Approach to Investigation of Hyperandrogenism in a Postmenopausal Woman

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Abstract

Postmenopausal hyperandrogenism is a condition caused by relative or absolute androgen excess originating from the ovaries and/or the adrenal glands. Hirsutism, i.e., increased terminal hair growth in androgen-dependent areas of the body, is considered the most effective measure of hyperandrogenism in women. Other symptoms can be acne and androgenic alopecia or the development of virilization including clitoromegaly. Postmenopausal hyperandrogenism may also be associated with metabolic disorders like abdominal obesity, insulin resistance and type 2 diabetes. Mild hyperandrogenic symptoms can be due to relative androgen excess associated with menopausal transition or polycystic ovary syndrome, which is likely the most common cause of postmenopausal hyperandrogenism. Virilizing symptoms, on the other hand, can be caused by ovarian hyperthecosis or an androgen-producing ovarian or adrenal tumor that may be potentially malignant. Determination of serum testosterone, preferably by tandem mass spectrometry, is the first step in the endocrine evaluation providing important information on the degree of androgen excess. Testosterone > 5 nmol/L is associated with virilization and requires prompt investigation to rule out an androgen-producing tumor in first instance. To localize the source of androgen excess, imaging techniques are used like transvaginal ultrasound or magnetic resonance imaging (MRI) for the ovaries and computed tomography (CT) and MRI for the adrenals. Bilateral oophorectomy or surgical removal of an adrenal tumor is the main curative treatment and will ultimately lead to a histopathological diagnosis. Mild to moderate symptoms of androgen excess are treated with anti-androgen therapy or specific endocrine therapy depending on diagnosis. This review summarizes the most relevant causes of hyperandrogenism in postmenopausal women and suggests principles for clinical investigation and treatment.

Key words: Hyperandrogenism, Hirsutism, Virilization, Postmenopausal women, Ovarian hyperthecosis, Androgen-producing tumor

Case presentation

A postmenopausal 66-year-old nulliparous woman with type 2 diabetes and hyperlipidemia is being referred to a specialist clinic at a university hospital due to suspected androgen-dependent hair loss that has developed over the years. She has frontotemporal baldness and has been using wig for a couple of years. The woman first sought medical help many years ago but was told that it is normal with hair loss after menopause. When examining the patient, it is noted that she is overweight with body mass index (BMI) 29 and she has abdominal fat distribution. Furthermore, she has so called "Hippocratic baldness", corresponding to grade 3 on the Ludwig Scale (1) (Figure 1), oily skin, increased body hair and blood pressure 160/90 mm Hg. The most marked finding in laboratory analyses is a clearly elevated testosterone level of 5.6 nmol/L (Table 1). Furthermore, androstendione (A4) of 6.8 nmol/L is above normal for a postmenopausal woman (2). Dehydroepiandrosterone sulfate (DHEAS) (4.0 micromol/L) and sex hormone-binding globulin (SHBG) (28 nmol/L) are within the reference range. Gynecological examination shows clitoromegaly and a greatly enlarged uterus on palpation, as well as bilateral ovaries of significant size detected by transvaginal ultrasound. She is referred to a Doppler ultrasound examination confirming large uterine fibroids and enlarged ovaries with normal blood flow. The patient undergoes hysterectomy and bilateral salpingo-ophorectomy. Histopathological examination reveals benign uterine fibroids and bilateral ovarian stromal hyperplasia with the presence of nests of luteinized theca cells in agreement with ovarian hyperthecosis. No signs of malignancy. Postoperatively, testosterone levels normalize within a couple of weeks (0.8 nmol/L). The symptoms subside spontaneously resulting in weight loss, reduced abdominal obesity, hirsutism and oily skin. However, androgenic alopecia and clitoromegaly remain.

Menopausal transition and circulating testosterone

Menopausal transition is associated with a decrease in the number of antral follicles and ovarian volume, as well as a decline in serum anti-müllerian hormone (AMH) as a marker for antral follicle count and

ovarian reserve. When the number of antral follicles and ovarian granulosa cells decrease, estradiol levels decline, and follicle-stimulating hormone (FSH) levels increase. Menopause, the last spontaneous menstruation, occurs on average at age 51 years when circulating estradiol has decreased to a level insufficient to stimulate the endometrium to grow and then shed. During this period, menopausal symptoms including hot flushes, sweating and sleep problems are common and associated with the gradual decline in estradiol.

In contrast to the decrease in estradiol, circulating levels of testosterone decline as a consequence of age-related, and not menopause-related reductions in secretion by both the adrenal gland and the ovary (3). This means a 50% reduction in testosterone in women aged 40-45 compared to women in the age group 18-24. In premenopausal women, about 50% of circulating testosterone arises by direct secretion from the ovary and the adrenal gland of equal amount by the pituitary control of luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH), respectively (4). The remaining 50% of testosterone is produced from peripheral conversion by ovarian and adrenal inactive androgen precursors (A4, dehydroepiandrosterone (DHEA) and DHEAS). Testosterone is further converted in target tissues to dihydrotestosterone (DHT) by the enzyme 5α reductase, and together these hormones constitute the two classical bioactive androgens that bind to the androgen receptor. In postmenopausal women, a larger part of these active androgens is synthesized in peripheral tissue from DHEA within the cell according to the concept of intracrinology (5).

Lately, it was demonstrated that the androgen derivates 11-ketotestosterone and 11-ketodihydrotestosterone from the adrenal glands also are potent agonists of the human androgen receptor (6). In contrast to the classical androgens (DHEA, DHEAS, A4 and testosterone), 11-keto androgens do not decrease by age (7). Furthermore, these androgens have shown to be predominant in several disorders of hyperandrogenism including polycystic ovary syndrome (PCOS) (8) and congenital adrenal hyperplasia (CAH) (9). However, determination of 11-keto androgens is yet not available as a clinical routine method.

SHBG, a protein secreted by the liver, regulates the bioavailability of testosterone. Around 65–70% of circulating testosterone is bound and inactivated by SHBG, 30–35% is loosely bound to albumin and only 0.5–3% represents freely circulating testosterone (10). Since the binding of testosterone to albumin is rather weak, the free and albumin-bound fractions are defined as bioavailable testosterone. The ratio of total testosterone to SHBG multiplied by 100, i.e. free androgen index (FAI), is used as a measure of circulating free testosterone. However, this measure is less relevant when total testosterone is pathologically increased.

During menopausal transition, ovarian theca cell production of testosterone decreases due to follicle depletion, but this loss is compensated by increased LH stimulation of stroma cell production of testosterone. Consequently, ovarian androgen production does not change significantly in relation to menopause. At the same time, SHBG decreases due to the decrease of the ovarian estrogen production, and subsequently FAI increases (11-12). Overall, this will result in a physiological shift from estrogen dominance to a relative predominance of androgens during menopausal transition (13). Besides typical menopausal symptoms, it is not uncommon for healthy postmenopausal women to experience androgen-dependent symptoms, such as increased facial hair growth and hair thinning due to relative androgen excess.

The most common cause of absolute androgen excess in postmenopausal women is PCOS causing mild to moderate symptoms of hyperandrogenism (2), whereas virilizing symptoms including e.g. clitoromegaly, deepening of the voice and breast atrophy besides severe hirsutism and possibly androgenic alopecia (Table 2) are rare and should be carefully investigated. The most relevant causes of hyperandrogenism, of either ovarian or adrenal origin, in postmenopausal women are described below (Table 3).

Etiology of hyperandrogenism in postmenopausal women

Polycystic ovary syndrome

PCOS is considered the most frequent endocrine disorder in women of reproductive age with a prevalence between 8-13% depending on diagnostic criteria and population studied (14-15). According to the Rotterdam criteria, at least two of the following three criteria are required for a diagnosis: oligomenorrhea or amenorrhea; biochemical or clinical hyperandrogenism such as hirsutism and acne; and polycystic ovarian morphology (16). There are no specific criteria to diagnose PCOS after menopause. The Endocrine Society Clinical Practice Guideline has therefore suggested that a diagnose of PCOS in a postmenopausal woman could be based upon a history of oligo/amenorrhea and hyperandrogenism during reproductive years (17).

The reproductive phenotype of PCOS usually improves by age due to loss of ovarian follicles leading to more regular cycles and decreased ovarian volume (18). However, the decrease in ovarian volume and serum AMH during menopausal transition may be relatively less in women with PCOS compared to other women (19-20). Consequently, the average age of menopause is approximately two years later in PCOS than in healthy controls (21). As androgen levels gradually decrease by age, symptoms of hyperandrogenism like hirsutism may improve in women with PCOS (22). Still, the prevalence of hirsutism was significantly higher in postmenopausal women with PCOS compared to control women (33% vs 4%) at mean age 81 years in a Swedish long-term follow-up study (23).

PCOS is considered a relatively mild form of hyperandrogenism since circulating levels of testosterone usually are within the upper normal female range, whereas SHBG is low resulting in increased levels of free and bioavailable testosterone. Today, liquid chromatography-tandem mass spectrometry (LC–MS/MS) is recognized as the golden standard method for testosterone determination in serum in comparison to immuno-based clinical methods, which are burdened with cross-reactivity against structurally similar steroid hormones and, moreover, are not sensitive enough for the determination of steroids at relatively low concentrations (24). Available measurements based on LC–MS/MS indicate that the normal range of testosterone in premenopausal women is 0.1–1.8 nmol/L, whereas the upper limit in women with PCOS is 3.1 nmol/L (95% CI, one-sided) (25). Although testosterone levels decline with

increasing age, most studies have shown higher testosterone levels in postmenopausal women with PCOS than in control women (26-28). However, testosterone levels in postmenopausal women with PCOS are seldom exceeding 2 nmol/L (27-30).

PCOS is also a metabolic disorder with increased occurrence of obesity, which aggravates all symptoms of the syndrome, including hirsutism (31). Especially abdominal obesity is associated with insulin resistance leading to secondary hyperinsulinemia (32). Hypersecretion of insulin stimulates ovarian androgen production in synergy with LH (32). In addition, insulin inhibits the hepatic synthesis of SHBG leading to increased levels of FAI (33). In this way, obesity and insulin resistance contribute to hyperandrogenism in women with PCOS. Testosterone may in turn induce hepatic insulin resistance by facilitating catecholamine-stimulated lipolysis in visceral fat tissue, as well as peripheral insulin resistance in muscle tissue by inducing decreased capillary density (31, 34). Women with abdominal obesity often have a more pronounced PCOS phenotype and remain hyperandrogenic after menopause or may even have worsening symptoms. In the long run, PCOS is associated with an increased risk of type 2 diabetes and metabolic syndrome (15), whereas the risk of cardiovascular disease seems not to be increased after menopause (29).

Although hirsutism may be severe in PCOS, virilizing symptoms including clitoral enlargement is not associated with PCOS (Table 1 and 2). In cases where hyperandrogenic symptoms increase and develop into virilization, other conditions of androgen excess must be ruled out.

Ovarian hyperthecosis

Ovarian hyperthecosis is a relatively rare disorder presenting with slow progress of severe symptoms of hyperandrogenism in a peri- or postmenopausal woman (Table 3) (35). It is likely the second most frequent cause of hyperandrogenism in postmenopausal women. The prevalence of ovarian hyperthecosis was reported to 9.3% in postmenopausal women undergoing investigation for symptoms of androgen excess (2).

The condition is often described as an extreme form of PCOS, however there is no clear evidence of a link between ovarian hyperthecosis and PCOS, and most women with PCOS will never develop ovarian hyperthecosis. In contrast to PCOS, ovarian hyperthecosis will progress into virilizing symptoms including severe hirsutism, androgenic alopecia, deepening of the voice, breast atrophy, and clitoromegaly (Table 2, Figure 2). In addition, the ovaries are bilaterally clearly enlarged with a volume up to 10 cm^3, compared to a volume between 1 to 5 cm^3 of a normal postmenopausal ovary in women with or without PCOS (36-38). Ovarian hyperthecosis is furthermore strongly associated with metabolic symptoms including abdominal obesity, hypertension, hyperlipidemia, insulin resistance, and acanthosis nigricans, i.e., the metabolic syndrome and type 2 diabetes (36, 39-40). The metabolic symptoms are often more severe compared to women with PCOS. Due to peripheral conversion of androgens to estrogens via aromatase, women with ovarian hyperthecosis also have an increased risk of endometrial pathology including polyps, hyperplasia and cancer (41-42), as well as breast cancer (43).

The condition is caused by nests of luteinized theca cells in the ovarian stroma producing high amounts of testosterone in the absence of other elevated androgens (44). Serum testosterone is usually increased above 5 nmol/L (35, 38), which differs this condition from PCOS (Table 3). The etiology of ovarian hyperthecosis is not known although a genetic disposition and association with PCOS have been suggested (45). Several mechanisms behind the increased testosterone production have been proposed. One is related to the "two cell hypothesis" where ovarian testosterone production by the theca cells is uncovered in a postmenopausal woman by the loss of granulosa cell-mediated aromatization of testosterone to estradiol (46). Another mechanism involves increased gonadotrophin stimulation by the elevated levels of LH after menopause (47). Thirdly, there is support that insulin resistance and hyperinsulinemia may induce stromal luteinization causing androgen overproduction (48).

The most important differential diagnosis for ovarian hyperthecosis is an androgen-producing tumor, which can potentially be malignant. In both cases, the patient has virilizing symptoms, but the progress is usually slow for ovarian hyperthecosis but rapid for androgen-producing tumor. In addition, testosterone is

greatly elevated (> 5 nmol/L) in both disorders, but mostly higher in women with androgen-producing tumor than in those with ovarian hyperthecosis (38). Furthermore, other androgens are usually not elevated in ovarian hyperthecosis, while androgen-producing adrenal tumors are associated with high levels of DHEAS and A4, and ovarian tumors with high levels of inhibin B. Almost all women with ovarian hyperthecosis have obesity and insulin resistance, whereas this is not the case of women with androgen-producing tumor. Ovarian hyperthecosis is also characterized by bilateral increase in ovarian stroma, whereas an ovarian tumor usually is presented as a unilateral enlargement. Still, it can be difficult to distinguish ovarian hyperthecosis from an androgen-producing tumor. In the end, the diagnosis of ovarian hyperthecosis is confirmed by histopathology.

Androgen-secreting ovarian tumors

Androgen-secreting ovarian tumors originate from sex cord-stroma and include Sertoli cell tumors, Sertoli-Leydig cell tumors, Leydig cell tumors, Thecoma and Granulosa cell tumors (49-50). These tumors are predominantly benign and occur at any age but approximately 25% present after menopause (51). Together they comprise 5-8% of all ovarian neoplasms (26). The presentation is often rapid progress of clinical manifestations of excessive androgen and/or estrogen production (Table 3). Androblastomas (Sertoli cell tumors, Sertoli-Leydig cell tumors, Leydig cell tumors) are those primarily secreting androgens, while Thecoma and Granulosa cell tumors mainly secrete estrogens, which can lead to postmenopausal bleeding, endometrial hyperplasia or cancer. However, around 10% of Granulosa cell tumors secrete androgens and may cause virilization (52). The prevalence of androgen-secreting ovarian tumor in postmenopausal women with symptoms of hyperandrogenism has been reported to 2.7% (2).

Endocrine characteristics of androblastomas are clearly elevated testosterone, not seldom in the lower male range (8-29 nmol/L) (25), with accompanying increase in A4 and 17-hydroxyprogesterone (17-OHP), whereas DHEAS and cortisol levels usually are normal (49). In the case of granulosa cell tumors, they usually co-secrete AMH and inhibin B, besides estradiol and/or testosterone (53-54).

Ovarian tumors are often small but can be identified using transvaginal ultrasound with color Doppler or magnetic resonance imaging (MRI). Asymmetry of the ovaries may suggest a tumor.

Androgen-secreting adrenal tumors

Androgen-secreting adrenal tumors are less common than the corresponding ovarian tumors. Benign adrenal adenomas include non-secretory (incidentalomas) and secretory adenomas, of which the latter can cause hyperandrogenism. Adreno-cortical carcinomas, on the other hand, are usually highly malignant tumors and approximately 25% of the cases are associated with severe symptoms of hyperandrogenism leading to virilization (Table 3) (2, 55-57). The incidence is 1-2 cases/million population per year. There is a bimodal age distribution with peaks before the age of five and in the fourth and fifth decade of life (58).

Adrenocortical carcinomas are considered gonadotropin independent and are manifested by an increase in adrenal androgens, DHEA and DHEAS (2). DHEAS concentrations are often more than twice the upper limit, and a value above 19 µmol/L is indication for further evaluation. Furthermore, testosterone is highly elevated in the male range, as well as A4 (55-56). Cortisol secretion may be increased both in androgen-secreting adenomas and carcinomas leading to a clinical image of Cushing syndrome in addition to hyperandrogenism.

Adrenal tumors are best visualized by computed tomography (CT scan) as a unilateral mass. Adenomas are usually small, 2 to 2.5 cm, whereas adrenocortical carcinomas are larger, between 4 to 21 cm (57).

Nonclassical congenital adrenal hyperplasia

CAH is an autosomal recessive disease characterized by low or absent production of cortisol and aldosterone with concomitant overproduction of androgens due to an enzyme deficiency in the adrenal cortex steroid biosynthesis (59). The most common type is 21-hydroxylase deficiency caused by a mutation in the gene (CYP21A1) encoding the adrenal 21-hydroxylase enzyme (60). There are different clinical forms of CAH depending on the degree of enzyme deficiency: the severe salt wasting form (SW

CAH), the simple virilizing form (SV CAH), and the less severe nonclassic form (NC CAH). SW CAH and SV CAH, often referred to as classic CAH, are usually diagnosed in infancy via newborn screening programs if available, due to varying degrees of virilization in females, or in the most severe cases due to life-threatening salt wasting (60). The treatment of CAH consists of substitution therapy with glucocorticoids and mineralocorticoids, which will reduce the overproduction of androgens (60).

In contrast to classic CAH, women with NC CAH are usually diagnosed later in life due to mild symptoms of androgen excess, such as hirsutism, menstrual disorders, and infertility (Table 3) (61). The symptoms are very similar to PCOS, and in agreement with PCOS, NC CAH is not associated with virilizing symptoms. Since some of these women may be undiagnosed or have worsening symptoms by age (62), NC CAH should be considered in postmenopausal women with hyperandrogenism. The NC form of CAH is estimated to be one of the most common autosomal recessive disorders with a prevalence of 1% to 10% in women with hyperandrogenic symptoms (61).

Elevated serum 17-OHP, due to accumulation before the enzyme block, is indicative of CAH (63). It should be further investigated by adrenocorticotropic hormone (ACTH) stimulation test. Serum concentrations of testosterone and adrenal androgen precursors (A4, DHEA and DHEAS) are also increased. The diagnosis is confirmed by genetic testing and detection of a mutation causing enzyme deficiency and impaired corticosteroid synthesis.

Cushing's syndrome

Cushing's syndrome is a rare disorder, which can be either ACTH dependent and caused by pituitary hypersecretion of ACTH (Cushing's disease, 70%) or ACTH independent due to adrenocortical adenoma or carcinoma (20%) (64). ACTH dependent Cushing is associated with elevated ACTH levels causing bilateral adrenocortical hyperplasia and hypersecretion of cortisol. In contrast, ACTH independent Cushing is related to suppressed ACTH secretion due to negative feedback by increased cortisol secretion

(64). The overall incidence of Cushing's syndrome is estimated to 1.8-3.2 cases per million population (65). Cushing's disease occurs mainly in women aged 25 to 45 years.

The major clinical manifestations of Cuching's syndrome are moon face and facial plethora, abdominal obesity, striae, buffalo hump, proximal muscle weakness, bruising, hypertension, glucose intolerance, depression and other neuropsychological symptoms (66). About 50% of women with Cushing also have symptoms of hyperandrogenism like hirsutism due to adrenal androgen excess (A4, DHEA and DHEAS) (Table 3) (67). Furthermore, free androgen index is increased by endogenous hypercortisolism probably due to a decrease in SHBG. However, signs of hyperandrogenism are usually mild to moderate and seldom lead to virilization.

The diagnosis of Cushing's syndrome is established by hypersecretion of cortisol as measured by 24-h urinary free cortisol, late-night salivary cortisol or 1 mg dexamethason suppression test (68). Further evaluation is needed to determine ACTH dependence or independence by measurement of plasma ACTH, as well as pituitary, adrenal or ectopic etiology by using MRI of the pituitary and CT scan of the adrenal glands or other relevant imaging. First line of treatment is surgical removal of the ACTH- or cortisol-secreting tumor (68).

Iatrogenic

Iatrogenic causes of hyperandrogenism due to overuse or abuse of androgenic drugs should be considered. Systemic testosterone and DHEA treatment of hypoactive sexual desire disorder or other androgen-deficient-related symptoms in postmenopausal women may lead to overtreatment if not carefully monitored by measurement of serum testosterone (69). Furthermore, treatment with the antiepileptic drug valproic acid has been shown to increase the risk of a PCOS-like phenotype in epidemiological studies (70). The mechanism is attributed to direct stimulation of ovarian androgen production by valproic acid (71). The anabolic steroid Danazol, previously used for treatment of endometriosis and still used as

therapy for hereditary angioedema, has been reported to induce hirsutism (72). It is well known that anabolic steroids can cause virilization in women when abused (73).

Evaluation

Clinical symptoms

The patient's history of onset and development of symptoms should always be a guide for further investigation. Late onset and rapid development of virilizing symptoms suggest hormone-producing tumor, whereas slow development of virilizing symptoms in a peri- or postmenopausal woman is typical of ovarian hyperthecosis. In contrast, early symptom onset and slow progression of mild to moderate hyperandrogenic symptoms are more consistent with PCOS or another endocrine disorder (Figure 3).

Clinical signs of mild to virilizing symptoms of hyperandrogenism are shown in Table 2. Hirsutism has been considered the most effective measure of androgen excess in women (74). It is defined as excessive facial and body terminal hair in androgen-dependent body areas. Evaluation of hirsutism can be assessed by the modified Ferriman-Gallwey (mFG) score 0 (no terminal hair) to 4 (marked hirsutism) in nine body areas: upper lip, chin and cheeks, upper chest, upper abdomen, lower abdomen, upper arms, thighs, upper back, and lower back (Table 4) (75-76). A cut-off score of ≥ 4-6 on mFG was suggested to indicate hirsutism, depending on ethnicity (77). However, this method has not been validated in postmenopausal women.

Acne is associated with increased androgen levels, although the predictive value of acne for hyperandrogenism has been questioned (74, 78). Furthermore, there is no universally accepted classification tool for evaluation of acne (74). Still, it is recommended to assess acne in a patient undergoing evaluation of hyperandrogenism (Table 3) (74).

Androgenic alopecia, or female pattern hair loss, is characterized by thinning of hair in the fronto-parietal region of the scalp (Figure 2) (79). The disorder is dependent on androgens, particularly DHT and 5α

reductase activity in hair follicles (79). Hair loss on the scalp can be assessed using the Ludwig scale (Table 4) (Figure 1) (1).

Clitoromegaly is probably the most recognizable sign of virilization (Figure 2). It was defined as > 1.5 x 2.5 cm (26, 80). However, signs of clitoromegaly must be carefully investigated, otherwise easy to miss, especially in an obese woman. Other signs of virilization could be breast atrophy and severe hirsutism (Figure 2).

Endocrine evaluation

Symptoms of hyperandrogenism and particularly virilizing symptoms (including e.g. clitoromegaly, deepening of the voice and breast atrophy besides severe hirsutism and possibly androgenic alopecia) in a postmenopausal woman should be evaluated by endocrine screening, preferably serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, SHBG, A4, DHEAS, estradiol, 17-OHP and inhibin B (Table 4). For steroid hormone determination, it is highly recommended to use LC–MS/MS instead of immunological methods as mentioned above. By ROC analysis, the diagnostic threshold for serum testosterone as measured by LC–MS/MS to identify an androgen-producing tumor was defined as testosterone ≥ 5.1 nmol/L (sensitivity, 90%; specificity, 81%) (30). Serum testosterone > 5 nmol/L is clearly associated with virilizing symptoms (Table 2) (35, 81). It is therefore suggested to use this value of testosterone as cut-off in the first step of investigation to rule out a hormone-producing tumor or a non-tumor cause of severe hyperandrogenism such as ovarian hyperthecosis in a postmenopausal woman (Figure 3).

Women with virilizing symptoms - Testosterone levels tend to be higher (in the lower male range) and gonadotropin levels lower in association with a virilizing ovarian tumor than in ovarian hyperthecosis, however no cut-off value of testosterone has been proposed for discriminating between a hormone-producing tumor and ovarian hyperthecosis (26, 38, 82). Next step is therefore to proceed with diagnostic imaging (Figure 3), see below. Further endocrine evaluation may still be helpful for distinguishing between tumorous and non-tumorous causes of virilizing symptoms.

Ovarian hyperthecosis is typically associated with an isolated increase in testosterone, while other androgens usually are within the reference values (2), see the patient case above. Furthermore, insulin resistance is characteristic for ovarian hyperthecosis (36, 39-40), and therefore fasting insulin and glucose for calculation of Homeostatic Model Assessment (HOMA) or HbA1c should be considered. In the case of hormone-producing ovarian tumor, other hormones than testosterone may also be elevated including inhibin B, AMH, A4, 17-OHP and estradiol, whereas DHEAS and cortisol are normal (49, 82). In contrast, androgen-producing adrenal tumors are associated with increased DHEAS levels, often higher than twice the upper normal limit (> 19 µmol/L), together with increases in testosterone, A4 and cortisol (2).

Gonadotropin-releasing hormone (GnRH) agonist test can be used for distinguishing androgen-producing ovarian and adrenal tumor and successfully confirm ovarian source from adrenal source by suppression of testosterone (38). However, the test cannot differentiate between ovarian hyperthecosis and ovarian tumor, as both disorders are gonadotropin-dependent and will respond to GnRH with testosterone inhibition (83).

Selective ovarian and adrenal venous catheterization can localize an androgen-producing tumor by demonstrating differential gradients in androgen levels between ovarian, adrenal, and peripheral veins. However, success rates are poor, and this invasive method carries potential risks. A recent systematic review and meta-analysis concluded that there is limited evidence for the use of selective venous sampling in identifying androgen-producing tumors in postmenopausal women (84).

Women with mild to moderate symptoms of hyperandrogenism - In postmenopausal women with mild to moderate symptoms of hyperandrogenism and testosterone < 5 nmol/L, PCOS is the most likely diagnosis as supported by a premenopausal history of menstrual irregularities, hyperandrogenism and/or polycystic ovaries (Figure 3). In these women, androgen levels are slightly higher than controls, but serum testosterone is in most cases below 2 nmol/L (27-30). Transvaginal ultrasound is recommended (see below) but further endocrine evaluation is not needed if screening samples are normal, and the diagnosis is supported by medical history.

In the case of increased morning value of 17-OHP (> 9 nmol/L), ACTH stimulation test should be performed to rule out NC CAH (Figure 3) (60). A significant increase in ACTH in response to the test requires further investigation with genetic testing to confirm a CAH diagnosis (60). Cushing's syndrome can be suspected by typical symptoms and should be investigated with 24-hour urinary free cortisol and/or dexamethasone suppression test, as well as plasma ACTH followed by imaging, see below (Figure 3).

Imaging

Transvaginal ultrasound is used to investigate a possible ovarian cause of hyperandrogenism, primarily an androgen-producing ovarian tumor. The mean size of a normal postmenopausal ovary is estimated to 2.2 ± 0.01 cm^3 with a 95% upper confidence interval < 5.0 cm^3 (85). An ovarian tumor can be very small and difficult to identify but asymmetry of the ovaries could be suggestive of such a tumor. Furthermore, color Doppler ultrasound can identify a hypervascularized region suggesting a tumor (82). Failure to identify a tumor with ultrasound does not rule out this possibility, it is therefore important to investigate further with MRI (38, 82). Ovarian hyerpthecosis is associated with bilateral homogenous enlargement of ovarian stroma on ultrasound without hypervascularization (37-38). The average ovarian volume may be up to 10 cm^3 (36-38). Postmenopausal women with PCOS usually have larger ovaries than control women although significantly less than 10 cm^3 (19).

MRI has shown a higher positive and negative predictive value (78%) and (100%), respectively, for detection of an androgen-secreting ovarian tumor than transvaginal ultrasound (82). In addition, good sensitivity (83%) and specificity (80%) were reported for MRI in differentiating between virilizing ovarian tumors and ovarian hyperthecosis (38).

CT scan is the preferable imaging technique for detection of adrenal tumors, which will detect nodules > 5 mm (86). MRI could be an alternative imaging method for detection of adrenal tumors.

The majority of Cushing’s syndrome is ACTH-dependent, and the next step of evaluation is to identify a possible pituitary tumor by MRI (68). If no mass is identified, petrosal sinus sampling and/or further imaging to identify an ectopic source of ACTH should be considered.

Treatment

Treatment strategies depend on the cause, symptoms, and distress of androgen excess (Figure 3). Patients with severe symptoms and testosterone > 5 nmol/L should be managed urgently, and especially those with suspected malignancy. A postmenopausal woman with virilizing symptoms and indication of ovarian source of androgen excess (ovarian tumor, ovarian hyperthecosis) is primarily treated with surgery, i.e., laparoscopic bilateral oophorectomy, with or without hysterectomy (35, 38, 87). As in the patient case presented, testosterone levels will normalize rapidly within a couple of weeks after surgery while symptoms of androgen excess, including hirsutism and oily skin and acne, will gradually resolve. Although swelling of the clitoris may regress, clitoral hypertrophy usually persists. The same applies to androgenic alopecia, severe hair loss usually remains even after normalized testosterone levels. Deepening of the voice caused by androgen excess, is also a symptom that does not regress after treatment. It is therefore important to diagnose at an early stage for prompt management of potentially severe causes of hyperandrogenism although androgen-producing ovarian tumors seldom are malignant (49-50), and to avoid persistent symptoms despite elimination of androgen excess. Metabolic symptoms may improve to some extent but do usually not disappear (40, 88).

When a patient is not a suitable candidate for surgery of a benign ovarian cause of severe hyperandrogenism, treatment with GnRH analogs is an alternative (36, 40, 89). Depending on the menopausal status of the patient, she may experience symptoms of estrogen withdrawal, and in this case estrogen add-back therapy could be considered (90). Estrogen substitution is also important to consider during long-term treatment with GnRH analogs given the risk of accelerated bone loss (91).

The primary treatment for androgen-secreting adrenal tumors is adrenalectomy for histopathological diagnosis of malignant or benign tumor (92). Complete surgical resection can be sufficient therapy. In cases of malignancy, adjuvant therapy with mitotane, chemotherapy or radiation may be used. Adrenal

function must be closely monitored after surgery for detection of adrenal insufficiency or recurrent tumor (92).

Postmenopausal women with testosterone < 5 nmol/L and a history of PCOS with persistent or aggravating hyperandrogenic symptoms are treated with anti-androgen therapy (93). There are different types of anti-androgens with various mechanisms of action and potential side-effects (94). Ovarian androgen overproduction can be inhibited by GnRH analogues as mentioned above, androgen effects can be blocked by androgen receptor blockers (spironolactone, cyproterone acetate, flutamide) or by a 5α reductase inhibitor (finasteride) blocking the conversion of testosterone to DHT, which is the most potent androgen in peripheral tissue. However, treatment of metabolic symptoms with metformin or other substances has limited effect on hyperandrogenic symptoms (95). The efficacy of anti-androgens for treatment of hirsutism is moderate (96), and it must be explained to the patient that it takes time before any treatment effect can be observed (at least 6 months) and the effect disappears when treatment is stopped.

Postmenopausal women with NC CAH are usually treated with anti-androgens similarly to PCOS and cortisone is seldom needed (61). Cushing's syndrome is primarily treated with surgery of an ACTH-producing pituitary or ectopic tumor or cortisol-secreting adrenal tumor (68). In some cases, medical therapy or radiation may be necessary (68).

Conclusion

Postmenopausal hyperandrogenism can range from mild symptoms due to relative androgen excess during menopausal transition to virilizing symptoms caused by a hormone-producing ovarian or adrenal tumor that may be potentially malignant. A thorough investigation must be performed to determine the underlying cause and to offer appropriate treatment. Onset of symptoms, development and severity of symptoms are indicative for further investigation. Measurement of serum testosterone, preferably by LC–MS/MS, provides important information on the degree of androgen excess. Testosterone > 5 nmol/L is

associated with virilizing symptoms and should prompt further investigation with imaging modalities to rule out an androgen-producing tumor. There is no discriminatory method to distinguish an ovarian hormone-producing tumor from ovarian hyperthecosis although the latter is related to a slower development of virilizing symptoms and a bilateral ovarian enlargement. Surgery with bilateral oophorectomy or removal of an adrenal tumor is the main curative treatment and will ultimately lead to a histopathological diagnosis. GnRH analogues can be used as alternative treatment of ovarian hyperthecosis. PCOS is probably the most common cause of mild to moderate symptoms of hyperandrogenism and testosterone < 5 nmol/l in a postmenopausal woman. In this case, the recommended treatment is anti-androgen therapy using an androgen receptor blocker and/or a 5α reductase inhibitor. NC CAH could be treated in a similar way or if needed with cortisone, whereas Cushing's syndrome is primarily treated with surgery.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Table 1. Hormone values in the patient case.

Hormone	Patient value	Reference interval
FSH IE/L	30	30-150 postmenopausal
LH IE/L	16	15-65 postmenopausal
Testosterone nmol/L	5.6	0.3-3 premenopausal
SHBG nmol/L	28	35-350
Androstendione nmol/L	6.8	1.6-12 premenopausal
DHEAS micromol/L	4.0	0.5-4.1
Östradiol pmol/L	98	<40 postmenopausal

DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone-binding globulin

Table 2. Mild to moderate and virilizing symptoms of hyperandrogenism in postmenopausal women.

	Symptoms of hyperandrogenism
Mild to moderate symptoms	• Hirsutism • Acne and oily skin
Virilizing symptoms	• Severe hirsutism and acne • Androgenic alopecia • Deepening of the voice • Breast atrophy • Increased muscle mass • Enlargement of clitoris

Table 3. Characteristics of different conditions of hyperandrogenism in postmenopausal women.

Condition	**Prevalence/ Incidence**	**Testosterone levels**	**Presentation**
PCOS	Prevalence: 8-13% in the whole female population of fertile age	< 2 nmol/L	• Persistent or increased hirsutism but no virilizing symptoms • History of oligo/amenorrhea and hyperandrogenism during reproductive years • Later menopause • Overweight, abdominal obesity
Ovarian hyperthecosis	Prevalence: 9.3% in postmenopausal women with hyperandrogenism (2)	> 5 nmol/L	• Gradual development of virilizing symptoms in a peri- or postmenopausal woman • Isolated increase in testosterone • Severe insulin resistance, acanthosis nigricans, metabolic syndrome and/or type 2 diabetes • Bilaterally enlarged ovaries
Androgen-secreting ovarian tumor	Prevalence: 2.7% in postmenopausal women with hyperandrogenism (2)	> 5 nmol/L	• Rapid onset of virilizing symptoms • Serum testosterone often in the male range, accompanying increase in A4 and 17-OHP, but usually not DHEAS • Unilateral ovarian tumor
Androgen-secreting adrenal tumor	Incidence: 1-2 cases/million population/year (58)	> 5 nmol/L	• Rapid onset of virilizing symptoms • Serum testosterone in the male range, DHEAS and cortisol usually elevated • Unilateral adrenal tumor
Nonclassic congenital adrenal hyperplasia	Prevalence: 1-10% in women with hirsutism (61)	< 5 nmol/L	• Gradual increase in hirsutism since puberty • Similar symptoms as in PCOS • Serum testosterone moderately increased, 17-OHP elevated
Cushing's syndrome	Incidence: 1.8-3.2 cases/million population/year (65)	< 2 nmol/L	• New-onset hirsutism but seldom virilizing symptoms • Typical signs of Cushing such as moon face, abdominal obesity, striae, buffalo hump • ACTH-secreting pituitary adenoma or a cortisol-producing tumor

ACTH, Adrenocorticotropic hormone; A4, androstenedione; DHEAS, dehydroepiandrosterone sulfate; 17-OHP, 17-hydroxyprogesterone; PCOS, polycystic ovary syndrome

Table 4. Investigation of hyperandrogenism in postmenopausal women.

	Assessment
Clinical symptoms	• Hirsutism: modified Ferriman-Gallwey score • Acne • Androgenic alopecia: Ludwig score • Clitoromegaly: > 1.5 x 2.5 cm
Endocrine evaluation	• Serum analyses of FSH, LH, testosterone, SHBG, A4, DHEAS, estradiol, 17-OHP and inhibin B • HOMA-index or HbA1C as marker for insulin resistance can be considered • ACTH stimulation test to rule out NC CAH • 24-hour urinary free cortisol and Dexamethasone test to rule out Cushing's syndrome
Imaging	• Transvaginal ultrasound • MRI, CT

ACTH, Adrenocorticotropic hormone; A4, androstenedione; CT, computer tomography; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; HOMA, Homeostatic Model Assessment; 17-OHP, 17-hydroxyprogesterone; LH, luteinizing hormone; MRI, magnetic resonance imaging; NC CAH, nonclassical congenital adrenal hyperplasia; SHBG, sex hormone-binding globulin

Figures

Figure 1. The Ludwig scale of androgen-dependent frontotemporal baldness type 1 to 3, where type 3 is the most severe form of androgenic alopecia also called "Hippocratic baldness".

Figure 2. Clinical signs of severe hyperandrogenism and virilizing symptoms of hyperandrogenism in a peri- and postmenopausal woman including A. androgenic alopecia, B. breast atrophy and hirsutism, and C. clitoromegaly.

Figure 3. Algorithm for principles of investigation and treatment of different causes of hyperandrogenism in postmenopausal women. The cut-off of 5 nmol/L for serum testosterone is based on LC–MS/MS measurement. ACTH, adrenocorticotropic hormone; CT, computer tomography; GnRH, gonadotropin-releasing hormone; 17-OHP, 17-hydroxyprogesterone; MRI, magnetic resonance imaging; NC CAH, nonclassical congenital adrenal hyperplasia; PCOS, polycystic ovary syndrome

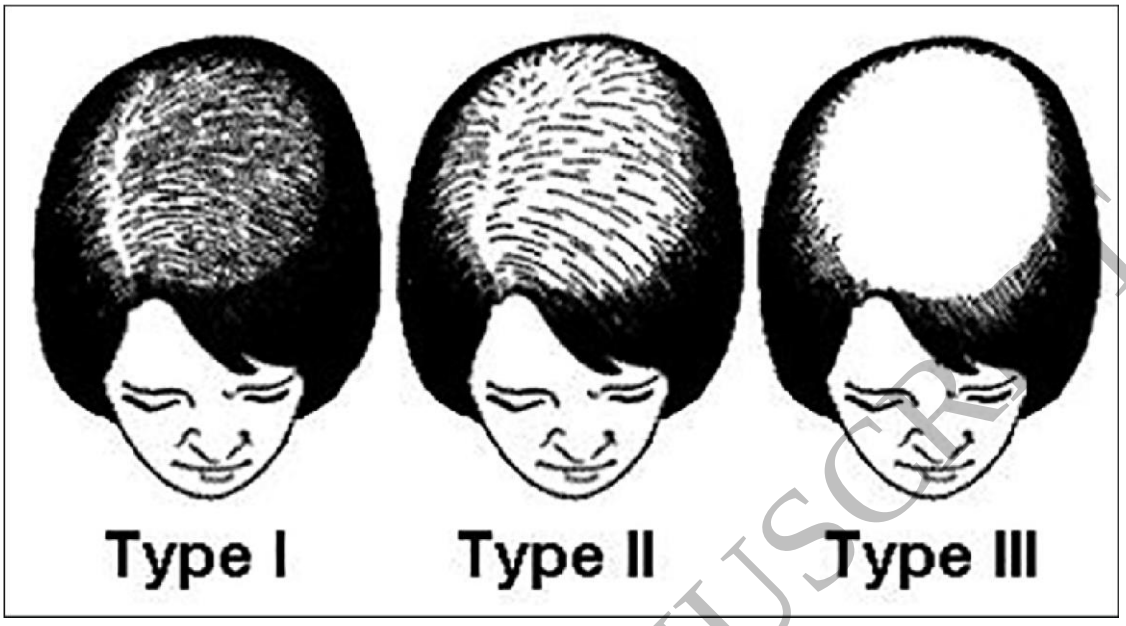

Figure 1
150x82 mm (.86 x DPI)

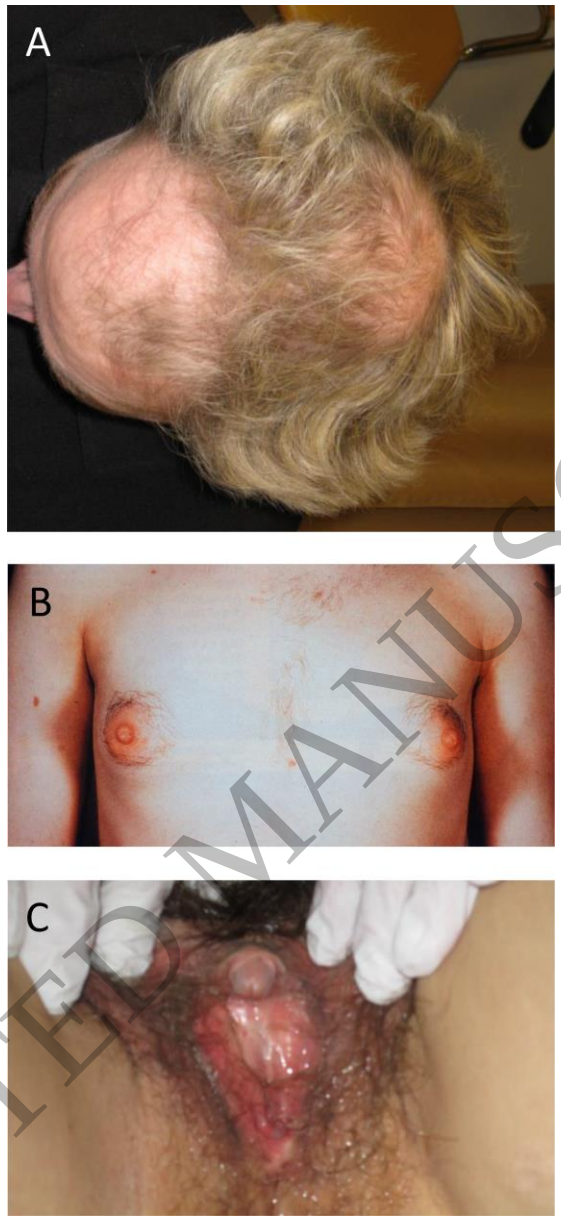

Figure 2
72x160 mm (.86 x DPI)

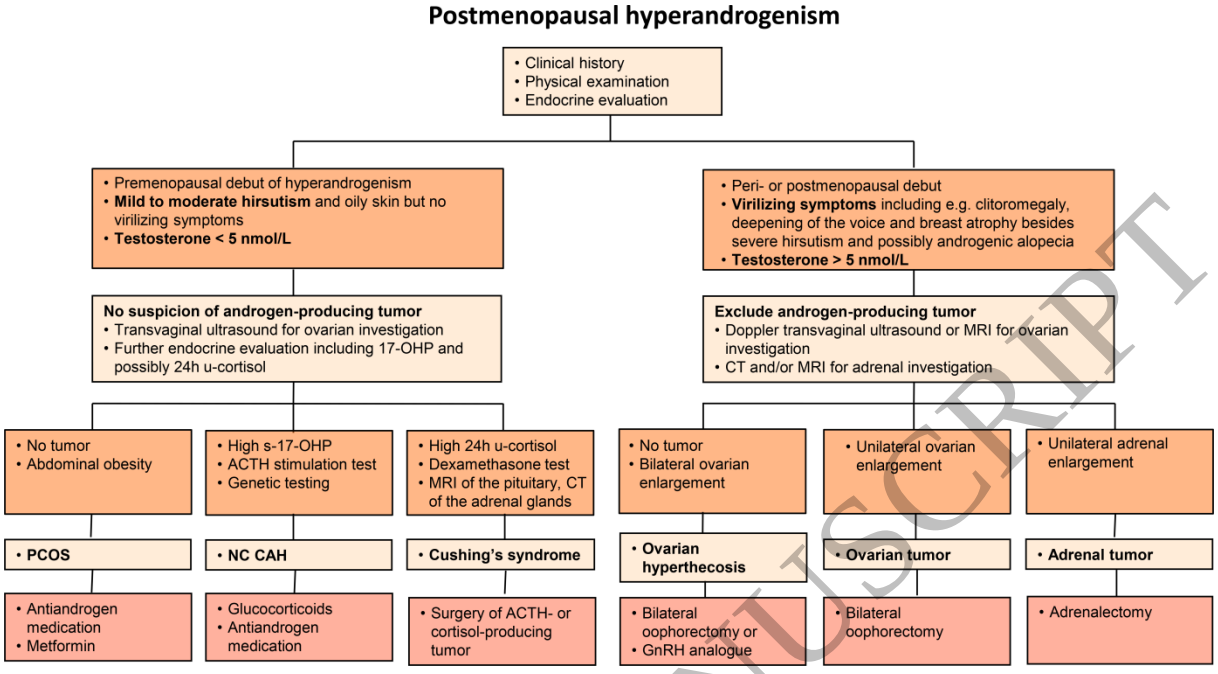

Figure 3
247x135 mm (.86 x DPI)