Laboratory diagnostics in polycystic ovary syndrome

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Overview

Polycystic ovary syndrome (PCOS) is a common endocrine and reproductive disorder of women, which can result in menstrual complications, fertility problems and other health issues. Laboratory measurement of levels of different sex hormones in blood samples is a key criterion for the diagnosis of PCOS and is particularly important for assessing patients who are struggling to conceive.

In pregnancy, women with PCOS are at increased risk for complications such as gestational diabetes

Disease background

PCOS is the most frequent endocrinopathy of women of reproductive age, occurring with a prevalence of between 6 and 20 percent in this demographic depending on the definitions used. It is a complex syndrome of unclear aetiology with a heterogenic phenotype. Symptoms encompass reproductive, metabolic and psychological disorders, with presentation varying by ethnicity. The three main characteristics of the disease are hyperandrogenism, polycystic ovaries and ovulatory dysfunction. Four phenotypes of PCOS are identified based on the different combinations of these features, although the clinical implications of this classification have not yet been clarified.

Clinical signs and diagnostic criteria

Diagnosis of PCOS is based on the exclusion of other diseases and the presence of at least two of three of the features, known as the Rotterdam criteria.

1. An overproduction of androgens (hyperandrogenism) such as testosterone and its precursors is the defining feature of PCOS, although 10 to 20 percent of patients may suffer from nonhyperandrogenic PCOS. The excess of androgens interferes with follicle maturation, delaying or hindering ovulation, and has a virilising (masculinising) effect, causing symptoms such as acne, alopecia (hair loss) and hirsutism (excess growth of hair in male-like patterns on the face, chest, abdomen, arms or legs). Hyperandrogenism is assessed using clinical and/or biochemical parameters.

2. Polycystic ovaries in PCOS patients contain a large number of harmless enlarged follicles. These are, however, are neither cysts nor tumours. They are underdeveloped, fluid-filled sacs, which are unable to release an egg. The size and morphology of the ovaries is assessed by pelvic ultrasound. The sonographic criteria for polycystic ovaries are, however, only valid eight or more years post-menarche.
3. Oligoovulation (irregular ovulation) or anovulation (lack of ovulation) is common in the initial years after menarche. The possibility of PCOS should be investigated if irregular periods, for example less than eight a year, continue beyond the first few years.

Comorbidities

A large proportion of PCOS patients, many of whom are overweight, exhibit glucose metabolism disorders, in particular insulin resistance, which is a risk factor for developing diabetes mellitus type 2. The increased insulin levels may cause a further build up of androgens, exacerbating the PCOS symptoms. PCOS patients also have an increased risk of cardiovascular diseases and are more likely to have hypothyroidism.

Women with PCOS often suffer from mental health issues due to their hirsutism or adipositas. Psychiatric disorders associated with PCOS include depression, anxiety, body dysmorphia, eating disorders and sexual disorders.

PCOS is, moreover, one of the most common causes of female infertility, Patients usually have a high ovarian reserve, but require medical interventions to stimulate ovum maturation and ovulation. Interventions include fertility drugs or the removal of androgen-producing tissue in the ovaries by laparoscopic ovarian drilling. In vitro fertilisation (IVF) is a further option if ovulation induction therapies are unsuccessful. In pregnancy, women with PCOS are at increased risk for complications such as gestational diabetes, preeclampsia or premature delivery and must be intensively monitored.

Hormone imbalances

It is currently debated if the primary event in PCOS originates in the ovary, pituitary gland or hypothalamus or is related to obesity and hyperinsulinism. The hyperandrogenism is caused by disruption of the normal ovarian or adrenal function, which is regulated by a complex interaction of different hormones. In women with PCOS, luteinising hormone (LH) is hypersecreted in the pituitary gland, which results in an imbalance in the ratio of LH to follicle-stimulating hormone (FSH). LH is involved in mediating ovulation, while FSH is responsible for ovum maturation. If there is too much LH to FSH, the eggs cannot mature and accumulate in the ovaries. Moreover, the imbalance of LH to FSH leads to increased production of androgens in the ovaries. Thus, the hormone imbalances reinforce each other and intensify, leading to the symptoms of PCOS.

Laboratory biomarkers

Measurement of hormone levels is an important part of the diagnostic workup for PCOS. Oral contraceptives should be stopped three months before the blood tests. According to international guidelines for PCOS, determination of testosterone (total or free/bioavailable) is the first-line recommendation for assessing androgen excess in women. Measurement of sex hormone-binding globulin (SHBG) is also recommended, with low values being indicative of PCOS. Further, the free androgen index (FAI), which is the ratio of total testosterone to SHBG, is considered a highly sensitive measure of hyperandrogenaemia.

Around 3 percent of testosterone in the blood is free, while the rest is bound. Testosterone is bound with high affinity to SHBG and with low affinity to non-specific proteins such as albumin. SHBG is a protein produced by the liver which binds stores and transports the males sex hormones testosterone and dihydrotestosterone and the female sex hormones from the groups of estrogens, inactivating them for the time of binding. A lower SHBG concentration thus leads to a higher concentration of active testosterone. Free and albumin-bound forms are considered as bioavailable. These forms can be measured directly or calculated from total testosterone, SHBG and albumin values.

The testosterone precursors androstendione, and dihydroepiandrosterone (DHEA) or its metabolite DHEA sulfate (DHEA-S) can also be determined. They play a more limited diagnostic role, but can increase the probability of detecting hyperandrogenaemia. DHEA is the most important androgen of the adrenal cortex. Since DHEA-S is less dependent on diurnal or cyclic deviations, determination of the DHEA-S level is often the preferred diagnostic choice. Elevated DHEA/-S levels are found in 20 to 30 percent of women with PCOS and indicate an organic cause of the hormonal imbalance in the adrenal cortex. In these cases, it is important to exclude an enzyme defect or a tumour.

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Other tests which are suggestive of PCOS but not diagnostic include measurement of FSH and LH. An increased LH secretion with an elevated LH/FSH ratio is frequently observed in PCOS. Anti-Mullerian hormone (AMH) is a marker of ovarian reserve and functionality. In PCOS the concentration of AMH is often two to three times higher than in healthy women of the same age due to increased secretion of AMH in the follicles. AMH is particularly relevant for women over 35 years old, in whom polycystic ovaries are less often detected by sonography.

Due to the association of PCOS with metabolic disorders, all patients should also be assessed for impaired glucose tolerance, as well as for cardiovascular risks associated with obesity. The vitamin D level can also be measured and optimised, since many women with PCOS are vitamin D deficient, and the vitamin D status may affect their metabolism and fertility.

Differential diagnostics

Endocrinological diseases affecting the pituitary gland, adrenal glands, ovaries or thyroid gland may lead to similar symptoms and must be excluded in the diagnostic workup for PCOS. Other causes of hyperandrogenism include congenital adrenal hyperplasia (CAH), Cushing syndrome and androgen-secreting tumours, while other causes of oligoovulation and amenorrhoea can be hypothyroidism, hyperprolactinaemia, or premature menopause.

CAH is caused in most cases by a deficiency of the enzyme 21-hyroxylase, resulting in accumulation of 17-OH progesterone and androgen excess. 17-OH progesterone is measured to indicate CAH. Cushing syndrome is an endocrine disorder characterised by excess levels of cortisol. Analysis of cortisol levels in saliva and 24-hour urine, as well as an overnight dexamethasone suppression test are performed in suspected cases. Measurement of DHEA-S can help to predict androgen-secreting adrenal carcinoma, with significantly elevated levels being suggestive of a tumour. Thyroid stimulating hormone (TSH) is examined to assess the function of the thyroid gland and prolactin to indicate hyperprolactinaemia. The ovarian reserve and fertility are evaluated using the parameters AMH, FSH, LH and estradiol.

Hormone measurement

The gold standard for evaluating steroid hormones is chromatography coupled with mass spectrometry (GC-MS or LC-MS/MS), which provides the most accurate measurements. Immunoassays are an alternative used by many laboratories. Enzyme-linked immunosorbent assays (ELISA) and chemiluminescence immunoassays offer the advantage of being economical, fast and easy to perform, and automatable for high-throughput analyses. Reference ranges should be established in each individual laboratory before implementation, as they may vary depending on the local population and laboratory conditions.

An extensive range of ELISAs for determination of hormones in serum and/or plasma is available from EUROIMMUN, with parameters encompassing total testosterone, free testosterone, androstendione, sex hormone-binding globulin (SHBG), 17-OH progesterone, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S). Assays for additional parameters such as estriol (free), total estrogens, estrone, pregnenolone, dihydrotestosterone (DHT) and 3alpha-androstanediol glucuronide (3alpha-adiol G) are also available for investigation of other hormone imbalances. All EUROIMMUN ELISAs can be fully automated to increase standardisation and efficiency.

Outlook

Since PCOS affects a significant percentage of women of childbearing age, it has a considerable impact on women's health, necessitating the need for a further understanding of its origins and phenotype presentation. Current hypotheses on the aetiology of PCOS postulate that genetic factors, low birth weight or in utero exposure to androgens might contribute to the development of the disease. PCOS is not curable, but reliable diagnosis can guide decision-making on treatment to reduce symptoms and prevent further health deterioration. Therapy options aimed at managing PCOS include combined oral contraceptives, diabetes medication, as well as exercise and dietary lifestyle changes. Moreover, identification of PCOS enables targeted fertility treatment in women who are trying to conceive. In the future, diagnosis of PCOS could potentially be improved by the use of new biomarkers of androgen excess and ovarian dysfunction.

References

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