

Polycystic Ovary Syndrome: Features, Diagnostic Criteria and Treatments

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorder that affects 6–15% of the female population. It is a very complex syndrome that involves the hypothalamus, the pituitary gland, the ovaries, the adrenal gland and the peripheral adipose tissue that together contribute to create a generally imbalance, associated with three characteristic symptoms: oligo-anovulation, hirsutism and infertility. PCOS has been studied over a prolonged period of time and yet is still not fully understood. In 2003, in Rotterdam, the European Society for Human Reproduction and Embryology (ESHRE) together with the American Society for Reproductive Medicine (ASRM), drafted a consensus document that, gathering the conclusions discussed and compared of various experts works, established a worldwide standard diagnostic criteria for the Polycystic ovary syndrome. Since then, other workshops focused on summarizing know-hows on PCOS by identifying gaps in various aspects. Most symptoms of PCOS first appear in adolescence, around the start of menstruation. However, some women do not develop symptoms until early to mid-20's. Although PCOS presents early in life, it persists through and beyond the reproductive years. This manuscript focuses on summarizing the current knowledge on PCOS, underlying features, diagnosis and treatment.

Keywords: Polycystic ovarian syndrome; Hyperandrogenism, hirsutism, hyperinsulinemia

Polycystic Ovary Syndrome

Definition and diagnosis

Polycystic ovary syndrome (PCOS) is one of the most common female endocrine disorders [1]. The difficulty in diagnosing PCOS is related to the intrinsic characteristics of the syndrome: the heterogeneity of the symptoms, their variability in different age ranges, the lack of overlapping biochemical parameters and shared cut-off useful in clinical practice [2]. PCOS is, in fact, a very complex disorder that involves the hypothalamus, the pituitary gland, the ovaries, the adrenal gland and the peripheral adipose tissue creating an imbalance associated with three characteristic symptoms: oligo-anovulation, hirsutism and infertility [3]. Unfortunately, as the onset of PCOS is the result of a complex series of the alteration of physiological mechanisms, the symptoms and clinical signs of this disease are not always evident, as already Stein and Leventhal described in 1935. They identified the syndrome on the increased dimension of the ovary, thickening of the capsule and the presence of multiple follicular cysts covered by abundant stroma [4]. Even then, however, the disorder appeared heterogeneous. On 7 patients with bilateral polycystic ovaries and oligo/amenorrhea, 4 were obese, 5 were hirsute (one obese), 1 long-limbed and having acne [4]. The coming of ultrasound and in particular transvaginal probe has revealed that the response of the micropolycystic ovaries doesn't necessarily imply a diagnosis of PCOS, since patients with normal ovaries present clinical symptoms and

laboratory features of PCOS, and vice versa [5,6]. In 1990, in Bethesda, as a result of the Conference of the National Institute of Health/ National Institute of Child Health and Human Development (NIH/ NICHD), anovulation and hyperandrogenism were established as criteria for PCOS diagnosis [7-10]. PCOS is mainly diagnosed on the exclusion of androgen excess or ovulatory disorders with clearly defined causes: the 21-hydroxylase deficiency is observed in non-classic adrenal hyperplasia (NCAH) and affects 1-10% of hirsute women [11]; Cushing's syndrome with hyperandrogenic characteristics [12], adrenal carcinomas, ovarian tumors and other adrenal androgen-secreting tumors present in 1:300-1000 androgenic women; exogenous anabolic drugs, whose prevalence is unknown, but it is generally present only in the minor androgenization [13]. Many authors also exclude disorders that may result in ovulatory dysfunction, such as thyroid dysfunction and hyperprolactinemia or hypothalamic amenorrhea, whose prevalence is in the order of 1 to 3% [14-17]. Other features present in women with PCOS, were then reported, such as obesity (found in 30-60% of patients, depending on the ethnic differences) [18], insulin resistance and hyperinsulinemia (present in 50-70%) [19-23], and the ratio between the Luteinizing hormone and the follicle-stimulating hormone (LH/FSH) >2 or 3 (frequency of 30-50%) [24-29]. In 2003, in Rotterdam, the European Society for Human Reproduction and Embryology (ESHRE) together with the 'American Society for Reproductive Medicine (ASRM), drafted a consensus document that, gathering the conclusions discussed and compared of various experts works, established a

worldwide standard diagnostic criteria for the Polycystic ovary syndrome. According to the Rotterdam Criteria, the PCOS diagnosis can be made in patients that present at least 2 of the 3 following criteria:

Oligo-anovulation; hyperandrogenism (clinical or biochemical); presence of 12 or more follicles with a diameter of 2 ± 9 mm in each ovary, and/or increased ovarian volume (>10 ml).

Even in this case all those disorders characterized by androgen excess or ovulatory disorders with clearly recognized causes must be excluded. On the basis of these criteria it is possible to identify four main PCOS phenotypes:

Hyperandrogenism, chronic anovulation, polycystic ovaries (PCOS classical phenotype I)

Hyperandrogenism, chronic anovulation and morphologically normal ovaries (PCOS classical phenotype II); Hyperandrogenism, polycystic ovaries, the presence of ovulatory cycles (ovulatory PCOS); Chronic anovulation, polycystic ovaries, no signs of hyperandrogenism (PCOS normoandrogenica).

In 2004, the Expert Commits of Androgen Excess PCOS Society (AEPCOS) considered more correct to include only the first three phenotypes thus implying the necessity of the presence of hyperandrogenism for the definition of PCOS [9].

Diagnostic sonographic criteria

Stein and Leventhal described PCOS hallmarked by oligomenorrhea or amenorrhea, infertility and the presence of cystic ovaries, first identified at laparotomy and then histologically confirmed at biopsy [4,30]. With the advent in the 1980s of high-resolution and real-time scanners, more discrete criteria for PCOS were established. Transabdominal 2-D ultrasound (TAUS) has largely been superseded by (TV) scanning because of greater resolution and in many cases patient preference [31]. The transabdominal route is of course required in girls and women who are virgo intacta or for patients who decline a transvaginal scan. A transabdominal scan offers a panoramic view of the pelvic cavity, and may be useful if there are associated uterine or ovarian developmental abnormalities. By the way, transvaginal technique doesn't require fullness bladder and performs the examination in less time [32]. Transvaginal approach allows greater resolution and provides a more accurate view of the internal structure of the ovaries especially in obese women. The high-frequency probes (>6 MHz) can be used in virtue of the proximity of the ovaries and uterus to the vagina and the relief of ultrasound undergoes less interference from the adipose tissue. However the sonographic criteria for polycystic ovary diagnosis has never found unanimous consent, even if an increased volume of the ovaries compared to normal ovaries are considered the main features of the polycystic ovary [33,34]. The ultrasonographic examination allows to evaluate both external (volume or area) and internal (number of follicles, stromal echogenicity) ovary aspects.

External aspects

Volume: According to the Rotterdam Consensus criteria, an ovarian volume $>10\text{cm}^3$ is diagnostic of PCO. According to recent studies it is more appropriate to lower the limit to 7cm^3 in order to have a greater sensitivity of the examination. In fact, the limit of 10cm^3 has a specificity of nearly 100%, but a sensitivity lower than 50% [35]. To assess the ovary volume it is necessary to measure its maximum

diameter on the following parameters: longitudinal, transverse and anteroposterior. Traditionally, the ovarian volume (OV) is calculated using ellissoide formula: $\pi/6 \times D1 \times D2 \times D3$, D1, D2 and D3 being the three maximal longitudinal, antero-posterior and transverse diameters respectively. Whereas these criteria guarantee a specificity of 98%, it does not offer the best sensitivity [36].

Ovarian Area: In 2-D US ovarian area (OA) is calculated by using the formula $\pi/4 \times \text{length} \times \text{width}$ (area of an ellipse), by adapting an ellipse to the ovary, whose area is calculated from ultrasound or by hand drawing the ovarian outline, automatically calculating the area below the line. Although OA is less frequently used in research protocols, it has better diagnostic power than those OV. The limit for this parameter is 5cm^2 [37].

Internal aspects

Number of follicles. To evaluate the number of follicles each ovary is scanned in cross section by the interior edges to the external ones in order to achieve to the total number of cysts/follicles. The number of follicles should be estimated on two levels of the ovary so as to calculate its dimension and position. According to the Rotterdam Consensus criteria polycystic ovary should contain 12 or more follicles of 2-9 mm in diameter. This is a useful parameter to distinguish PCO cases from multi ovarian follicular (MFOs), a transitory condition generally associated to delayed puberty, hyperprolactinemia, hypothalamic anovulation, amenorrhea related to weight [10]. The multifollicular ovary is morphologically characterized by a number of follicles, lower than PCO (between 6 and 10), distributed throughout the ovary and by the absence of hypertrophy of the stroma. The presence of multiple follicles is due to an incomplete stimulation of the Gonadotropin-releasing hormone (GnRH) follicular development. Moreover, unlike what occurs in PCOS, patients suffering from ovarian multifollicular show normal levels of LH and T, but reduced levels of FSH. Generally, the ovary resumes its normal aspect after therapy and/or change of body weight [38].

Stroma: volume and echogenicity

Stromal echogenicity: The increased echodensity of the polycystic ovary is a key histological feature [39] but is a subjective assessment that may vary depending upon the setting of the ultrasound machine and the patient's body habitus. Stromal echogenicity has been described in a semi-quantitative manner with a score for normal (=1), moderately increased (=2) or frankly increased (=3) [40]. Echogenicity has been quantified by one group [41] as the sum of the product of each intensity level (ranging from 0 to 63 on the scanner) and the number of pixels for that intensity level divided by the total number of pixels in the measured area. They have found that women with PCOS had greater total ovarian volume, stromal volume and peak stromal blood flow compared with normal ovaries, yet mean stromal echogenicity was similar. The stromal index (mean stromal echogenicity: mean echogenicity of entire ovary) was higher in PCOS, due to the finding of a reduced mean echogenicity of the entire ovary [42]. Increased stromal echogenicity is due to either increased stromal volume alongside either reduced echogenicity of the multiple follicles. Ovarian volume correlates well with ovarian function and is both more easily and reliably measured in routine practice than ovarian stroma. Thus, in order to define the polycystic ovary, neither qualitative or quantitative assessment of the ovarian stroma is required [36].

Hyperandrogenism

One of the most immediate common symptoms of PCOS is the excess of androgens [43]. In animals it was possible to reproduce a syndrome PCOS similar through exposure in utero to an excess of androgens [44]. It's therefore correct to acknowledge that the clinical and biochemical features of PCOS may be the consequence of genetically determined hypersecretion of androgens secreted by the ovary during early childhood or puberty [45]. One of the mechanisms that seems to be the basis of hyperandrogenism in PCOS is the hypersecretion of LH, accompanied by normal or reduced levels of FSH. The LH, that in physiological conditions is the main regulator of the production of androgens by the ovarian theca, tends to be produced in larger amounts, especially for increased amplitude and frequency of its secretory peaks, and changes in its glycosylation [46]. Due to the increase in the production of LH and the concomitant presence of normal or reduced levels of FSH, the LH/FSH ratio is greater than 2.5 [47]. Pituitary sensitivity to acute stimulation with GnRH never reveals deficient or absent answers, but mostly normal, or exaggerated in 25-30% of cases. This would indicate a retained and sometimes increased sensitivity on part of the LH-secreting cells [48].

Ovarian and adrenal hyperandrogenism

The detectable hyperandrogenism in PCOS is not only determined by a defect in ovarian steroidogenesis, but involves a second "disintegration" mechanism of ovarian level and adrenal gland. We know that the P450c17 is a key enzyme in the regulation of hormone synthesis, and is the only known enzyme capable of converting the precursors of C21 in the pre-steroid hormones, that is the 17-ketosteroids. It presents, in fact, a double catalytic activity: 17-hydroxylase and 17,20-ligase [49]. Its regulation is a significant factor in the expression of hyperandrogenism [50]. The secretion of androgens in the ovary is LH dependent, while in the adrenal gland it is Adrenocorticotrophic hormone (ACTH) dependent [49]. The androgen response to each of these two hormones seems to be modulated by internal autocrine and paracrine tissue mechanisms. The insulin, Insulin-like growth factors (IGFs) and inhibin are counted among the many growth factors capable of increasing the cellular response to the action of LH and ACTH [51]. In particular, it has been shown that the insulin/IGF system stimulates the gene expression of P450c17 and the activity of the enzyme both at ovarian and adrenal gland level. However, experimental evidence shows that an alternative androgen biosynthetic pathway exists [52].

Ovarian hyperandrogenism

Studies on ovarian theca hormone production show that patients with PCOS have an increase in ovarian production of progesterone and androgens. This observation has directed interest in studying the CYP11a gene, a key gene in the metabolism of steroids, as its product (cytochrome P450sc), catalyzes the first step in the synthesis of steroid hormones [53]. Recent studies have confirmed that the expression of this gene increases in the ovarian theca cells of women with PCOS compared to those of the control group, as a positive correlation between the presence of certain polymorphisms of the gene and the presence of PCOS [54].

Diagnostic criteria in hyperandrogenism in PCOS

The diagnosis of hyperandrogenism is performed through laboratory investigations, by looking for increased serum levels of

androgens, or through clinical examination, by looking for signs of hyperandrogenism, like hirsutism, even in the presence of normal levels of androgens in the blood [55].

Biochemical parameters: In patients with PCOS it is possible to detect high levels of all androgens, from the most powerful (Testosterone T; Dihydrotestosterone DHT, 5-Androstenediol D5-A-diol), to the weakest (4-Androstenediol D4-A; Dehydroepiandrosterone DHEA, Dehydroepiandrosterone sulfate DHEA-S). The levels of Estrone (E1) are increased, those of Estradiol (E2) are normal or reduced by a decreased follicular production with an inversion of the E2/E1ratio. The SHBG levels are reduced. The selective catheterization of the ovarian and adrenal veins, as well as test stimulators and inhibitors specific for adrenal and ovary, have revealed that the adrenal gland is the main source of DHEA and DHEAS, while the major contribution to the production of T, D4-A and 17-hydroxyprogesterone (17-OH-P) comes from the ovary, in agreement with the high enzymatic activity of the P450c17 at the theca cells level [56]. The quantity of free testosterone (T) or the index of free T (free androgen index, FAI) are the most sensitive methods to assess the hyperandrogenemia [57]. Only 30-50% of patients with PCOS have elevated levels of adrenal androgens and only a small fraction can have an isolated elevation of dehydroepiandrosterone sulfate (DHEA-S), without showing, however, the suppressibility to dexamethasone and stimulation to ACTH, typical in adrenogenitalica syndrome. The increase in the LH/FSH ratio, generally >2.5, is present in many but not in all women with PCOS. A normal value of this ratio does not therefore exclude the diagnosis of this disease [58]. Finally, in recent years the use of diagnostic tests involving GnRH show an exaggerated production of 17-OH-progesterone due to a dysregulation of 17-lyase (cytochrome p450). However it is not a highly specific test and it is certainly impractical to perform [59].

Clinical parameter: The skin is an organ target of androgens. On the skin are localized specific receptors for these hormones and enzymes capable of metabolizing testosterone and its secondary products [60]. In particular, the pilosebaceous unit, the structure of the skin that contains the sebaceous gland and hair follicle, is the more sensitive structure to the action of androgens. As a result of the binding of these hormones to the receptors in the skin, the pilosebaceous unit is stimulated and such stimulation produces sebum with possible development of acne and hairs. Therefore, the skin manifestations of hyperandrogenism in women is seborrhea, with concomitant development of acne, ipertrocosi, or hirsutism [61]. In most important cases of hyperandrogenism, these clinical symptoms are extremely evident and are accompanied by the presence of alopecia, increased muscle mass, clitoromegaly, changes in the voice tone and other organs and tissue manifestations that are affected by the action of androgens [62].

Physiology pilosebaceous unit

The pilosebaceous unit is a skin structure that contains two distinct functional units, the sebaceous gland and hair follicle, and it is strongly controlled by the action of androgens [63]. The sebaceous gland is greatly stimulated during sexual maturation by androgens, and, in fact, the increase in sebum production is one of the clinical signs during prepuberty. The hair follicle is the structure where the hair formation occurs. In men and women, there are two types of hair: the pixie hair (or fleece) and the terminal hair. The pixie hair is characterized by a very small diameter and the absence of pigmentation, while the terminal hair is thicker, harder, pigmented and generally longer than

the pixie hair. The pixie hair is typical in prepubertal age. In this period of life only eyelashes, eyebrows and hair are terminal type (non-sexual hair that is not under the control of sex hormones) [63]. During sexual maturation, due to androgens action, in certain areas, the pixie hairs turn into terminal hairs: in female, this happens in the pubic zone and underarms; in male, in addition to the previous, face, trunk and limbs (sexual hair). Although head hair is terminal non-sexual hair (not dependent on androgens), it can be influenced by the increase (or post-pubertal changes in steroid metabolism) in plasma concentrations of testosterone, increment that can lead in both sexes to the involution of the hair as from terminal hair to pixie hair and later can cause atrophy of the hair follicle (male and female androgenic alopecia). In addition to this general genetic type determination (belonging to the female sex) the location, density and type of hair follicles are also determined by breed and by individual genetic features. However, generally, woman hair terminals are only present in eyelashes and eyebrows as well as in axillary hair, pubic hair and the scalp; in the rest of the body they're pixie type. Disregulation in the production of androgens in various etiology, or increased sensitivity to their action at the level of skin receptors, can cause the transformation of the pixie hair into terminal hair [63].

Hypertrichoses

Hypertrichoses, indicates the increase of sexual hair (terminal hair) and amount of hairs (pixie hair) in women where physiologically present, or non-sexual hair present on the forearms, legs and lumbosacral areas. The increased hair indicates a higher density of hairs per area unit [64].

Hirsutism

Hirsutism is defined as the development of male pattern terminal hair growth in women and affects approximately 5-8% of the population [14,65]. It is often associated with androgen excess disorders including non-classic adrenal hyperplasia, androgen secreting tumors, and polycystic ovary syndrome (PCOS) [14,66]. Family history and physical exam are particularly important in evaluating excess hair growth in women, because there is no absolute clinical distinction between physiologic and pathologic hirsutism. For clinical evaluation and diagnosis, it is important to have a standardized system to evaluate hirsutism [65]. The most widely used scoring system was first developed by Ferriman and Gallwey in 1961, and involves subjective tabulation of terminal hair growth in various areas of the body [67]. The original Ferriman-Gallwey system involved the scoring of 11 body areas including the lip, chin, chest, upper abdomen, lower abdomen, upper arm, forearm, thigh, lower leg, upper back, and lower back. This was later modified (i.e. the modified Ferriman-Gallwey or mF-G method) to include only nine body areas, excluding the forearm and lower leg as these areas were found not to correlate with androgen excess. In the mFG scoring system, each individual body area is visually scored on a scale of zero to four, where zero indicates no terminal hair growth and four indicates full male pattern terminal hair growth [65]. However, this system has its limitations. First, it involves a detailed, full body exam assessing and scoring all nine body areas [68] which may be considered invasive by many patients and is cumbersome when performing epidemiologic studies. For these reasons, it would be prudent to identify a simpler and more widely applicable screening system for hirsutism. Cook et al. have recently identified a simpler and widely applicable screening system for hirsutism. They have indicated that the best single body area

predictor of total body hirsutism is the lower abdomen. Using only the lower abdomen as a predictor, 55% of the variability in the data set is explained ($p < 0.001$). This study has examined all subset combinations of the nine body areas included in the mFG scoring system, and has found that the best single body area correlating to the full mFG score is the lower abdomen. They have demonstrated in two large cohorts that examination of only the chin and abdomen is a simple and reliable screening method for detecting hirsutism. Furthermore, reducing the number of body areas assessed may increase the willingness of study participants or patients to be evaluated. [69].

Virilization

The term virilization indicates a complex clinical picture due to the presence of an important hyperandrogenism. It involves besides the skin, other organs and tissues targets of androgens. At skin level it is possible to identify a strong hypertrichosis and hirsutism, seborrhea and/or acne, while in a general symptom context it is possible to identify androgenetic alopecia, increased muscle mass, clitoromegaly (clitoral index above 35 mm²), changes in the tone of the voice (for hypertrophy of the larynx), breast reduction, modification of fat deposits in the body as male (reduction of deposits in the gluteal-femoral level and increase at the thoraco-abdominal), together with disturbances of the sexual cycle. Virilization is generally indicative of androgen-secreting tumors [70].

Seborrhea and acne

Seborrhea, overproduction of sebum, is for the woman not only an aesthetic problem (shiny skin, oily, greasy), but also a predisposing factor to the most annoying and pathological disorder, such as the presence of comedones, development of acne vulgaris or other types of infectious diseases of the skin [71]. The overproduction of sebum is due to abnormal stimulation of the sebaceous glands induced by overproduction of androgens. This alteration, however, could also be the result of an increased sensitivity of the skin receptors to male hormones, or to an increase of androgen metabolism in the skin (increased transformation of testosterone into dihydrotestosterone) [71]. Functional alteration of the sebaceous gland is also the basis for the development of acne. Acne (acne vulgaris) is a cutaneous condition featuring pathological occlusion of the pores and skin with consequent inflammation and pus formation inside the pore itself. It is usually localized to the face and trunk area [72] The sebaceous glands secrete lipids, mainly triglycerides, fatty acids, cholesterol and cholesterol esters, squalene. After hormone stimulation, you can attend to an overproduction of secreted and/or changes in its chemical composition leading to hyperkeratosis of the excretory duct with congestion of sebum within the duct itself. The result is the formation of comedones (point-like skin bumps made of the material that obstructs the glandular orifice). The following transfer of free fatty acids as a result of the action of esterase produced by *Corynebacterium acnes* (a bacterium normally present within the excretory duct) on the neutral fats of the product leads to a perifocal glandular inflammation of the surrounding tissues and the consequent formation of acneic pustule. Actually acne has a multifactorial complex etiology and not yet fully understood. In addition to the influence of androgens, thyroid and genetic predispositions have a defined role. In particular, the hereditary predisposition to acne seems to be polygenic or dominant with variable penetrance. It occurs in less than 1-3 women with PCOS. Unlike androgenic alopecia and hirsutism, women's main problem

with acne is an increase in sebum production while serum levels of androgens often remain unaffected [72].

Depression

PCOS is a multifaceted disorder with multiple potential risk factors and a great impact on the lives of women affected. For this reason it is not surprising that a high percentage of women with PCOS also report symptoms of mood disorders like depression, anxiety and bipolar disorder [73]. Even though anxiety and depression often coexist, most of the past research on mood in PCOS is limited to prevalence of depression [73,74] with few data on prevalence and severity of anxiety or severity of depression [75]. These mental health differences are noted across the lifespan, including adolescents, and across the different PCOS phenotypes [76]. In a recent meta-analysis, studies that measured anxiety and depression in women with PCOS, listed in Pubmed and Medline published up 2010, were identified and compared. Results have highlighted that women with PCOS tend to experience mildly elevated anxiety and depression, significantly more than control groups [77]. These differences are slightly lower when both groups have a similar BMI, suggesting that mood in PCOS might be improved to some degree through weight control. In fact it was not surprising that both BMI and PCOS were significantly associated with depression as it has been found in previous research [78]. In a recent study was found that the low glycemic index diet has some advantages for women with PCOS and it was also evidenced that hypocaloric diets significantly led to reduced body weight and androgen levels in women with PCOS [79]. Moreover also physical activity could be an effective therapeutic option for the reproductive and metabolic features of PCOS. Even if the specific interaction between physical activity and mental health has not been explored in depth in PCOS, preliminary data including a recent clinic-based study of women with PCOS, have evidenced that physically inactive women suffer from higher depression scores than physically active women, and that there is an association between lower physical activity and mild depression [80].

Insulin Resistance

Insulin resistance is another metabolic characteristic frequently associated with PCOS [81]. This metabolic defect is attributable to a reduced functionality of the insulin receptor due to hyper-phosphorylation of serine residues of the same receptor by a serine/threonine kinase. This hyper-phosphorylation diminishes ligand affinity, with the result of the attenuation of the endocrine hormone signal and the onset of the insulin-resistance condition. It is essentially the same mechanism that, acting at the level of cytochrome P450c17, induces ovarian and adrenal hyperandrogenism. Only one molecular mechanism is then able to explain two of the major disorders of PCOS [81].

Hyperinsulinemia

Excessive pancreas insulin production is often present in PCOS and a serious risk factor in the onset of non-insulin dependent diabetes (NIDDM). Excessive activity of the pancreas beta cells does not depend on obesity and is not generally associated with reduced glucose tolerance. Hyperinsulinemia would provide a significant contribution to premature arrest of follicle growth, characteristic occurrence of anovulation in PCOS, and the interaction of insulin with LH would be a key element in this process [82].

Insulin and the regulation of steroidogenesis

Indirect stimulation mechanisms of the production of androgens: It has been shown that insulin has an active role in amplifying the stimulated production of androgens by the LH at a theca cell level. This would explain the prevalence of hyperandrogenic symptoms in obese subjects with PCOS [83].

From data obtained in vitro by stromal tissue from normal women and women with PCOS, it has also been demonstrated that the potentiating response of insulin on LH is much greater in the tissues of women with PCOS compared to those of normal women [84]. A second mechanism which alters the normal values of androgen depends on the reduction of plasmatic protein binding sex hormone (sex hormone binding globulin or SHBG). SHBG is a protein produced by the liver and acts as blood conveyor of sex hormones. Obviously, as more the hormone molecules are bound to this protein, as smaller is the concentration of the "free" and physiologically active hormone. The effect would precisely be due to the blockage of the synthesis of SHBG. This reduction raises blood levels of free testosterone, by increasing its bioavailability [85].

Direct mechanism of stimulation of the production of androgens: Besides positively modulating the action of gonadotropins in the ovary, amplifying the steroidogenic signal, insulin has a direct stimulatory effect on steroidogenesis in granulosa cells, both in normal and in the polycystic ovary. These actions appear to be mediated by insulin binding to two different receptors, specific receptors present in the ovary and IGF-I receptors, the latter having a structure similar to the insulin receptor [86]. The use of the PCOS insulin-sensitizing drugs such as metformin and troglitazone has highlighted that the improvement in insulin sensitivity follows a significant reduction of basal concentrations of testosterone (total and free), as well as an increase in blood levels of SHBG, especially in obese women with PCOS [87,88]. The hypothesis of the presence of a defect in the transduction signal of insulin has focused attention on the function of the second messengers of insulin signals, such as IPG (inositol fosfo glycans). In particular, studies show that a deficiency of myo-inositol can underlie, besides a non-insulin response, also a reduced answer to ovarian FSH, being the myo-inositol the second hormone messenger predisposed to follicular growth [89,90]. Insulin acts as a real gonadotropic hormone in the ovary. It is, in fact, an important factor in the regulation of ovarian androgen synthesis and metabolism and, therefore, the hyperinsulinemia may be responsible, at least in part, of hyperandrogenism in women with PCOS.

Diagnostic Criteria of Insulin Resistance in PCOS

Biochemical parameters

The assessment of hyperinsulinemia status is not always practicable through the evaluation of simple basic insulin. More accurate results are obtained from stimulation tests which assess the values of blood glucose and insulin in response to the glycidic stimulus administered (orally - OGTT, or intravenously - IVGTT). The insulin response is considered index of a metabolic problem when, already in the first two, three minutes of execution of the analysis reveals a response equal or greater than 50 ng/ml. In patients with PCOS, it is frequent that an increase response to the test occurs already in the first phase (60 min after administration of glycidic stimulus). Some authors state that the finding of a relationship glucose/insulin under 3 is indicative of insulin resistance [91,92].

Clinical parameters

When evaluating a patient for a possible diagnosis of PCOS, it is important to look for signs of insulin resistance such as obesity and acanthosis nigricans. Obesity usually appears in premenarche period and is more frequent in the presence of hyperinsulinemic hyperandrogenism. For a correct diagnosis also anthropometric features are important, since 50-60% of women with PCOS are obese: this is an androgenic obesity with prevalent deposition of fat in the abdomen, identifiable by a ratio waist circumference/ hip circumference greater than 0.85 [93]. Women with PCOS have altered lipid profile, with higher cholesterol levels (both total and LDL), lower HDL cholesterol compared with healthy women [94]. Dyslipidemia is common in PCOS and occurs independent of BMI [95], however there is a synergistic deleterious effect of obesity and insulin resistance in PCOS analogous to that seen in type 2 diabetes. This could explain the association observed between the triglycerides levels and the variables that usually describe obesity, such as BMI and waist circumference. The causes of dyslipidaemia in PCOS are again multifactorial. Insulin resistance appears to have a pivotal role mediated in part by stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase [96]. Cupisti et al. evaluated differences among the 8 most frequent phenotypes of PCOS women with regard to metabolic changes, in terms of markers of lipid profile (LDL, HDL, cholesterol, and triglycerides). Only HDL was significantly lower in women with hyperandrogenemia, hirsutism, and oligomenorrhea than in controls and in comparison with women with oligomenorrhea and polycystic ovaries [97]. In another study Fruzzetti et al. concluded that hyperandrogenemia is a risk factor for dyslipidemia, which was altered only in the phenotypes with elevated androgen levels [98]. The mechanism by which hyperandrogenism may contribute to development of lipid abnormalities in PCOS is not clear. Hyperandrogenism may lead to the abnormalities in lipoprotein profile by working directly at the liver, or it may alter body composition by favoring central adiposity [99].

It is possible to identify in a small percentage of patients Acanthosis Nigricans, a mucocutaneous eruption characterized by thickened skin, in appearance hyperpigmented and velvety that occurs more frequently in the area of the axillas, the skin cracks and the nape of the neck and is thought to be caused by excessive exposure of the dermis to insulin [100,101]

PCOS and age

More and more evidences indicate that PCOS appears to be a pathology that involves "the whole" life of the woman, that begins in the intrauterine life in genetically predisposed individuals, occurs at the time of puberty, endures in child bearing age and exposes, especially after menopause, to a higher risk of developing endometrial cancer, cardiovascular disease, hypertension, diabetes mellitus type 2 [102]. For these reasons it is clear that a correct and early diagnosis of this syndrome is fundamental, as it allows to perform the most appropriate treatments and check-ups thus reducing the risk of developing all the complications related to it.

Adolescence

Menstrual irregularity is a common feature of PCOS, occurring in more than 75% of the adult PCOS population, and is often the earliest clinical manifestation in the adolescent [103,104]. van Hooff and colleagues observed polycystic ovaries in 9% of adolescent girls with

regular menstrual cycles, in 28% with irregular cycles (average cycle length between 22 and 41 days), and in 45% with oligomenorrhea and found a similar association with higher androgen levels. On the basis of these considerations it is possible to make the diagnosis of PCOS in adolescents only in the presence of all 3 Rotterdam criteria: the presence of amenorrhea/oligomenorrhea for at least two years after menarche and the presence of polycystic ovary syndrome, characterized by ovarian size $>10\text{cm}^3$, and most important a hyperandrogenism test rather than only the clinical signs of androgen excess as acne [105]. Acne is a common element of the young women, both in the presence and absence of PCOS, and hirsutism develops later. For all these reasons, the criteria of PCOS diagnosis in adolescents differs from the ones considered for women in adulthood [106,107]. According to some authors, hyperandrogenism could be the most important marker of PCOS among adolescents. Which is why it is important to recognize and decrease androgen levels in adolescence in order to reduce the associated risk of developing metabolic syndrome (MBS), diabetes, and infertility in adulthood [108].

Adult age and pregnancy

Menstrual irregularities are common events during reproductive adulthood, not only, as women with PCOS may experience spontaneous ovulation, it is not always easy to give a correct diagnosis. In 90% of cases, young women with menstrual irregularities will be diagnosed as PCOS women: 95% of adult women with PCOS present oligomenorrhea [109]. PCOS women with amenorrhea present a more severe hyperandrogenism than women with irregular menstrual cycles, or oligomenorrhea. Furthermore, women with PCOS tend to have a more regular cycle as they get closer to menopause [110]. Another important factor to evaluate in adulthood is that PCOS is one of the most common endocrine causes of female infertility. Infertility is frequent in PCOS patients and is usually associated to ovarian dysfunction, dysfunctional uterine bleeding, oligomenorrhea and amenorrhea [111]. The presence of regular menstrual bleeding doesn't exclude anovulation. The causes of infertility or reduced oocyte quality are attributable to ovarian hyperandrogenism and hyperinsulinemia, which would alter the intrafollicular environment through a premature luteinization of the granulosa cell and a paracrine dysregulation of growth factors [112]. The most evident neuroendocrine feature regulating abnormal ovarian follicle development in PCOS is increased luteinizing hormone (LH) pulsatility regarding both frequency and amplitude, with relatively low FSH secretion [113]. The ovarian dysfunction of PCOS involves both these morphological features of polycystic ovaries, described as an accumulation of small antral follicles of size 2-9 mm, as well as the clinical consequence of oligo-/anovulation. The prevalence of menstrual irregularities oligo-/amenorrhea in PCOS depends on the used diagnostic criteria but is approximately 75% [114]. The ovulatory dysfunction in PCOS can be ascribed to disturbed follicular development with excessive early follicular growth and abnormal later stages of arrested follicle growth well before expected maturation [115]. Ovulatory cycles are usually obtained after correcting overweight or immediately after the discontinuation of estrogen-progestin. If this does not occur, ovulation must be pharmacologically induced [116]. Clomiphene is one of the drugs that is normally used for this purpose: it is a weak estrogen that also behaves as antiestrogen. It is likely that the drug interacts with the hypothalamic estrogen receptors, confounding the endogenous estradiol and creating, given its biological activity almost absent in this district, a contrived hypogonadism condition. The hypothalamic centers responsible for

the release of GnRH are thus stimulated to greater activity. After administration of clomiphene, in fact, the frequency of the pulsatile secretion of LH and FSH is increased, while the amplitude remains unchanged. The ovulation in PCOS is induced in 80% of cases, while pregnancy is achieved in 20% of cases. Even if you start a pregnancy it is necessary to consider a whole range of gestational complications that women with PCOS are exposed to [117]. Indeed, there is a higher incidence of gestational diabetes (GDM; 40-50%) and associated fetal macrosomia, hypertensive disorders, such as pre-eclampsia and pregnancy-induced hypertension (5%), as well as miscarriages and premature births (10-15%). Metformin is commonly used to reduce gravidic complications both before and during pregnancy having no valid scientific evidence that the use is not recommended [118]. In cases where there was no response to treatment with clomiphene, in vitro fertilization or intracytoplasmic sperm injection (IVF; ICSI) is the next step necessary to achieve ovulation by administering gonadotropins. The purpose of therapy with gonadotropins, or rather with FSH, act on the follicles in the last stage of their maturation process that, in physiological conditions, is limited to the first two weeks of the menstrual cycle. It is proven that high levels of myo-inositol in follicular fluid are markers of a good oocyte quality. Numerous studies also show that supplementation with myo-inositol is able to improve the response to the pharmacological stimulation and in IVF protocols: reduces days of stimulation, number of germinal vesicles and degenerated oocytes during pick-up, while it increases the number of mature oocytes [119]. Being the myo-inositol a second messenger pathway for insulin, it enables a control of hyperinsulinemia and of gravidic complications associated to it [120].

Menopause

The transition to menopause involves a series of androgen changes in women. Some studies show that testosterone levels are reduced between the third and fifth decade and more evident in women with PCOS, especially regarding T free and DHEAS levels. When advancing in age, it seems that the clinical picture of the disease improves, increasing reproductive possibilities before menopause: the morphology and size of the ovaries become normal and there is often also a restoration of menstruations. According to some authors during perimenopausal there is a partial resolution of hyperandrogenism with a shift from the PCOS I-II-III phenotype to phenotype IV, characterized by anovulation, and polycystic ovaries, but lacking hyperandrogenism [121]. Unfortunately, the data of hyperandrogenism in postmenopause remain unclear. So, although it is impossible today to talk of specific PCOS phenotype in menopause, we can sense that the diagnostic criteria taken into account in reproductive age, is no longer valid [122]. In fact, although a decrease in secretion of ovary androgens has been detected in advanced age, the secretion of adrenal androgens is still evident in women affected by PCOS also in menopause, indicating that in these women the exposure to hyperandrogenism persists for a long time amplifying the risk of cardiovascular disease compared with non-PCOS in post-menopausal women [123]. At the same time insulin resistance, chronic inflammation, abdominal adiposity and dyslipidemia worsen, especially with BMI increase. In obese PCOS women, in fact, an IR liver is established, as result of an alteration of the entire insulin pathway generated by the synergy between PCOS and obesity [124]. In summary, although the data for the overall health status of women with PCOS after menopause is still few, we can speculate that these women will be exposed to greater health risks than healthy women.

Obstructive Sleep Apnea (OSA)

Obstructive sleep apnea (OSA) is a highly prevalent, chronic condition that is characterized by recurrent episodes of complete (apnea) or partial (hypopnea) obstruction of the upper airway leading to intermittent hypoxia, cortical microarousals, sleep fragmentation and chronic sleep loss [125]. Although gender is known to impact upon the distribution (REM vs. NREM) of obstructive events, the physiologic basis for this gender-based difference has not been established, and more recent large population-based studies have demonstrated that the prevalence of OSA is only 1.5–3 times higher in men than in women and this gender gap narrows even further after menopause [126]. Interestingly, the prevalence of OSA increases significantly, after the menopause, in women receiving no gonadal hormone replacement therapy (men-to-women ratio, 1.4:1) [125]. Additionally, recent data indicate that reproductive-age women with PCOS are at very elevated risk for obstructive sleep apnea (OSA) compared with women without PCOS, and it appears to be strongly associated with insulin resistance. Indeed, in some studies, insulin resistance was present only in obese women with PCOS who had OSA [127], meanwhile non-obese women with PCOS do not seem to be at increased risk of OSA [128].

Treatment of PCOS

Insulin sensitizing agents such as metformin, ioglitazone and troglitazone, have been proposed as treatment for PCOS-associated hyperinsulinemia [129,130]. Metformin may antagonize some hyperandrogenic signs, by reducing total and free testosterone concentrations [131,132]. Nevertheless commonly used insulin-sensitizing drugs induce gastrointestinal side effects, and this could likely reduce patients' compliance [133]. The discovery that the impairment in the insulin signaling could be due to a defect in the inositolphosphoglycans (IPGs) second messenger pathway [134,135] opened a new horizon in the clinical management of PCOS. IPGs are known to have a role in activating enzymes that control glucose metabolism [136,137]. When insulin binds to its receptor, two distinct inositol phosphoglycans (IPG) are released by hydrolysis of glycosyl-phosphatidylinositol lipids located at the outer leaflet of the cell membrane. IPGs are then internalized and they affect intracellular metabolic processes, namely by activating key enzymes that control the oxidative and non-oxidative metabolism of glucose [138]. In PCOS women, a defect in tissue availability or altered metabolism of inositol or IPGs mediators may contribute to insulin resistance [139]. Myo-inositol (MI) supplementation improved features of dysmetabolic syndrome, including insulin sensitivity, impaired glucose tolerance, lipids levels and diastolic blood pressure [140]. In addition, it has been noticed that MI improve glucose tolerance in rhesus monkeys [141], meanwhile Schofeld and Hackett demonstrated that MI-containing IPG from *P. falciparum* also had insulin-like effects in vitro and in vivo [142]. Usefulness of MI supplementation has since been assessed by several reports. Morgante et al. have evidenced that MI supplementation in insulin resistant-PCOS patients produces significant results with consequent improvement in clinical pregnancy rate (33.3% vs. 13.3%) obtained with INS treatment [143,144]. Overall, these data demonstrated that MI is equally effective than its stereoisomer D-chiro-inositol (DCI) in normalizing metabolic and endocrine features commonly associated to insulin resistance and PCOS. However, by considering both the systemic and the ovary hallmarks of PCOS, INS supplementation should preferably include both the isomers: MI and DCI. Given that physiological values of the

MI/DCI ratio, evaluated both in the plasma as well as in the follicular fluid, range from 40:1 to 100:1, it seems reasonable that INS should be administered jointly respecting a proportion that should reflect the natural balance among the two stereoisomers. Therefore, as proposed by a recent paper [145], the combined administration of MI and DCI in the physiological plasma ratio (40:1), could be considered as a first line approach in PCOS overweight patients, being able to reduce the metabolic, hormonal and clinical alteration of PCOS.

Conclusions

Despite the controversial thoughts regarding both the etiopathogenesis and the treatment of this syndrome, the scientific community agree in considering PCOS as a long term disease whose first symptoms are usually irregular menstrual and acne (during adolescence) and the last one are diabetes mellitus type 2 and CVD (during menopause). Indeed, further studies are required in order to clarify all links and the hierarchy of the pathophysiological pathways within the PCOS. On our opinion, in order to do this, we should start considering that PCOS might have two different roots, the first might be the metabolic one i.e. insulin resistance and the compensatory hyperinsulinemia and the subsequent hyperandrogenemia. The second might be the reproductive root leading to alteration of ovarian response to gonadotropins oligomenorrhea and hyperandrogenemia. This would likely help in redesigning to the management of PCOS and eventually improve new therapeutic approaches that will be able to cut both roots in order to improve quality of life as a whole of the women that are affected by this endocrine disorder.

Declaration of interest

SP, GG, GP, MB declares no conflict of interest. GC is LO.LI. Pharma employee. V Unfer is the president of LO.LI Pharma s.r.l.

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