotegerin (OPG), which is known to inhibit development of vascular calcifications (19), is decreased in PCOS women and its changes are positively correlated to concentrations of free testosterone (20, 21). Surprisingly, OPG is inversely related to vascular injury (21), probably as a protective mechanism, as newer data show that OPG stabilizes the atherosclerotic plaque.

Despite clearly increased cardiovascular risk, evidence of increased prevalence of cardiovascular events remains elusive, mainly due to lack of prospective population-based studies and phenotypic heterogeneity of the study populations. In the Women’s Ischemia Syndrome Evaluation study, quartiles of free testosterone predict cardiovascular events; postmenopausal PCOS women have shorter cardiovascular event-free survival compared to those without PCOS (22). Two recent meta-analyses including almost similar study populations reported significantly increased risk of stroke and coronary heart disease in PCOS patients, irrespective of the body mass index (23, 24). Still, many studies are biased by the fact that PCOS women are more obese, insulin resistant and dyslipidemic in comparison to controls, all of these attributes increasing cardiovascular risk. On the opposite, a small, prospective clinical study failed to demonstrate increased number of cardiovascular event in PCOS patients versus controls (25).

A consensus statement of the AE/PCOS Society published in 2010 (26) and reinforced by the Endocrine Society Guidelines 2013 on PCOS (27) recommended cardio-metabolic risk staging in PCOS based on American Heart Association (AHA) criteria in at risk and at high-risk categories (Fig. 2 and Table 2). Parameters to be obtained from PCOS patients to allow staging are waist circumference, insulin resistance, blood pressure measurement, a lipid profile, and depression and life quality questionnaires.

**Table 2. 75g-Oral Glucose Tolerance Test (OGTT)**

<table>
<thead>
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<th>Indication</th>
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<tr>
<td>Body mass index ≥30 kg/m² (≥25 kg/m² in Asian women)</td>
</tr>
<tr>
<td>Body mass index &lt;30 kg/m² and age &gt;40 years or familial history of type 2 diabetes or history of gestational diabetes</td>
</tr>
<tr>
<td>Acanthosis nigricans*</td>
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</table>

* ±HbA1c

$repeat every 2 years or yearly if abnormal

#ESHRE/ASRM Consensus Workshop 2012 (1)

**Management of Endocrine and Cardio-Metabolic Abnormalities in PCOS**

Management of cardio-metabolic complications in PCOS is challenging and needs to be individualized to the phenotype and other factors such as age or the desire for pregnancy.

Life-style measures are mandatory in obese PCOS patients by calorie-restricted diets and exercising...
however they are not sufficient in normal weight patients. Advanced Glycation Endproducts (AGE)-rich food that results by grilling, roasting and frying should be avoided. There is evidence of elevated AGE levels in PCOS women; moreover, these levels are related to dysovulation thus being considered as a potential pathogenetic factor in PCOS (28). Bariatric surgery may represent a therapeutic option in morbidly obese women (body mass index ≥ 50 kg/m²), in PCOS patients with body mass index ≥ 35 kg/m² and metabolic complications (i.e. type 2 diabetes) as well as in obese (body mass index ≥35 kg/m²), anovulatory PCOS women that fail to respond after 6 months of conservative treatment (29, 30).

According to guidelines, metformin, a traditional oral anti-diabetes drug is a first-line treatment in insulinresistant and hyperinsulinemic PCOS patients as illustrated by Figure 3. In a 2.8 years follow-up of incidence of diabetes in a large, randomized group of pre-diabetic subjects, metformin was able to decrease the incidence of diabetes by 31% (31). In PCOS patients, metformin exerts add in insulin-lowering “effects” in non-obese but particularly obese subjects with best results obtained in women with metabolic syndrome; indirectly, metformin has androgen-lowering effects (32). The more pronounced insulin resistance and compensatory hyperinsulinemia, the more efficacious is metformin not only in lowering insulin levels but also in promoting weight loss and alleviating dyslipidemia (33-35). There is plenty of evidence of metformin favorably influencing cardiovascular risk in PCOS, in monotherapy (36) and combined to oral contraceptives as well (37). According to 27, metformin is not recommended as first-line treatment in PCOS ovulatory dysfunction but may act beneficial as pre- or combination therapy with clomiphene citrate.

Pioglitazone, a thiazolidinedione, administered to hyperinsulinemic versus normoinsulinemic PCOS patients improves glucose uptake and effectively reduces insulin, which indirectly results in decreased testosterone (38), although a direct inhibition of steroidogenesis by thiazolidinedione’s cannot be excluded. Pioglitazone may alleviate anovulation in obese PCOS women that failed to respond to metformin (39); in summary, it may represent an alternative to metformin or can be used in combination with metformin in selected cases of PCOS patients, paying attention to its tolerance profile (27).

The use of glucagon-like peptide (GLP)-1

![Figure 3. Medical management of insulinresistance and androgen excess in PCOS (27, 42, 43).]
receptor agonists’ exenatide and liraglutide in PCOS is in its infancy, however, promising effects on weight loss have been obtained in obese PCOS receiving liraglutide either in monotherapy or combined to metformin (40). Statins (simvastatin, atorvastatin) lower testosterone levels in observational studies (41) but long-term effects in PCOS are not determined and current recommendation is to prescribe statins in women with PCOS who meet current indications for statin therapy (27).

Low-dose combined oral contraceptives alleviate clinical hyperandrogenism and menstrual irregularities and represent a therapeutic alternative, particularly in non-insulinresistant PCOS. Avoidance of nor-testosterone derivatives is preferred. Cyclical micronized progesterone or progesterin treatment may be used in women not needing contraception (9). Despite potential adverse effects of combined oral contraceptives by promoting insulin resistance and increasing vascular and thromboembolic risks, overall benefits of combined oral contraceptives appear to be greater than risks in PCOS patients (27).

Anti-androgens (42, 43) (Fig. 3) should always be used in combination with contraceptive methods to avoid fetal male pseudo-hermaphroditism and as the lowest efficacious dose, paying attention to the safety profile.

In conclusion, in the evaluation and management of PCOS patients following main steps have to be completed: measurement of both total testosterone and SHBG and calculation of free testosterone to assess testosterone excess; carefully documentation of polycystic ovarian morphology by transvaginal ultrasonography and, optionally, AMH measurement; definition of PCOS clinical phenotype; evaluation of metabolic and cardiovascular risk according to current guidelines; adjustment of the medical management to the clinical phenotype, age and desire of pregnancy of the patient.

Conflict of interest

The author declares that he has no conflict of interest concerning this article.

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