A comprehensive approach in diagnosing the polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is the commonest hyperandrogenic disorder in women that, by definition, may present with different phenotypes, including the classic forms and those with a milder presentation. Its diagnosis is mainly based on careful clinical judgment, although it may require additional investigation by blood testing or imaging techniques in the differential diagnosis of androgen excess. This article summarizes the most important aspects of the diagnostic procedure and suggests how to apply them in clinical practice.

Keywords: androgens • infertility • insulin resistance • obesity • polycystic ovary syndrome • Type 2 diabetes

Current criteria to define polycystic ovary syndrome

The polycystic ovary syndrome (PCOS) is the commonest hyperandrogenic disorder in women and one of the most common causes of anovulatory infertility. Its prevalence ranges from 6 to 10%, with different rates according to ethnicity and geographical areas [1]. The first description by Stein and Leventhal in 1935 [2] summarized all major characteristics of women with PCOS, including infertility, signs of androgen excess and typical ovarian morphology. As astute surgeons of that era, Stein and Leventhal performed abdominal surgery on these women and an unexpected benefit of this surgery was the resumption of menstrual cycles, and even pregnancies in some of these patients. Many decades later, the definition has been readdressed several times by a number of consensus conferences, aimed at achieving agreement on diagnostic criteria. In 1990 the NIH established that the presence of hyperandrogenism and chronic oligoamenorrhea (which implies chronic oligoanovulation) should be used as major criteria to diagnose PCOS, provided other causes of hyperandrogenism (such as classic congenital adrenal hyperplasia [CCHAH], Cushing’s syndrome, hyperprolactinemia and androgen-secreting neoplasms, or treatment with anabolic steroids) have been appropriately excluded [3]. The inclusion of polycystic ovarian morphology (PCOM) by ultrasound as a further potential criterion to define the syndrome was proposed by the Rotterdam consensus conference [4], which definitively reviewed the original findings by Stein and Leventhal. However, the decision by the Rotterdam consensus expanded the potential number of different phenotypes describing PCOS, since it was decided that at least two of the major criteria (namely oligo- and/or an-ovulation, clinical and/or biochemical hyperandrogenism and PCOM) were required for the diagnosis [4]. Undoubtedly, according to the Androgen Excess and PCOS Society (AEPCOS) it should be considered that androgen excess, either clinical or biochemical or both, represents the fundamental criteria to define PCOS [5].

An important issue is embodied by the fact that PCOS aggregates other pathological conditions such as insulin resistance, obesity, dyslipidemia and low-grade inflammation. These comorbidities may disrupt the phenotype and may also favor the susceptibility to
develop, later in life, Type 2 diabetes (T2D) and, possibly, cardiovascular diseases (CVDs) [6]. Finally, PCOS by itself may also have some genetic component, as documented by familial aggregation and recent genetic studies [7].

The PCOS & its different phenotypes
Conceptually, any syndrome may present with different phenotypes, according to the presence/absence of the main criteria included in the definition. PCOS represents a typical example of a syndrome presenting with different phenotypes, on the basis of the combination of the criteria mentioned above. The presence of metabolic comorbidities confers additional complexity in the definition of the phenotype in each individual patient. This may have some importance in the decision-making procedure to define the proper treatment and a follow-up strategy. A recent workshop at the NIH-US held in December 2012 [8] recommended maintaining the broad, inclusionary diagnostic criteria of the Rotterdam criteria while specifically identifying the major phenotypes on the basis of the presence of: androgen excess and ovulatory dysfunction; androgen excess and PCOM; ovulatory dysfunction and PCOM; and androgen excess and ovulatory dysfunction and PCOM. In addition, it was also suggested that major metabolic disorders should be addressed in the clinical workup while defining the PCOS in each individual patient. In fact, particular attention should be paid to the presence of excess weight or obesity, particularly during childhood and adolescent years. In fact, an early increase in bodyweight may specifically favor testosterone excess and menses abnormalities in adolescent girls [9]. In addition, there is sufficient scientific evidence that obesity may exacerbate the PCOS phenotype, particularly ovarian dysfunction and androgen excess, other than insulin resistance and compensatory hyperinsulinemia [10]. Both the Amsterdam consensus conference [11] and position statements by the European Society of Endocrinology [12] suggested that major metabolic disorders should be addressed in the clinical workup while defining the PCOS phenotype in each individual patient.

Another important issue may be represented by severe insulin resistant states, that are not so uncommon in women with PCOS and may require specific diagnostic procedures (e.g., genetic assessment) and treatment. Partial lipodistrophy is a typical example of these rare disorders, which may in fact present with metabolic abnormalities, a phenotype of PCOS and severe insulin resistance states [12]. Massive obesity may be associated with a secondary form of PCOS, since massive weight loss may totally overcome the phenotype of the disorder [12].

It is worthy of mention that PCOS is a lifelong disorder [10], however, most women may change their phenotype with increasing age [12] and in most cases clinical signs may partially or totally disappear. This may depend, among other factors, on the previous treatment (particularly, estrogen–progestin compounds, dieting or insulin sensitizers), on any weight loss and on changes in lifestyle behavior. In these cases, clinical history can help the clinician to define the appropriate diagnosis and, hopefully, the proper treatment. On the other hand, it should be considered that the criteria used to define PCOS intrinsically relate to fertile women. The specific phenotype of PCOS in postmenopausal women is still poorly defined. However, some of the available reports imply that PCOS dismetabolic sequelae continue postmenopause [12], namely an increased prevalence of cardiovascular risk factors, although more recent prospective studies do not support these alarming findings [14].

The most important aspect showing the vulnerability of women with PCOS regarding metabolic derangements is T2D. The current perspective is that these women may develop alterations of glucose metabolism, such as impaired glucose tolerance (IGT) and T2D over the years [14]. This susceptibility is highly associated with the presence of obesity, particularly the abdominal phenotype [15]. Available studies have shown that the incidence of T2D may be 2–5-times higher than in the general population [15,16] and that obesity may represent the most important predictive factor. In fact, studies performed in normal weight women with PCOS failed to indicate any increased evolution from normoglycemia to T2D, although occasional cases have been reported [14].

How to approach the patient
Major concerns of women with PCOS in asking for the doctor’s help are represented by hirsutism, abnormal menses and anovulation, infertility, or obesity. During adolescence, menses irregularities and/or the discovery of altered ovarian morphology (PCOM) are the most common causes of concern. Women with signs and symptoms suggesting PCOS may therefore consult different specialists, including dermatologists, gynecologists or endocrinologists. However, it has been shown that endocrinologists may be particularly interested in hirsutism and androgen excess, whereas menses abnormalities and chronic anovulation together with PCOM may attract the gynaecologist’s attention, since they may primarily focus on ovarian dysfunction, as shown by a study performed in Australia comparing the perspectives of gynecologists and endocrinologists involved in PCOS diagnosis and treatment [17]. A recent paper focused on a survey on European endocrinologists [18] showed that their choices may depend on the fact that they pay particular attention to the treatment of androgen excess
and metabolic disorders, as well as to the prevention of T2D and cardiovascular diseases. On the other hand, it is important to emphasize that there are no substantial different opinions in the diagnosis of PCOS between the Endocrine Society Guidelines [18] and the position paper published on behalf of the European Society of Endocrinology [12]. As expected, most ecologists are more focused on ovarian dysfunction (menses and anovulation), whereas endocrinologists are more focused on hormonal issues and metabolic alterations [9]. In any case, these aspects imply that, based on the individual needs of each patient, cooperation between ecologists and endocrinologists might improve the achievement of therapeutic benefits, particularly in the long term.

In any case, relevant clinical aspects should be investigated according to age, these being: if the patient has clinical hyperandrogenism (hirsutism, acne or alopecia); if there are potential signs and symptoms suggesting an alternative cause of androgen excess; if she has irregular menses, amenorrhea and anovulation; if she has problems of infertility; if excess weight or obesity are present; if metabolic alterations are detectable; if psychopathological aspects are present and quality of life is compromised; and, finally, if a familial genetic background should be investigated.

Past medical history should also include information on prior pelvic surgery, and a complete history of previous therapies must be documented, including topical treatments for hirsutism and acne and, most importantly, oral contraceptives (type of progestins, how long and potential side effects, among others). Finally, a list of all cosmetic therapies is necessary for the interpretation of physical findings. A simplified diagnostic flow-chart for PCOS is depicted in Figure 1.

How to identify women at risk for PCOS

It should be emphasized that PCOS is a diagnosis by exclusion, so it is mandatory that all known causes of androgen excess should be carefully evaluated by a detailed clinical approach and if necessary by appropriate diagnostic tests. On the other hand, the clinician should also consider all potential factors representing a risk for a woman of having PCOS. Family history of clinical hyperandrogenism should be investigated (see ‘Family history’ section), since a familial trait may be present. During adolescence, the presence of severe menses irregularities alone should be considered as a potential feature of PCOS, independent of the presence of hyperandrogenemia or dermatological signs of androgen excess. This aspect will be discussed in a subheading included in the ‘Evaluation of menstrual irregularities & ovulatory disorders’ section. Early pubarche should also be considered as a potential early sign of PCOS, although in many girls it represents a distinct clinical entity [12]. Another potential condition risk is represented by the onset of excess weight or obesity. In fact, it is well known that PCOS features may appear together with increasing body fat, particularly during adolescence [12,18], and that this is often associated with the appearance of menses irregularities. In these cases, low birth-weight and a subsequent exaggerated catch up can be identified [12]. All these aspects should be considered in the diagnostic workup independent of the reasons for the consultation by the patients.

Clinical picture of PCOS

The clinical evaluation should include a complete medical history and physical examination, and arguments regarding differential diagnosis. Most patients do not meet the strict criteria for diagnosis of PCOS mentioned above, and careful examination, blood tests and other diagnostic procedures are therefore required. In addition, doctors should pay particular attention to metabolic disorders and excess bodyweight, since they may represent a potential target for therapeutic intervention.

Evaluation of menstrual irregularities & ovulatory disorders

Menstrual irregularities and ovulatory disorders should be primarily evaluated according to the patients’ age. In fact, age is an important factor in the clinical evaluation of women suspected of having PCOS. In the perimenarcheal phase, adolescent women may in fact exhibit a transient state of anovulation and irregular menses. Therefore, making a correct clinical diagnosis of ovarian dysfunction may be difficult. Two decades ago, Apter and coworkers [20] found that, by using sequential progesterone measurements in adolescent girls, more than 80% of cycles are anovulatory during the 1st year after menarche, 60% during the 3rd and 25% after the 6th year are still anovulatory. Notably, the same authors found that anovulatory otherwise normal menstruating adolescent girls are often characterized by elevated total testosterone and LH levels [21]. These findings were confirmed in a more recent study providing liquid chromatography plus mass spectrometry (LC-MS/MS)-based, menstrual phase-specific reference intervals for the circulating androgen profile in young females, showing that a subgroup of anovulatory otherwise normal late adolescent females were characterized by elevated total testosterone and LH levels [19]. In the absence of clinical hyperandrogenism, it cannot be excluded that even ‘physiological’ anovulation after menarche may be due to an incomplete level of maturation of the hypothalamic-pituitary-ovarian axis leading, in turn, to increased androgen production [20]. On the other hand, this might represent an early phase of a potential susceptibility to develop PCOS later in life, particularly in those develop-
Flow chart in the diagnostic algorithm

Clinical presentation and physical examination

General

Personal history
- Include family history
- Obtain detailed information on weight history, dietary habits, lifestyle, etc.
- Obtain information on early signs/symptoms and of previous pharmacological and cosmetic treatment
- When appropriate, investigate psychological aspects and quality of life (by questionnaires)

Physical examination:
- Perform anthropometry
- Check for acanthosis nigricans

Criteria to define PCOS

In the presence of hyperandrogenism, investigate all potential causes of androgen excess

History of menses and definition of menses alteration
Exclude secondary causes of anovulation
Score hirsutism (or acne, or alopecia)

Check for anovulation,
Perform a progestin test, if needed

Perform ovarian ultrasound

Blood samples to measure total testosterone, androstenedione, SHBG and FAI

Define the specific phenotype of PCOS and other relevant clinical features and comorbidities

Define the therapeutic strategy, based on individual needs

Figure 1. Flow chart algorithm for the diagnosis of polycystic ovary syndrome.

FAI: Free androgen index; PCOS: Polycystic ovary syndrome; SHBG: Sex hormone binding globulin.

In the presence of hyperandrogenism, the increase in LH blood levels reflects altered LH pulsatility, with an increased number and amplitude of the hormone pulses [20]. Interestingly, it has been found that in adolescents with disordered LH pulsatility and increased testosterone blood levels these alterations may persist in many of them, whereas in others they may totally recover [23]. Currently, no clear guideline criteria to diagnose PCOS during adolescence exist, therefore specific attention on the diagnostic conclusions is required, and a careful follow-up should be recommended to evaluate persistent oligomenorrhea or amenorrhea as a potential early clinical sign of PCOS, especially when it persists 2 years beyond menarche [18].

In adult women, menses abnormalities and chronic anovulation are important criteria in the diagnosis of PCOS, although some women with hyperandrogenic symptoms may have regular menstrual cycles. In those with severe oligomenorrhea or amenorrhea, chronic anovulation is usually present, whereas occasional ovulation may occur in women with mild-to-moderate oligomenorrhea. Most young adult women may have a long term history of the use of oral contraceptives (OCs), often with different preparations, and this may mask or delay the recognition of menstrual dysfunction or hyperandrogenic symptoms. Obviously, in the presence of recent unexplained amenorrhea, pregnancy should be excluded by appropriate testing [18]. Moreover, weight- and exercise-related causes, hyperprolactinemia and subclinical or overt thyroid dysfunction, all causes of premature ovarian failure (particularly in young women), should be investigated.

Evaluation of clinical hyperandrogenism

Clinical hyperandrogenism (hirsutism, acne or alopecia) represents a relevant cornerstone in the diagnostic work up [24]. The onset of hirsutism often occurs during adolescence and may worsen in time. The evaluation of hirsutism can be performed by the modified Ferriman and Gallway score [25,26]. Although it became very popular, being easy, convenient, cheap and fast, it has been shown to present large interobserver variability [27]. Its reliability may therefore be questioned, particularly in patients with borderline representation of hirsutism. In addition, the cutoff values are probably inappropriate, and how to interpret predominant facial hirsutism, the most distressing concern for many women, still represents a matter
of interpretation [28]. The cutoff values should obviously be interpreted according to race and ethnicity. At present, it has been suggested that the cutoff value to define whole body hirsutism should be 8 or above, whereas a cutoff of 3 or above is suggested for far-east Asian women, who rarely develop hirsutism [29]. Specific metabolic pathways of testosterone in the air follicles may be involved to explain the distinct features in Asian women populations [29].

Acne may be a typical manifestation of hyperandrogenism during adolescence and, rarely, in adult age. In some cases there is a family history of acne. The typical acne lesions vary in increasing order of severity. Androgenic alopecia is relatively less frequent, particularly in adolescent age. Androgenic alopecia may be graded by well known subjective methods, such as the Ludwig score [30].

Virilizing symptoms are very rare in women with PCOS; however, they should be investigated. Their presence (particularly increased muscle mass, deepening of the voice or clitoromegaly) may suggest an underlying ovarian or adrenal neoplasm, or a classic form of previously undiagnosed congenital adrenal hyperplasia [5].

Evaluation of infertility
PCOS is a typical condition of anovulatory infertility. However, in some cases, even the presence of PCOS does not rule out other pathological conditions, such as tubal factors or endometriosis. A careful evaluation of infertility factors should be performed with the help of a gynecologist or a reproductive endocrinologist. In any case, specific attention should be paid to the presence of excess bodyweight and metabolic abnormalities, specifically insulin resistance. In fact, obesity has been found to significantly reduce fertility rates in women with PCOS [31]. It is important to point out that ovulation rates after appropriate treatment with clomiphene citrate (CC) or exogenous gonadotropins are significantly reduced in women with PCOS, but also that in many cases they can be improved by weight loss achieved by lifestyle intervention programs with or without insulin sensitizers (specifically metformin) [12,18]. Their effectiveness seems to improve after a period of treatment of at least 6–12 months [32]. An increased rate of miscarriages or early pregnancy loss has been documented in patients with PCOS, particularly in the presence of obesity [31].

Evaluation of excess weight & obesity
Excess weight and obesity are very common in women with PCOS. For many years it was suggested that their prevalence was significantly higher in the PCOS population with respect to the general population [33]. In general, there is no doubt that there is a strong association between excess bodyweight and PCOS, and some evidence that the prevalence of PCOS may increase with increasing BMI has been reported [34]. On the other hand, recent studies provided some evidence of referral bias in the PCOS phenotype, primarily driven by obesity and the severity of disease burden, and that women with PCOS seeking clinical help generally have a more severe PCOS phenotype and are more obese [35]. Therefore, a more accurate picture of the association between PCOS and obesity might arise from the studies where PCOS is detected through the screening of unselected or minimally biased population.

What we actually know is that obesity, particularly the abdominal/visceral phenotype, worsens the metabolic and also the reproductive features of PCOS [36]. An interesting concept from available studies is that androgen excess might be responsible for visceral fat tissue enlargement in women [37]. Accordingly, there are convincing data that support a role for testosterone excess in the development of adipose tissue and muscle insulin resistance [36,37]. In addition, it is important to outline that obesity is the major risk factor for the development of T2D in women with PCOS [14]. Finally, it is worth mentioning that incoming studies in the last few years have shown that the adipose tissue of women with PCOS has aberrant morphological and functional characteristics [38], and that chronic inflammatory factors [39] are likely to be involved in these processes. This area of research seems to support the potential role of androgen excess in determining the association between excess body fat and PCOS.

From the clinical point of view, obesity may have profound negative effects on the clinical features of PCOS, including a likelihood to present with a more severe hyperandrogenic phenotype and more severe menstrual disturbances [34,35]. Among others, one of the factors responsible for increased testosterone levels in an obese PCOS woman is represented by a decrease in hepatic production rate and blood levels of the sex hormone-binding globulin (SHBG), which, in turn, may favor an increased availability of the free testosterone fraction in the target tissues [37]. Therefore, the androgen profile can be further negatively affected in PCOS women by the presence of abdominal body fat distribution [37].

In adolescent girls, weight gain often precedes the onset of menses abnormalities [12,18], therefore a careful weight history should be recorded, including potential factors associated with or responsible for weight gain. In this context, major stressful events should also be investigated, regardless of whether they precede weight loss or, frequently, weight gain. Finally, previous dietary treatments or eating disorders should also be investigated. Birthweight and subsequent catchup should also be recorded, since there are convincing data supporting that these very early events may predispose to the devel-
opment of obesity later in time \[12,18\]. Definitely, these data can help to understand the pathophysiology and development of obesity and PCOS.

An anthropometric assessment should always be performed in all women, particularly in those with excess weight, by simple measures such as BMI and fat distribution by waist and hip circumferences, according to the standard rules \[40\].

**Dietary habits & food intake**

Dietary habits may play a role in the development of the PCOS phenotypes. Useful information can be obtained with the help of dieticians or using standardized questionnaires. Available studies have shown that an excess in energy and particularly fat intake is not uncommon in PCOS patients, although the data are still controversial \[12\].

By contrast, a potential role of advanced glycation end-products (AGEs) has been suggested as playing a potential role in the pathophysiology of insulin resistance and ovarian dysfunction in PCOS \[41\]. Diet is an important source of AGEs and other oxidants. There are studies providing evidence that AGE blood concentration in PCOS women may be significantly higher than in the control population \[41\]. In addition, it has been shown that a reduction in dietary AGE intake is associated with parallel changes in serum AGEs, and with an improvement in metabolic, hormonal and oxidative stress biomarkers in women with PCOS \[42\]. An accurate evaluation of dietary habits may have some relevance in the treatment of PCOS women with excess bodyweight, since lifestyle intervention may significantly improve body composition, metabolic abnormalities and possibly ovarian dysfunction \[43\].

**Psychological aspects & quality of life**

Women with PCOS may have a reduced quality of life and psychological disorders which in turn may have significant implications on the quality of life \[12\]. These conditions may be specifically related to both clinical signs of androgen excess as well as to menses abnormalities and infertility. Anxiety and depression have been shown to be relatively common in these women, and infertility and excess weight or obesity are specifically associated with these psychological disorders. Both long-term OCs use \[44\] and weight loss \[45\] have been shown to improve psychological disturbances and quality of life. A specific questionnaire has been developed for PCOS women that involves analysis of emotions, hirsutism, menstrual problems, infertility and excess bodyweight \[46\]. Although there are very few available studies, it appears that similar psychological profiles exist not only in the classic phenotypes of PCOS but also in those with milder forms of the disorder \[12\], suggesting that psychological aspects and quality of life should be investigated in all women with PCOS.

**Sleep disorders**

Sleep disorders can be associated with PCOS, particularly but not exclusively in obese women. Some studies have shown that PCOS women may have an increased risk of the obstructive sleep apnea syndrome (OSAS) \[47\], which, in turn, may be strongly associated with insulin resistance \[48\]. This disorder should be diagnosed either by questionnaires or by overnight polysomnography \[47\].

**Insulin resistance, metabolic syndrome, T2DM & risk for CVD**

Women with PCOS may have a positive family history for T2D, obesity and CVDs. As reported above, insulin resistance is a common finding in all overweight or obese women with PCOS and in approximately two-thirds of those who are normal weight \[49\]. The presence of abdominal fat distribution and acanthosis nigricans are phenotypic biomarkers of insulin resistance, dysmetabolic disorders and, therefore, of cardiovascular risk. Due to their increased risk for developing T2D in adult age \[14\], a history of glucose intolerance during pregnancy should also be investigated, since gestational diabetes is a risk factor for T2D \[50\]. This risk can be increased especially in women with a first degree relative with T2D \[18\].

Women with PCOS should also be investigated for the presence of nonalcoholic fatty liver disease, a benign condition of ectopic fat deposition, and nonalcoholic steatohepatitis. In fact, there is evidence that their prevalence is significantly increased in women with PCOS \[18\]. In these cases, liver dimensions may be increased and can be determined by physical examination.

Finally, there is increasing evidence that PCOS status is a condition of increased risk for cardiovascular diseases \[12,18,51\]. The dysmetabolic milieu, chiefly insulin resistance, impaired glucose intolerance states and lipid disorders and obesity, particularly the abdominal phenotype (all clustering the metabolic syndrome). There is also evidence that in women with PCOS hypertension is significantly more prevalent than in non-PCOS women, independently of age, BMI or the presence of T2D \[12\]. In addition, it has also been shown that the nocturnal drop in blood pressure may be lower even in obese adolescents with PCOS \[12\]. Additional nonclassic risk factors that have been found to be significantly associated with PCOS status, particularly androgen excess by itself \[51\], are impaired coagulation, anatomical and functional endothelial injury and vascular dysfunctions (such as increased carotid artery intima-media thickness), and a state of subclinical inflammation \[12\]. However, in spite
of a large amount of literature supporting an increased risk, very few data support an increased prevalence of cardiovascular events in PCOS, and no long-term longitudinal studies are available at present. By contrast, The Nurses’ Health Study noted an adjusted relative risk of 1.53 for coronary heart disease in women with a history of menses abnormalities [52]. In addition, clinical features of PCOS have been associated with more angiographic coronary artery disease among postmenopausal women investigated for suspected ischemia [53].

**Family history**

Family history may have an important role in the clinical approach of PCOS. In fact, an increased risk of PCOS in sisters and daughters of women with PCOS has been documented. Familial hirsutism, acne, menstrual irregularity, obesity and T2D are all potential factors suggesting a risk of developing PCOS [12]. It should be remembered that the same signs and symptoms may also indicate disorders such as nonclassic congenital adrenal hyperplasia (NCCAH).

**Laboratory analyses**

**Serum androgens**

Although the diagnosis of hirsutism does not necessarily reflect high circulating androgen levels, the investigation of androgen blood levels is mandatory to define PCOS. It is important to acknowledge that the current assay performance of analytical methods is relatively imprecise and their specificity and accuracy may be poor, particularly in the common range of androgen levels in females, which is lower than 1 ng/ml [54]. By contrast, modern technologies, such as liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) display good precision, sensitivity and high accuracy [55–57]. LC-MS/MS has recently been introduced in many laboratories and in the next few years it is expected that its use will become widespread around the world.

Testosterone has been accepted as the major androgenic biomarker to define biochemical hyperandrogenemia [4,5]. We recently produced reference values for many androgens measured by LC-MS/MS, in large and well defined groups of healthy normal late adolescent (aged 16–19 years) [22] and premenopausal [58] women. In these women (n = 133), testosterone blood levels, a key biomarker of hyperandrogenemia in women with PCOS, never exceeded 0.55 ng/ml, whereas in postmenopausal women (n = 53) the highest values were 0.45 ng/ml. Reference values for androstenedione did not exceed 2.2 ng/ml and 1.0 ng/ml, respectively. Finally, in healthy, normal-weight ovulatory late-adolescent girls (Tanner stage 4–5) we found that the low and high reference limits in the follicular phase were 0.124 ng/ml (0.102–0.148) and 0.438 ng/ml (0.398–0.482), respectively, for testosterone, and 0.393 ng/ml (0.323–0.469) and 1.546 ng/ml (1.381–1.727) for androstenedione [22].

Free testosterone evaluation can be useful to establish hyperandrogenemia. Although there are assays available for its measurement, they should not be used due to their poor reliability [55]. Therefore, calculated free testosterone, based on the ratio between testosterone and SHBG blood levels, should be preferred [59]. In women with PCOS, SHBG blood levels are invariably lower than normal, particularly in the presence of excess body fat. This is primarily due to the inhibiting effect of high circulating insulin on its hepatic preproduction [37,49]. The decrease in SHBG leads, in turn, to increased availability of unbound testosterone to target tissue. This is because elevated insulin levels (a frequent concomitant of PCOS) and elevated androgen levels both act to inhibit hepatic production of SHBG. Thus both low SHBG and increased testosterone production result in elevated free testosterone levels. Measurement of androgens should also be performed to exclude any other potential disorders responsible for a hyperandrogenic phenotype, which is required to diagnose PCOS. Interested readers can refer to a recent overview of the diagnostic procedures needed to achieve this goal [12].

**Other biochemical tests**

Since lipid abnormalities are very common in women with PCOS, monitoring of fasting lipids is prudent. In addition, an oral glucose tolerance test should be performed in the presence of risk factors for T2D and in obese women with PCOS [18,50]. Although indices of insulin resistance can be obtained by the ratio between glucose and insulin blood concentration, in both fasting and after a glucose tolerance test [50], it should be considered that they are relatively inaccurate on an individual basis [60]. In the presence of normal fasting glucose values, fasting insulin levels can however predict insulin resistance by approximately two thirds when measured by the clamp technique [50].

**Ovarian ultrasound morphology & anti-Müllerian hormone**

By definition, according to the current diagnostic guidelines [4] the evaluation of a typical PCOm by ultrasound (possibly transvaginal) is required to complete the full diagnostic workup. It should be noted that this largely depends on available technologies and subjective evaluation by the operator, therefore the collaboration of a skilled gynecologist is often needed. In the last 20 years, the consensus for the diagnosis of PCOS suggested a threshold to define PCOm by the presence of 12 or more follicles of 2–9 mm in diameter [4]. Recently, given the exciting improvement in resolution
by modern technologies, it has been defined that the most accurate way to define PCOM in clinical practice should be represented by the evaluation of follicle number per ovary with a threshold at ≥25, provided new technologies are used, whereas increased ovarian volume should be evaluated if such technologies are unavailable [61].

Serum anti-Müllerian hormone, measured by an appropriate assay, might be a more sensitive and specific biomarker of the ovarian dysfunction typical of PCOS [62]. However, a threshold value for its use as a surrogate marker of PCOM has not yet been defined.

**PCOS after menopause: a still undefined problem**

The definition of PCOS after menopause still represents an unresolved challenge, due to the disappearance of menses and ovulation. Undoubtedly, the transition to postmenopause involves a marked decrease in estrogen production by the ovaries, whereas androgens are still synthetized [10]. Using the LC-MS/MS technique [58], we defined that in 65 normal-weight (BMI: 23.2 ± 1.7) postmenopausal women, aged 45–76 years, blood levels of testosterone ranged from 0.077 to 0.392 ng/ml (median: 0.147 ng/ml) and those of androstenedione from 0.095 to 0.773 ng/ml (median: 0.299 ng/ml). These data confirm previous old findings by radioimmunoassay or immunenzymatic methods, supporting that androgen blood levels are slightly reduced after menopause because of a decreased ovarian steroidogenesis.

In addition, it should be recognized that there are very few data on ovarian morphology of PCOS women after menopause. One study found that approximately half of PCOS women may still present ultrasound features consistent with PCOM, and that these women have higher testosterone blood levels than their counterpart with normal ovaries had [62]. Much more effort should be dedicated to identifying appropriate criteria to define PCOM after menopause.

**Physical examination**

**Anthropometry**

Height and weight should be measured to calculate BMI (kg/m²). Body fat distribution can be assessed by measuring waist and hip circumferences. In fact, waist alone and the waist-to-hip ratio (WHR) are good predictors of abdominal (visceral) fat enlargement [40]. In addition, they may add additional information as to the cardiovascular risk profile for individual women. In addition to truncal obesity, a buffalo hump and supraclavicular fat deposition may suggest the presence of Cushing’s syndrome.

**Dermatological manifestations**

Hirsutism, acne and androgenic alopecia should be investigated in all patients with PCOS using available scores, as described in the ‘Evaluation of clinical hyperandrogenism’ section. It is mandatory that hirsutism is distinguished from hypertrichosis, the excessive growth of androgen-independent hair which is vellus, prominent in nonsexual areas, and most commonly familial or caused by systemic disorders (hypothyroidism, anorexia nervosa, malnutrition due to other long-term eating disorders, porphyria and dermatomyositis) or medications (phenytoin, penicillamine, diazoxide, minoxidil or cyclosporine) [24]. As reported above, it has been pointed out that hair growth on the face may be more relevant than in other parts of the body [24,28]. On the other hand, this requires more specific research. In many cases the help of a dermatologist should be advocated, being much more confident with more extensive diagnostic methods, including pulling and weighing hairs in a defined region, standardized photographs and assessing hair density in defined regions of the scalp.

Other dermatological findings that should be investigated include seborrhea, acanthosis nigricans and striae, thin skin or bruising, which suggests possible Cushing’s syndrome. Acanthosis nigricans is particularly relevant in the clinical evaluation of PCOS [49]. It is quite common, particularly in obese women with PCOS and in those with severe insulin resistant states. It can be found on the nape of the neck and in the axillary region, and sometimes in other parts of the body (elbows, folds of the skin, hands, etc.). Notably, acanthosis nigricans represents a skin marker of insulin resistance and the metabolic syndrome. It should be considered that skin examination by a clinician may be relatively insensitive for detecting acanthosis nigricans, as documented by a study comparing clinical staging with histological examination [63].

**Reproductive system**

A complete reproductive system examination should be conducted at the time of diagnosis. The breast exam should include a check of galactorrhea or pathologic masses. The presence of clitoromegaly should also be investigated. The examination should also verify that the internal genitalia (vagina, uterus and ovaries) are present. Pelvic ultrasound may assist in the physical examination and, therefore, in the diagnosis of PCOM, as summarized in the ‘Ovarian ultrasound morphology & anti-Müllerian hormone’ section. Most of these aspects should be investigated with the help of a gynecologist.

**General**

Arterial blood pressure should always be measured, and a careful investigation of the cardiovascular sys-
From the practical point of view, it should be borne in mind that the diagnosis of polycystic ovary syndrome (PCOS) is a diagnosis of exclusion; therefore, other causes of hyperandrogenism including hyperprolactinemia, drugs (danazol and androgenic progestins, and valproate), nonclassic congenital adrenal hyperplasia, Cushing’s syndrome and androgen secreting tumors (ovarian or adrenal) should always be ruled out by appropriate blood testing and imaging techniques. On the other hand, a skilled clinician may approach the diagnosis of PCOS based only on medical history and a clinical perspective.

Conclusion & future perspective

In the years to come, it is hoped that doctors will improve their skills in identifying the different phenotypes of PCOS, which may require a specific therapeutic approach. The incoming era of LC-MS/MS will provide more sensitive values of blood androgen levels, which is expected to considerably improve the diagnosis of hyperandrogenemia in women with PCOS and, in particular, much more attention should be paid to sensitive areas of the body, such as the face and the trunk.

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Executive summary

Diagnosis of polycystic ovary syndrome

- From the practical point of view, it should be borne in mind that the diagnosis of polycystic ovary syndrome (PCOS) is a diagnosis of exclusion; therefore, other causes of hyperandrogenism including hyperprolactinemia, drugs (danazol and androgenic progestins, and valproate), nonclassic congenital adrenal hyperplasia, Cushing’s syndrome and androgen secreting tumors (ovarian or adrenal) should always be ruled out by appropriate blood testing and imaging techniques.

The need to identify different phenotypes of PCOS

- Since the diagnosis of PCOS may be applied to different phenotypes, an effort should be made to identify the specific phenotype affecting the patient, since this may have an important impact while planning the therapeutic strategy.

The definition of hyperandrogenism

- According to the current diagnostic criteria, hyperandrogenism can be defined by either the presence of hirsutism and/or biochemical hyperandrogenemia. However, not all women with PCOS are hirsute and not all have higher than normal testosterone or calculated free testosterone. The quantification of hirsutism is largely subjective and its proposed cutoff values of the modified Ferriman and Galwey (mF-G) score are based on inadequate scientific basis. There are studies showing no correlation between basal testosterone and hirsutism score measured by both radioimmunoassay (RIA) or LC-MS/MS and the hirsutism score. In addition, commonly used immunoassay systems may lack accuracy in the evaluation of testosterone levels at the usual female blood concentrations, often yielding values higher than those obtained using gas chromatography coupled with mass spectrometry or liquid chromatography plus mass spectrometry.

The role of obesity

- Particular emphasis should also be given to the presence of excess weight or obesity, and to the pattern of fat distribution, particularly when its onset has occurred during adolescence. In fact, excess body fat can have an important role in the pathophysiology of PCOS and associated metabolic comorbidities, particularly Type 2 diabetes.

The follow-up is strategic in women with the classic PCOS phenotype

- It should be considered that the existing literature supports the concept that women with the classic phenotype are more prone to manifest metabolic abnormalities, particularly in adult age. These women should therefore be followed up for a long period, in order to prevent the development of metabolic comorbidities, such as Type 2 diabetes and, possibly, cardiovascular diseases, later in life.
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