

ORIGINAL ARTICLE OPEN ACCESS

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Society for Endocrinology Clinical Practice Guideline for the Evaluation of Androgen Excess in Women

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Received: 27 January 2025 | **Revised:** 9 April 2025 | **Accepted:** 26 April 2025

Funding: Contributors in this work receive research funding from the following: MWOR: Health Research Board Emerging Clinician Scientist Award (ECSA-2020-001); A.A.: NIHR Clinician Scientist Award (CS-2018-18-ST2-002); S.C. received research funding from the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre; P.K.: NIHR Advanced Clinician Scientist Award (NIHR303671); R.K.S.: Wellcome Trust (WT210572).

Keywords: adrenal | androgen excess | ovarian | severe insulin resistance | testosterone | virilisation

ABSTRACT

Context: Androgen excess is common in women and refers to clinical or biochemical evidence of elevated androgenic steroids such as testosterone. It is associated with underlying polycystic ovary syndrome in the majority of cases. However severe androgen excess is less common and may indicate the presence of underlying adrenal or ovarian neoplasms, genetic disorders or severe insulin resistance syndromes. Currently there are few consensus guidelines to assist clinicians with a standardised management approach to the patient with severe androgen excess.

Design: Clinical practice guideline.

Methods: This guideline has been developed with expertise from colleagues in endocrinology, gynaecology, clinical biochemistry and nursing, and furthermore provides a unique patient perspective to guide clinicians.

Results: The Society for Endocrinology commissioned this new guideline to collate multi-disciplinary guidance for clinical practitioners in the investigation of severe androgen excess. Recommendations have been made in the areas of clinical assessment, biochemical work up, dynamic testing and imaging, informed where possible by the best available evidence.

Conclusion: This guideline will provide guidance for clinicians in their approach to patients with severe androgen excess.

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1 | Introduction and Methodology

Androgen excess is defined as clinical or biochemical evidence of increased production of androgenic steroids in women. It is observed in up to 10% of women of reproductive age and less commonly in postmenopausal women [1]. Androgen excess typically manifests clinically as hirsutism or acne, but more severe and prolonged exposure can lead to overt virilisation, including clitoromegaly, deepening of the voice, female pattern hair-loss (FPHL) or erythrocytosis [2]. Most adolescents and women of reproductive age who present to secondary or tertiary care with androgen excess will have underlying polycystic ovary syndrome (PCOS) [3]. However, other primary underlying pathologies must be excluded in a subset of patients harbouring red flag clinical features. These include severe biochemical disturbances, rapidly progressive symptoms or signs or presentation in the postmenopausal phase of life. This cohort of patients have increased likelihood of underlying neoplastic ovarian, adrenal or pituitary disease, or occasionally monogenic disorders of insulin signalling or androgen metabolism, compared to those with milder disturbances in the PCOS range [4]. A detailed clinical history, targeted physical examination and careful interrogation of the pattern and severity of biochemical disturbances are critical to rationalise the requirement and strategy for further biochemical, radiological and, where appropriate, genetic investigations. Although androgen excess in women with PCOS is closely correlated with adverse metabolic health outcomes throughout their life course [5, 6], severe biochemical disturbances or overt virilisation are rarely observed in PCOS. Therefore, a systematic diagnostic approach is required to investigate patients with typical red flag signs and symptoms.

The Society for Endocrinology (SfE) is a professional scientific organisation that promotes the advancement of scientific knowledge and best clinical practice in endocrinology, with over 3000 members across the United Kingdom, Ireland and internationally. This clinical practice guideline was commissioned by the Clinical Committee of the SfE, and MWOR was nominated as chair of the guideline group. A working group was established to reflect the breadth of multidisciplinary expertise required to support the guideline. Representatives from endocrinology, gynaecology, clinical biochemistry, nursing and patient support groups were nominated by the chair after a formal application process. The guideline scope was agreed at an early meeting and specific writing tasks were assigned to core group members based on their respective areas of expertise. A series of virtual meetings were held between March 2023 and September 2024. The group members performed narrative reviews of the literature in their assigned area and provided written reports in each case. Each report underwent internal peer review followed by subsequent revisions. In circumstances where consensus on content could not be reached a decision was made by the chair. An advanced draft of the manuscript was reviewed by the SfE Clinical Committee and underwent further revision before submission for publication. Grading of recommendations was not undertaken due to a paucity of high-quality data in the area of investigation of severe androgen excess in women.

2 | Androgen Metabolism in Women

Androgen metabolism in women involves complex interplay between the ovaries, adrenal glands and peripheral tissues (Figure 1). In the *classic* androgen pathway, androgenic precursors such as dehydroepiandrosterone (DHEA) and androstenedione (A4) are produced in large quantities by the zona reticularis of the adrenal cortex [7]. DHEA can be inactivated to its sulphated ester DHEAS by the action of DHEA sulfotransferase 2A1 (SULT2A1) which requires 3'-phosphoadenosine-5'-phosphosulfate (PAPS) for its catalytic activity. Mutations encoding the gene for PAPS synthase 2 (PAPSS2) are associated with impaired DHEA sulfation, resulting in a monogenic syndrome that is phenotypically similar to PCOS, featuring androgen excess and oligomenorrhoea [8]. DHEAS circulates in the micromolar range and represents the most abundant steroid in the human circulation; DHEAS may also be converted back to DHEA by the action of steroid sulfatase (STS), thereby releasing DHEA into the androgen pool for peripheral activation into more potent metabolites.

The zona reticularis of the adrenal cortex is responsible for adrenal androgen precursor synthesis [9], which is under the control of pituitary corticotropin (ACTH) [10]. The adrenal gland secretes several 19 carbon (C19) steroids, with DHEA and DHEAS produced in the greatest quantity [11]. The majority of DHEA is converted to A4 via oxidation of the 3-beta-hydroxyl group through the action of 3 β -hydroxysteroid dehydrogenase type 2 (3 β HSD2). Small quantities of the active androgen testosterone (T) can also be produced from A4 in the adrenal glands through the activity of aldoketoreductase type 1 C3 (AKR1C3) [12], but the bulk of circulating T in women is of ovarian origin (see below). DHEA and DHEAS are therefore considered biomarkers of adrenal androgen secretion, while A4 can originate from both ovaries and adrenals.

Androgen synthesis in the theca cells of the ovarian follicular unit is under tonic stimulation by pituitary luteinising hormone (LH). Ovarian thecal cells can generate DHEA *de novo* from cholesterol and pregnenolone through the actions of cholesterol side-chain cleavage enzyme and 17 α -hydroxylase, and thereafter it is converted to A4 and T [13]. Venous sampling studies have confirmed significant gradients between ovarian vein and peripheral samples for both T and A4, even after the menopause, with a significantly greater relative production of T from the ovary [14]. Serum T in women is therefore considered an accurate biomarker of ovarian androgen generation, particularly if adrenal precursors such as DHEA or DHEAS are not simultaneously elevated. Ovarian androgen generation may be amplified by insulin, which can act directly on theca cells to drive T production; it may also potentiate the steroidogenic response to LH [15]. Most T in women circulates bound to sex hormone binding globulin (SHBG) and other binding proteins such as albumin, such that only the free component is physiologically active and available to impact on target tissues. Insulin suppresses hepatic SHBG output, thereby increasing the pool of unbound active T. The relationship between insulin and androgen metabolism is therefore highly relevant in hyperinsulinaemic conditions such as PCOS, obesity and monogenic insulin resistance.

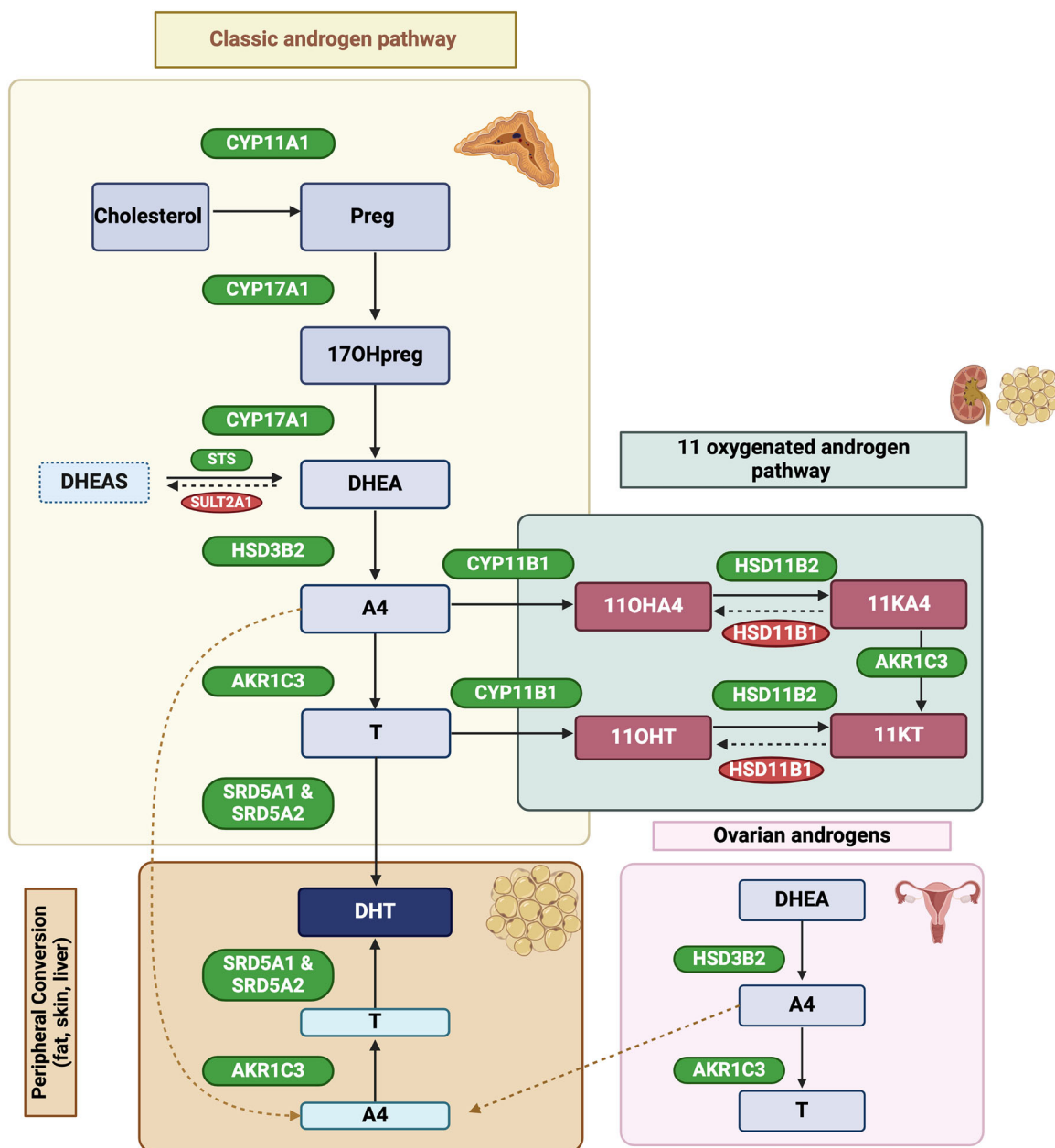


FIGURE 1 | Adrenal, ovarian and peripheral androgen metabolism. Both classic and 11-oxygenated androgen pathways are demonstrated. Androgenic precursors are secreted predominantly by the adrenal glands and activated to potent androgens in the ovaries and peripheral tissues. 11KA4, 11-ketoandrostenedione; 11KT, 11-ketotestosterone; 11OHA4, 11 β -hydroxyandrostenedione; 11OHT, 11 β -hydroxytestosterone; 17OHpreg, 17-hydroxypregnenolone; A4, androstenedione; AKR1C3, aldoketoreductase type 1C3; CYP17A1, cytochrome P450 17A1; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; HSD11B1, 11 β -hydroxysteroid dehydrogenase type 1; HSD11B2, 11 β -hydroxysteroid dehydrogenase type 2; HSD3B2, 3 β -hydroxysteroid dehydrogenase type 2; SRD5A1/2, 5 α -reductase type 1/2; STS, steroid sulfatase; SULT2A1, sulfotransferase family 2A member 1; T, testosterone.

Androgenic precursors such as DHEA and A4 can be activated to more potent active androgens in the periphery, depending on the expression of androgen-activating enzymes in local tissues. The enzymes HSD3B1 and HSD3B2 are expressed in skin, adipose, liver, brain and breast and can convert DHEA to A4 [16]. A4 is converted to potent T by the action of AKR1C3 in peripheral tissues [17]; AKR1C3 is highly expressed in subcutaneous adipose tissue, and its expression and activity are increased in women with PCOS and simple obesity [18, 19]. T can bind directly to the androgen receptor, but its effect can be markedly amplified by enzymatic reduction to

5 α -dihydrotestosterone (DHT), the most potent androgen, by isoforms of the enzyme 5 α -reductase. Systemic 5 α -reductase activity is upregulated in women with PCOS [20, 21]. SRD5A1, which encodes 5 α -reductase type 1, is expressed in the liver, kidney, skin and brain. SRD5A2 is highly expressed in genital skin and more efficiently reduces T to DHT compared to the type 1 isoform.

In addition to the classic pathway of androgen metabolism outlined above, there is an increasing focus on the poorly understood 11-oxygenated pathway in human health and disease.

11-oxygenated androgens have been shown to constitute a significant proportion of circulating androgens in a number of disorders of androgen excess including PCOS, congenital adrenal hyperplasia (CAH) and premature adrenarche [22]. A4 can be converted to the 11-oxygenated androgen precursor 11 β -hydroxyandrostenedione (11OHA4) by the activity of adrenal cytochrome P450 11 β -hydroxylase type 1 (CYP11B1, Figure 1). In tissues with 11 β -hydroxysteroid dehydrogenase type 2 (11 β HSD2) activity such as the kidney, 11OHA4 is converted to 11-ketoandrostenedione (11KA4), which then undergoes activation to 11-ketotestosterone (11KT) by AKR1C3 in peripheral tissues, particularly adipose; indeed, 11KA4 is a preferred substrate for AKR1C3 compared to A4 [23, 24]. 11KT binds and activates the androgen receptor (AR) with equivalent affinity to T [25], and circulating 11KT levels do not decline with age in women, a phenomenon that may have implications for metabolic health in the postmenopausal phase of life [26].

Adrenarche is the process of maturation of the adrenal zona reticularis with subsequent secretion of adrenal androgens derived from both the classic and 11-oxygenated pathways [27]. In children expansion of the zona reticularis leads to an increase in secretion of DHEA and DHEAS around the ages of 6-8 years. Clinical features include development of pubic and axillary hair, mild acne and adult body odour. After onset of puberty, relatively high levels of T secretion are observed in young girls. In early puberty serum levels correlate closely with DHEAS, indicating that T may be predominantly derived from adrenal precursor activation or direct adrenal secretion; in later puberty serum T levels are closely correlated with circulating oestradiol, indicating a primary ovarian origin under the control of gonadotropin secretion at that point [28].

Serum classic androgen precursors (A4, DHEA and DHEAS) and active androgens (T and DHT) decline with age in women [29]. This is due to involution of the zona reticularis of the adrenal gland with ageing, which reduces the pool of androgen precursors for peripheral activation to testosterone. Ovarian testosterone secretion also declines into the perimenopausal phase of life with evolving ovarian failure, however this process is more gradual than that of oestrogen deficiency [30]. Conversely, 11-oxygenated androgens do not change significantly with advancing age [26]. The biological reasons for this are unclear. The previous dogma that serum testosterone concentrations change significantly across the menstrual cycle is not borne out in recent studies using mass spectrometry-based analysis [31]. Fluctuations in serum concentrations of 11-oxygenated androgens across the menstrual cycle also appear to be negligible [29].

3 | Causes of Androgen Excess in Women (Table 1)

3.1 | Polycystic Ovary Syndrome (PCOS)

PCOS, with a prevalence between 8% and 13% in women of reproductive age [32, 33], is the most common cause of androgen excess in women. Hyperandrogenism is one of the key features of PCOS and is directly responsible for most of its features. The 2023 evidence-based international PCOS guideline

based on the Rotterdam criteria require two out of three features for the diagnosis of PCOS: (1) ovulatory dysfunction with irregular menstrual cycles, (2) polycystic ovarian morphology (PCOM) with increased numbers of antral follicles detected by ultrasonography or increased anti-Müllerian hormone (AMH) and (3) clinical or biochemical features of hyperandrogenism [32–34]. Therefore, hyperandrogenism is not required for the diagnosis of PCOS and up to 20% of those with PCOS do not display features of androgen excess [35]. However, as androgens are thought to have a role in the development of PCOM, it is unclear if those women have increased androgen synthesis or action within the ovary [36].

Androgens are synthesised in the theca cells of the ovarian follicle under the influence of LH. Although the increased numbers of antral follicles means that there are increased numbers of theca cells in the polycystic ovary, in PCOS each theca cell also has an increased capacity to synthesise androgens [37–40]. The mechanism of this is unclear but it is likely to be linked to the increased LH concentrations seen in PCOS as well as increased concentrations of insulin which enhances LH-stimulated androgen synthesis [36]. The aetiology of the increased LH and insulin concentrations is not well understood but it is likely to be prenatally programmed. Free androgens in the circulation are increased when SHBG concentrations are reduced. This is associated with insulin resistance and obesity which are both common in PCOS thereby exacerbating the hyperandrogenic phenotype [36].

In PCOS, as well as increased ovarian androgens, there is also evidence of increased adrenal androgen secretion [41, 42]. Distinct ovarian and adrenal androgen excess phenotypes may therefore co-exist. Women with PCOS show a greater decline in androgen concentrations after the suppression of adrenal steroidogenesis using dexamethasone than controls without PCOS [43]. Adrenal-derived 11-oxygenated androgens are a significant androgenic subclass in women with PCOS [44]. In addition, androgen concentrations are increased more after synthetic ACTH-stimulation in women with PCOS than in controls without PCOS [45]. Animal models have shown that, like the theca cells of the ovary, the steroidogenic cells of the adrenal zona reticularis have an increased capacity to synthesise androgens. Insulin may also act as a co-factor in adrenal as well as ovarian androgen synthesis [46].

3.2 | Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) comprises a group of autosomal recessive disorders which result from monogenic defects in selected critical steroidogenic enzymes [47]. Mutations in the *CYP21A2* gene on chromosome 6 causes 21-hydroxylase deficiency (21OHD) that accounts for 95% of cases. 21OHD may be subclassified into classic and non-classic phenotypes according to the severity of loss in enzymatic activity. The classical form is characterised by severely reduced or absent enzymatic activity, resulting in cortisol deficiency which can be associated with impaired aldosterone production in 75% of cases. Classic CAH typically manifests clinically in the neonatal period, presenting with ambiguous genitalia due to intrauterine virilisation at birth in females, or

TABLE 1 | Causes of androgen excess in women.

Condition	Prevalence or incidence	Virilisation	Rapidity of onset	Supportive biochemical features	Other features
Polycystic ovary syndrome	Prev: 8%–13% in premenopausal women	Not observed	Insidious, often since puberty	Mild-to-moderate elevations of serum T (typically < 51/L) Elevated A4 and DHEAS observed in the mild-to-moderate range depending on assay employed	Oligo/amenorrhoea Increased AMH levels Increased metabolic risk with variable degrees of insulin resistance clinically depending on BMI
Ovarian hyperthecosis	Prev: 9.3% in postmenopausal women with AE	Often present	Insidious	Increased serum T ranging from mild to severe (sometimes > 101/L) elevation Adrenal androgens usually normal > 50% suppression of serum T on GnRH analogue test	Bilateral enlarged ovaries/ increased ovarian volume on ultrasound Histology-ovarian stromal hyperplasia with cellular luteinisation May be observed in patients with insulin resistance and T2DM
Virilising ovarian tumour	Prev: 2.7% in postmenopausal women with AE	Present in 50%	Variable	Increased serum T usually > 51/L T > 101/L more likely VOT than OHT. A4 and/or E2 may also be increased. Adrenal androgens (e.g., DHEAS) usually normal Inhibin B and AMH may be increased Variable suppression with GnRH analogue test with significant overlap with OHT; no suppression if fully autonomous or suppressed gonadotropins	Difficult to visualise on imaging/asymmetry may be suggestive. MRI has higher PPV (78%) and NPV (100%) than US for detecting VOTs. MRI has 83% sensitivity and 80% specificity for differentiating VOT from OHT. Role for FDG-PET in selected cases if other imaging equivocal or negative
Non-classic congenital adrenal hyperplasia	Prev: 1%–10% in women with AE depending on population studied	Virilisation at birth in classic cases only; overt virilisation unusual in non-classic cases	Insidious, often since puberty	Increased 17OHP (typically > 101/L basal) as diagnostic hallmark Variable elevation of serum A4 and T DHEAS usually low in treated patients	Basal morning follicular phase 17OHP > 51/L-progress to SST. Stimulated 17OHP on SST > 301/L is diagnostic

(Continues)

TABLE 1 | (Continued)

Condition	Prevalence or incidence	Virilisation	Rapidity of onset	Supportive biochemical features	Other features
Adrenocortical carcinoma	Incidence: 1–2 cases/million population/year	Often present	Usually rapid (3–6 months)	Severe elevation in serum T (> 51/L) may be observed. T rarely elevated in isolation. Severe but variable elevations in DHEAS and/or A4 may be present. Clinical and biochemical cortisol excess (ACTH-independent) with failed ONDST may be observed. Increased adrenal steroid precursors on steroid metabolome analysis.	Unilateral adrenal mass should be visible on cross-sectional imaging
Cushing's disease	Incidence: 1.8–3.2 cases/million population/year 1% of premenopausal AE, and 4% of PM AE	Seldom present	Variable	Highly variable. Serum T/DHEAS/A4 may be normal or elevated Failed ONDST with detectable or elevated ACTH levels at baseline. May co-exist with gonadotropin deficiency.	Cushing's syndrome features: Discriminant: Violaceous abdominal striae, proximal muscle weakness, osteoporosis Non-specific: abdominal obesity, interscapular fat pad, bruising, hypertension
Severe insulin resistance	Not clearly defined. May be congenital or acquired. Most common in severe obesity but also seen in lean to mildly obese people with lipodystrophy or insulin signalling defects.	May be present in severe cases	Most commonly insidious from puberty and worsened by weight gain. Rarely can be acute and fulminant in acquired autoimmune insulin resistance (Type B IR).	Serum T ranges from normal to extremely elevated (> 20 nmol/L) Severely increased plasma insulin and HOMA-IR and suppressed plasma adiponectin Usually high triglyceride, low HDL cholesterol, evidence of fatty liver (if not, then consider INSR mutation or anti-INSR Ab)	Ovulatory dysfunction PCOM Acanthosis nigricans Often family history of T2DM Lipodystrophy or centripetal obesity Wide range of syndromic features sometimes seen
Acromegaly	Prevalence: 2.8–13.7 cases/100,000 Incidence: ~ 0.21.1 cases/100,000 per year	Seldom present	Insidious onset	Elevated IGF1; failure to suppress growth hormone below 0.4 ng/mL on OGTT Often biochemical evidence of androgen excess in women	Coarsening of facial features; enlarged hands; interdigital separation; thyroid goitre; evidence of cardiomyopathy in advanced cases

Abbreviations: A4, androstenedione; AE, androgen excess; AMH, anti-Müllerian hormone; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; E2, oestradiol; INSR, insulin receptor; NPV, negative predictive value; ONDST, overnight dexamethasone suppression test; OHT, ovarian hyperthecosis; PCOM, polycystic ovarian morphology; PCOS, polycystic ovary syndrome; PPV, positive predictive value; SIR, severe insulin resistance; SST, short synthetic ACTH-stimulation test; T, testosterone; T2DM, type 2 diabetes mellitus; VOT, virilising ovarian tumour.

with a salt-wasting adrenal crisis in males during the first 2–3 weeks of life. In turn, cortisol deficiency leads to increased pituitary ACTH secretion and hyperplasia of the adrenal glands. Steroidogenic precursors proximal to the enzymatic defect accumulate and are shunted down the pathway of androgen synthesis, resulting in severe clinical and biochemical androgen excess, often with overt virilisation. New-born screening data report the incidence of classical CAH at 1:10,000 to 1:20,000 live births [48, 49] depending on the population studied. The non-classic form is milder due to relative preservation of cortisol and aldosterone production (25%–50% residual enzyme activity) [50], with varying degrees of postnatal androgen excess. The true prevalence of non-classic CAH is unclear; estimated at 1:200 to 1:1000 in the general Caucasian population [51], but up to 1:50 to 1:100 among populations with high rates of consanguinity [52]. CAH may be the cause in up to 10% of women with hyperandrogenism, as opposed to up to 80% for PCOS [53].

Women with non-classic CAH may present in adolescence or adulthood with hirsutism, ovulatory dysfunction, or infertility or may even be asymptomatic, and are often clinically indistinguishable from women with PCOS. Importantly, women with non-classic CAH may show a PCOM appearance on transvaginal ultrasound in 30%–40% [53–56]. Women with CAH are less likely to have oligomenorrhoea than those with PCOS (17%–30% vs. 80%) [53], whereas raised LH, insulin resistance and higher bodyweight are more common in PCOS.

A family history of CAH is an important clue to the diagnosis. Identifying women with non-classic CAH highlights the selective use of glucocorticoids as a treatment option for hirsutism, beside other measures such as the combined oral contraceptive pill, but also has wider implications, for example for fertility and pre-pregnancy genetic counselling. Therefore, 17OHP should be included in the initial evaluation of women with suspected androgen excess. Whereas a raised 17OHP above 30 nmol/L is considered diagnostic for CAH, a mildly raised 17OHP > 5–7 nmol/L taken in the early follicular phase should alert clinicians to the possibility of underlying CAH and trigger a synthetic ACTH-stimulated 17OHP measurement [48]. It should be noted that 17OHP levels may also be significantly elevated in patients with adrenocortical carcinoma and therefore adrenal imaging is recommended at diagnosis in adulthood.

3.3 | Ovarian Hyperthecosis (OHT)

OHT results from hyperplasia of androgen-secreting theca cells in the ovary. It is thought that some of the steroidogenically inactive ovarian stromal cells differentiate into theca-like cells capable of producing androgens. That means they are spread through the ovarian stroma and not purely associated with the ovarian follicles. These theca cells produce androgens under the influence of LH. This condition is usually seen after menopause [1], where there are high concentrations of LH, after the ovary has few, if any, remaining follicles able to aromatise androgens to oestrogen [45]. The cause of OHT is unknown, but genetic factors may be implicated. High insulin levels are also associated with ovarian hyperthecosis. Although androgens can increase insulin concentrations, hyperinsulinaemia is likely to

be the primary driver of hyperthecosis, albeit potentiated by positive feedback from increased androgens.

Another unusual and self-limiting cause of hyperthecosis is the rare luteoma of pregnancy [57]. The corpus luteum of pregnancy is formed from the cells of the post-ovulatory follicle, and its main role is the production of progesterone to support the pregnancy. However, as it contains theca cells, it also produces androgens. The pregnancy hormone human chorionic gonadotrophin (hCG) acts through the LHCG receptor to promote progesterone and androgen synthesis. The cause of luteoma of pregnancy is unknown, but the theca cell volume and activity expand markedly. A luteoma can grow in size to over 6 cm, produces large amounts of androgens [58], and regresses after delivery with normalisation of androgens. Although the mother can be virilised during pregnancy, a female foetus is relatively protected by the high expression of placental aromatase [58].

3.4 | Virilising Ovarian Tumours (VOTs)

VOTs are predominantly benign tumours that can present at any age, with 25% presenting postmenopausally [59]. The prevalence of VOTs in post-menopausal (PM) women with hyperandrogenism (HA) is around 2.7% [1]. Approximately 5%–8% of ovarian tumours are hormonally active and 1% secrete androgens [59–61]. Androgen-secreting tumours derive from sex-cord and stromal cells of the ovary (Figure 2) [62].

Sertoli-Leydig Cell Tumours (SLCT) are the most common type in premenopausal women, whereas Leydig cell tumours (LCT) are the most common pathology after the menopause [61]. Granulosa cell tumours (GCT) account for 70% of pure sex cord tumours and are the commonest cause of hyperoestrogenism [61].

3.4.1 | Pure Stromal Tumours

3.4.1.1 | Thecoma (Theca Cell Tumours). Only 10% of thecomas secrete androgens. They usually secrete oestrogens and, therefore, may present with abnormal menstrual bleeding. They are usually benign and occur in postmenopausal women [61].

3.4.1.2 | Sclerosing Stromal Tumours. These are rare (< 5%) and usually present in younger patients (< 30 years). These are benign and typically unilateral [61].

3.4.1.3 | LCTs. These are small tumours (< 3 cm), usually isoechogenic on ultrasound. They are mostly unilateral and benign, but 20% are associated with peritoneal metastases. They typically present in postmenopausal women; 75% are hormonally active with rapid virilisation in 30%. Reinke crystals are seen on histopathology. The non-hilar subtype has distinct resemblance to a lipid cell luteoma of pregnancy [61].

3.4.1.4 | Steroid Cell Tumours (SCTs). These are seen in premenopausal women, and 50% are androgen-producing. However, they can also produce other hormones. Reinke crystals are not seen on histopathology.

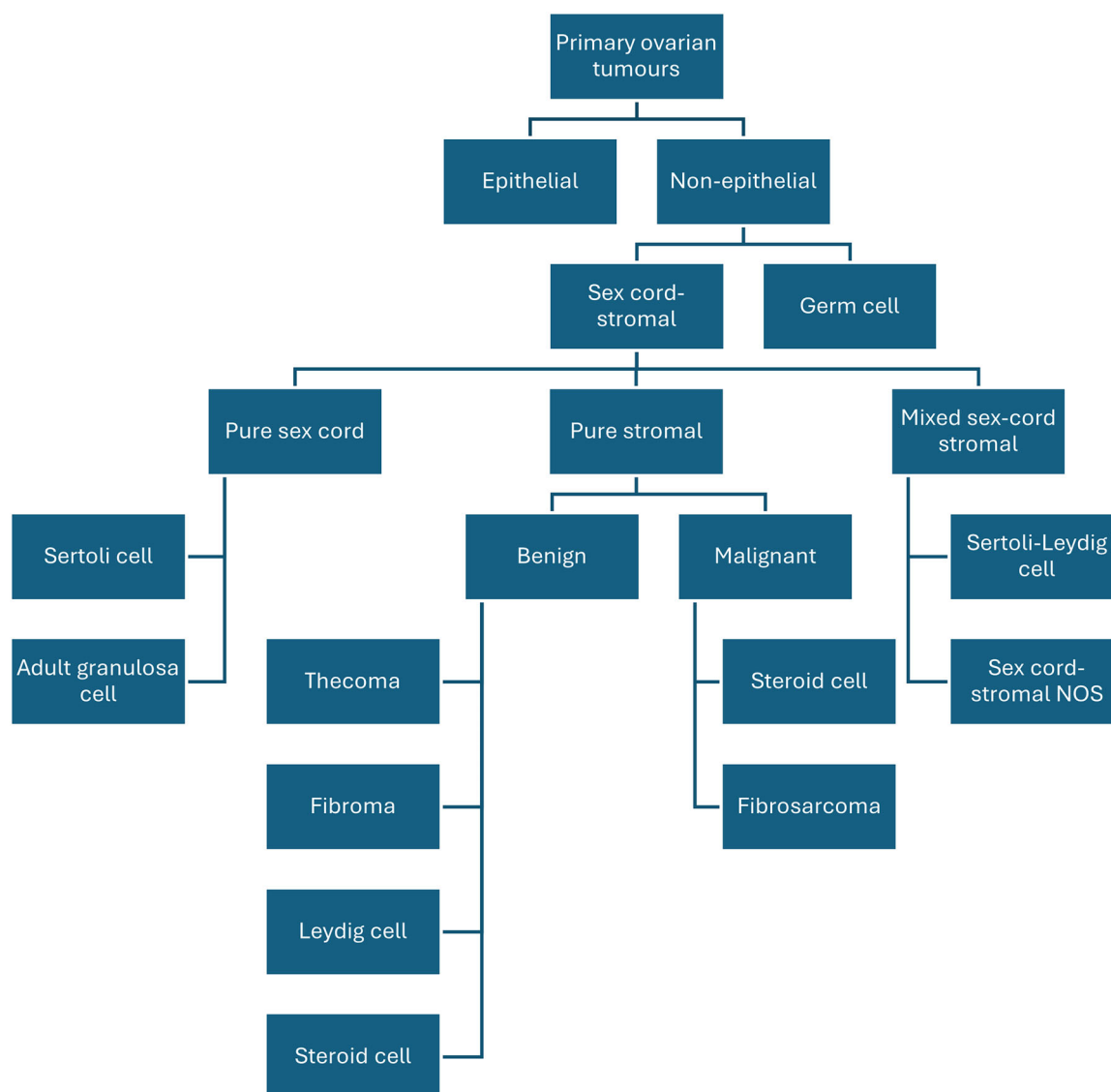


FIGURE 2 | Classification of primary ovarian tumours (VOTs): Primary ovarian tumours can be grouped according to the WHO classification [62] into epithelial and non-epithelial. Androgen-secreting tumours typically arise from sex-cord and stromal cells of the ovary.

3.4.2 | Pure Sex Cord Tumours

3.4.2.1 | Granulosa Cell Tumours (GCT). These malignant tumours represent 5% of all malignant ovarian tumours. Adult GCTs (95% of GCT) occur mainly in postmenopausal women. Juvenile GCTs occur in children and women under 30 years of age. They are mainly oestrogen-producing, but 10% secrete androgens; they frequently co-secrete inhibin B and AMH.

3.4.2.2 | Sertoli Cell Tumours. These are rare and usually nonfunctional.

are usually unilateral and detected at an early stage (Stage 1), posing a management dilemma as effective treatment to prevent recurrence is balanced with preservation of fertility. SLCTs can secrete oestrogen, or less commonly both androgens and oestrogens. Imaging findings include a mixture of a solid mass with multiple cystic spaces on imaging. They can be grouped as androblastomas (Sertoli cell tumours, Sertoli-Leydig cell tumours, Leydig cell tumours) that predominantly secrete androgens, and thecomas and granulosa cell tumours that predominantly secrete oestrogens [59]. Oestrogen excess causes postmenopausal bleeding, endometrial hyperplasia or cancer, although androgens from androblastomas can also be aromatised to oestrogens.

3.4.3 | Mixed Sex Cord-Stromal Tumours

3.4.3.1 | Sertoli-Leydig Cell Tumours (SLCT). These are rare and account for 0.5% of ovarian tumours. 50% are hormonally active, although this rises to 75% in women under 30 years. They

3.4.4 | Other Androgen Producing Tumours

Other tumours that can secrete androgens include metastases (mostly of gastric origin e.g., Krukenberg tumour), primary mucinous cystic tumours, teratomas, carcinoid and Brenner

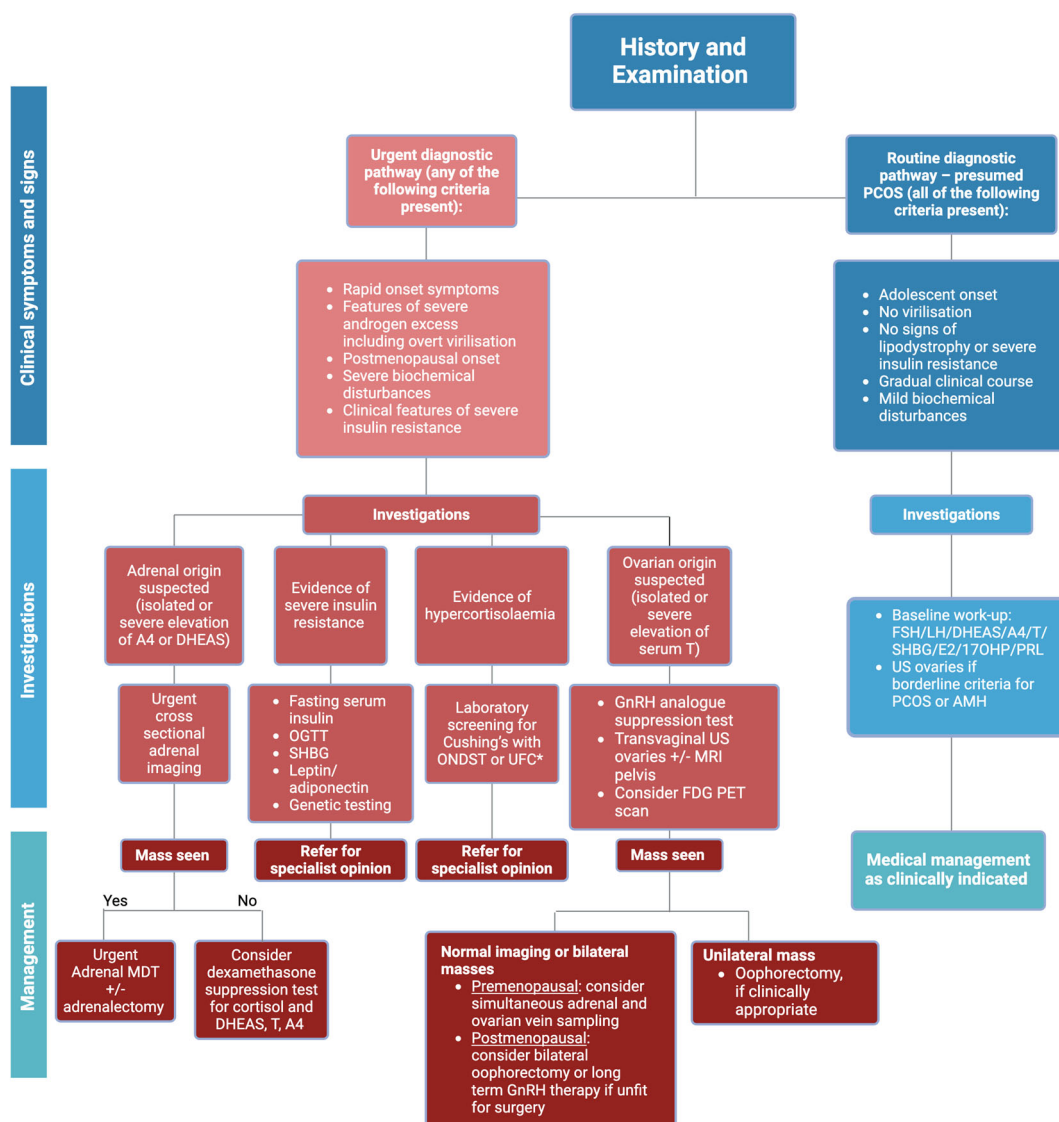


FIGURE 3 | Clinical algorithm for the investigation of androgen excess in women. The diagnostic approach should be divided into *routine* or *urgent* pathways based on the presence or absence a number of central clinical and biochemical factors. Baseline biochemical work up: FSH, LH, oestradiol, 17-hydroxyprogesterone (17OHP), testosterone (T), dehydroepiandrosterone sulphate (DHEAS), androstenedione (A4), sex hormone-binding globulin (SHBG) and prolactin (PRL). FSH, follicle-stimulating hormone; LH, luteinising hormone; OGTT, oral glucose tolerance test; ONDST, overnight dexamethasone suppression test; PCOS, polycystic ovary syndrome; PET, positron emission tomography; UFC urinary free cortisol.

tumours (usually benign, ovarian tumours of surface epithelium usually presenting in postmenopausal women) [61].

3.4.5 | Clinical Presentation of Ovarian Tumours

VOTs may present with rapid onset hirsutism or acne, and less commonly in severe cases with virilisation (male-pattern baldness, clitoromegaly, breast atrophy, muscle hypertrophy, deepening of the voice) [61]. Due to their small size, ovarian androgen-secreting tumours may not be detected on imaging by transvaginal ultrasound with colour doppler, or on CT/MRI/FDG PET [61]. Ovarian volumes ≥ 4.0 cm [63] with asymmetry ≥ 2.0 cm [64, 65] are suggestive of VOT. Bilateral increased ovarian stroma on ultrasound is characteristic of OHT [61].

Stromal content of SLCTs and Leydig cell tumours may be detected as signal intensity on T2-weighted MRI [61]. The prognosis is good with bilateral salpingo-oophorectomy with 92% survival if discovered at Stage 1 [61, 66].

3.5 | Cushing's Syndrome

Hyperandrogenism is present in up to 50% of cases of Cushing's syndrome, particularly due to ACTH-driven adrenal androgen excess. Clinical features of Cushing's syndrome include violaceous abdominal striae, abdominal obesity, proximal muscle weakness, interscapular fat pad, easy bruising, poor wound healing, osteoporosis, hypertension and glucose intolerance [67]. Excess cortisol secretion may be ACTH-dependent due to

either a corticotroph pituitary adenoma (Cushing's Disease, 70% of cases) or neoplastic ectopic ACTH secretion (10% of cases). Up to 20% of cases are ACTH-independent due to adrenal disease such as adrenocortical carcinoma (ACC, see below) [68]. ACTH-independent causes of androgen excess due to an adrenal mass must be presumed to be an ACC unless proven otherwise, as adrenal androgen excess in a woman in the presence of an adenoma is an unusual phenomenon, with some notable exceptions [69]. Detailed discussion of Cushing's syndrome is beyond the scope of this guideline.

3.6 | Adrenocortical Carcinoma

Adrenocortical carcinoma (ACC) is a rare malignancy with an incidence of 0.7–2 per million per year [70, 71]. Clinical and/or biochemical androgen excess is present in 40%–60% of patients with ACC [72]. Clinical features of glucocorticoid excess are also commonly present. The detection of a large (> 4 cm) radiologically indeterminate adrenal mass with concomitant ACTH-independent glucocorticoid and androgen excess should be regarded as ACC until proven otherwise. Serendipitous detection of an adrenal mass is also an important mode of ACC detection, and all such incidental masses should be evaluated for possible underlying malignancy [73]. Although ACC can occur in any age, the median age of diagnosis is in the fifth to sixth decade. In all age groups, there is a predilection for female sex (ratio 1.5–2.5:1).

The presence of rapidly progressive severe androgen excess, particularly with an isolated or predominant increase in adrenal androgens such as DHEAS and A4, should alert clinicians to the possibility of an underlying adrenal tumour. Cross-sectional imaging of the adrenal glands should be considered early in the evaluation of such cases, without necessarily waiting for the results of the full hormonal work up. If an adrenal mass is detected, patients should be urgently referred to expert centres for further evaluation and management.

3.7 | Syndromes of Severe Insulin Resistance (SIR)

Syndromes of SIR are a heterogeneous group of disorders that may be genetic or acquired. They are sometimes attributable to primary defects in insulin action, for instance due to monogenic changes directly affecting the insulin signalling pathways for example, in the insulin receptor (INSR), or to autoantibodies downregulating the insulin receptor (Type B insulin resistance). Far more commonly, however, they are caused by acquired or genetic defects in adipose tissue development or function, usually manifest as anatomical lipodystrophy [74], which sometimes occurs in the context of more complex developmental syndromes.

SIR most commonly presents around puberty in females (which may be precocious). Despite the severe metabolic derangements that define it, the sentinel complaint often relates to ovulatory dysfunction and symptoms of androgen excess. Consideration of SIR when evaluating a patient with hyperandrogenism is thus important in ensuring timely diagnosis. Suspicion of SIR should arise in patients with clinical or biochemical features of insulin resistance that is *disproportionate* to their body mass index (BMI) and/or a strong family history of insulin resistance or diabetes. Acanthosis nigricans and/or skin tags are important clinical clues to the presence of IR and should always be sought. Atypical distribution of adipose tissue, including a Cushingoid habitus, and unusually severe fatty liver, dyslipidaemia, history of pancreatitis and a muscular appearance of the limbs are strong clues to underlying lipodystrophy (Table 2).

Arbitrary biochemical definitions of SIR are sometimes suggested. These include a fasting insulin concentration > 150 pmol/l, or a peak insulin on glucose tolerance testing of > 1500 pmol/l in those with a BMI < 30 kg/m² and normal glucose tolerance. In those with absolute insulin deficiency, an exogenous insulin requirement of greater than 3 units/kg/day is also suggestive [75].

TABLE 2 | Clinical and biochemical features of severe insulin resistance in patients with severe androgen excess.

	Common insulin resistance	Lipodystrophy	INSR mutation
Body habitus	Obese	Lean, muscular limbs; absent adipose tissue globally or regionally. Centripetal pattern common ('Cushingoid'). Sometimes pseudoacromegalic.	Usually lean but may be overweight/obese. Sometimes pseudoacromegalic
Acanthosis nigricans	+	++	++
Fasting insulin ^a (pmol/l)	Highly variable. May be as severe as in SSIR, but in context of severe obesity	> 150 (> 1500 on OGTT)	> 150 (> 1500 on OGTT)
Androgen excess	+	++	++
Dyslipidaemia	+	++	–
Fatty liver disease	+	++	–
Adiponectin	↓	↓	Preserved or ↑
SHBG	↓	↓	Preserved or ↑

^aSevere elevation confirms IR but does not discriminate subtypes.

However, many patients have partial pancreatic decompensation, making it difficult to evaluate insulin concentrations precisely in the context of hyperglycaemia. Thus, diagnosis usually relies on a combination of clinical and biochemical features. Biochemical testing may also help to identify SIR subtypes. Patients with INSR mutations are protected from hepatic steatosis and dyslipidaemia, even though these are usually central features of lipodystrophy. While serum adiponectin concentration is preserved or increased in insulin receptor signalling defects, it is reduced in lipodystrophy or obesity (Table 2).

Female patients with SIR typically have PCOM on pelvic imaging and may sometimes develop large ovarian cysts. Androgen-secreting tumours are also reported in some extreme cases of IR, especially where it is longstanding. Biochemical androgen excess is commonly severe and may be characterised by extreme elevations in serum T [76] in excess of 10 nmol/L and overt virilisation [77]. Suppression of serum SHBG is observed in SIR related to lipodystrophy and obesity-related IR, while normal or elevated levels are observed in those with INSR mutations. Relationships between total and free androgens thus vary according to SIR subtype [77], and grossly elevated SHBG may sometimes make a major contribution to extreme total testosterone elevation in women with INSR dysfunction. Recent data further suggest that 11-oxygenated and adrenal androgens may help in differentiating SIR subtypes. Patients with SIR due to INSR mutations appear to be relatively protected from severe adrenal and 11-oxygenated androgen excess compared to patients with lipodystrophy, suggesting that adrenal INSR signalling is required for stimulation of adrenal androgen output [46].

Importantly, severe hyperinsulinemia appears to act synergistically with gonadotropins to drive ovarian androgen production as it does to a lesser extent in PCOS and OHT. Key evidence for this is that, in both pre- and post-receptor SIR, high doses of GnRH analogues result in near complete suppression of serum T, indicating that effects of hyperinsulinaemia on ovarian androgen production require intact LH action. This can be exploited both diagnostically and therapeutically: a single dose of GnRH-analogue may be used to demonstrate that elevated circulating T is ovary-derived, while repeated doses may be used therapeutically to suppress androgens. It should be noted that this will not suppress 11-oxygenated or adrenal androgen output in those with lipodystrophy, leading to speculation that the therapeutic benefit of GnRH-analogues is greatest in those with INSR mutations.

Patients with androgen excess and SIR should undergo diagnostic testing and management guided by national specialist centres. For those with lipodystrophy, lifestyle, pharmacological and sometimes surgical treatments aiming to reverse positive energy balance are the cornerstone of management even in women with BMI well below the commonly applied thresholds for these interventions.

3.8 | Acromegaly

Acromegaly is a disease caused by growth hormone (GH) excess resulting in excess hepatic insulin-like growth factor-1 (IGF-1) production, which causes enlargement of extremities, abnormal

glucose and lipid metabolism, as well as an impact on gonadal function. GH is a lipolytic hormone, and excess GH causes insulin resistance [78]. Previous studies have described that clinical and biochemical characteristics of oestrogen-sufficient women with acromegaly are consistent with a PCOS-like picture [79, 80]. These include features of hyperandrogenism, with or without menstrual irregularity, normal serum E2 levels, increased free androgen levels (normal total T and low SHBG) and LH hyperresponsiveness to GnRH stimulation [81–83]. IGF-1 may also directly impact on ovarian steroidogenesis [84]. There are IGF-1 receptors on thecal cells, and IGF-1 may directly stimulate the activity of 17-hydroxylase and 17,20 lyase, upregulating ovarian androgen production [85]. Insulin resistance can also cause increased adrenal androgen biosynthesis [86]. Excess GH is associated with lower SHBG alongside preservation of total T levels. This could be due to a direct effect of IGF1 on SHBG production or an indirect effect of raised insulin levels.

3.9 | Disorders of Sexual Development (DSD)

DSD may present with severe androgen excess in patients who are phenotypically female and who are assigned female sex at birth with either 46XX or 46XY karyotype [87]. The majority of patients with 46XX with androgen excess have 21OHD CAH and are diagnosed with ambiguous genitalia at birth. Therefore, 46XX DSD patients are less likely to present to adult services with severe androgen excess. However, non-classical 21OHD may present with mild androgen excess and ovulatory dysfunction in the late teenage years similar to PCOS. 46XX DSD due to 21OHD is discussed in more detail above.

Patients with 46XY DSD who are raised as females may present to either paediatric or adult services with severe clinical and/or biochemical androgen excess in adolescence [88]. A detailed discussion of 46XX or 46XY DSD disorders as an underlying cause of severe androgen excess in women is beyond the scope of this guideline, however, a number of relevant conditions are discussed briefly below.

Deficiency of 5 α -reductase due to mutations in the *SRD5A2* gene causes 46XY DSD with under-virilisation due to impaired conversion of T to DHT. Over 70% of patients are typically assigned female gender at birth [89]. The majority of patients have a significant increase in serum T at puberty, with subsequent conversion to DHT by the type 1 isoenzyme of 5 α -reductase (*SRD5A1*), which is only expressed significantly in the skin from puberty. This invariably results in marked virilisation and severe biochemical androgen excess in patients who were assigned female sex at birth. Similarly, mutations in the gene encoding 17 β -hydroxysteroid dehydrogenase type 3 (*HSD17B3*) result in impaired testicular conversion of A4 to T in 46XY patients, with under-virilisation at birth and frequent assignment of female sex depending on the severity of the mutation [90]. After the onset of puberty, 17 β -hydroxysteroid dehydrogenase type 3, which is then highly expressed in adipose tissue, can convert A4 to T, resulting in virilisation and potential sex reversal. Heterozygous mutations in *NR5A1* (encoding steroidogenic factor 1, SF1) are also one of the most common causes of 46XY DSD, with gonadal dysgenesis and

rarely adrenal failure [91]. Rare cases of sex reversal with overt virilisation and severe androgen excess in adolescence have been described in the literature in patients with underlying 46XY DSD due to this condition who had been assigned female sex at birth [92].

Androgen receptor (AR) gene mutations are the most frequent cause of 46XY DSD and encompass a spectrum of disease from complete (phenotypic female) to partial (assigned male or female sex depending on severity) [93]. The 46XY DSD due to complete androgen insensitivity syndrome (CAIS) is the clinical consequence of a dysfunctional AR, which cannot mediate the genomic effects of androgen binding. Female patients with CAIS cannot, by definition, present with virilisation or clinical signs of androgen excess. However severe biochemical elevation of serum T within or above the normal male reference range may be observed at diagnosis, typically with co-elevation of gonadotropins. Significant T elevations in phenotypic females presenting with raised gonadotropins and primary amenorrhoea without clinical features of androgen excess should strongly raise the clinical suspicion of this condition and chromosomal and genetic testing for DSD should be instituted.

4 | Health Consequences and Metabolic Associations of Androgen Excess

Androgen excess, regardless of aetiology, is strongly associated with metabolic disorders, encompassing insulin resistance, abdominal fat accumulation and obesity [33, 45, 94]. This association persists across a wide spectrum of severity, from severe monogenic disorders such as CAH to milder conditions such as PCOS. Whilst a minor subset of AE disorders, typified by virilising tumours, manifest abruptly and severely, most AE disorders are lifelong conditions that predispose to metabolic diseases [18]. Despite limited longitudinal studies on CAH, available research underscores adverse cardiometabolic outcomes, including hypertension, insulin resistance and dyslipidaemia among patients with non-classic CAH or milder glucocorticoid deficiency phenotypes identified in adulthood, that is, even in cohorts in whom supraphysiological glucocorticoid replacement doses cannot be implicated [95, 96]. PCOS, which constitutes the majority of cases involving AE in women, has been associated with conditions such as metabolic syndrome, sleep apnoea, depression and endometrial carcinoma in extensive longitudinal studies [97–99]. A 2019 systematic review, including 59 publications in the meta-analysis and 27 studies in the meta-regression, robustly confirms the association between PCOS and metabolic syndrome [100].

4.1 | Obesity and Body Composition

Compared with controls matched for age and BMI, women with non-classic CAH had higher rates of obesity in a Swedish cohort study [101]. In addition to higher rates of obesity, androgen excess is associated with visceral adiposity and centripetal fat distribution [102–105]. This may explain in part the associations between AE and metabolic dysfunction. Anthropometric studies and meta-analysis by Lim and colleagues further affirm an

augmented prevalence of central obesity in women with PCOS [100]. This relationship is likely to be bi-directional, with elevated androgens observed in patients with simple obesity, likely reflecting the endocrine role of adipose tissue as a site of androgen generation [18].

4.2 | Fatty Liver

The association between hyperandrogenism and metabolic dysfunction-associated steatotic liver disease (MASLD) is complex and difficult to disentangle from insulin resistance. Several cross-sectional studies have shown an increased prevalence of MASLD in women with PCOS independent of BMI [106–109] and this has also been described in a large epidemiological study comparing 63,000 women with PCOS and 121,000 matched controls [110]. Serum T levels greater than 3.0 nmol/L were linked to a higher risk of MASLD, with the risk increasing further with worsening biochemical androgen excess. There was an almost three-fold increased risk of MASLD at baseline, and even lean women with PCOS have an almost two-fold increased risk [110]. Androgens, including testosterone, have been implicated in influencing lipid metabolism and contributing to insulin resistance. This altered lipid metabolism may lead to increased fat accumulation in the liver, resulting in MASLD [111].

4.3 | Dysglycaemia

Hyperandrogenic conditions such as PCOS have been strongly associated with insulin resistance and glucose intolerance in women. Persson and colleagues highlighted a greater risk of type 2 diabetes mellitus (T2DM) among women with PCOS with a hyperandrogenic phenotype compared to those with a normoandrogenic phenotype [112]. This was also seen in a previous study whereby the severity of dysglycaemia increased with the severity of the androgen excess [113]. In a large retrospective cohort study, T2DM increased significantly with serum T concentrations greater than 1.5 nmol/L [114]. Furthermore, women with PCOS with glucose intolerance exhibited a higher free androgen index (FAI) compared to those with normal glucose tolerance, even after adjusting for confounders [115]. A 2019 systematic review and meta-regression analysis of 40 eligible studies found a significantly higher prevalence of impaired glucose tolerance and T2DM in women with PCOS [116]. The risk varied by ethnicity, with a five-fold increase in Asian women and three-fold in European women for impaired glucose tolerance. Additionally, the risk was more pronounced with obesity and doubled in studies using self-reported or administrative data for T2DM diagnosis. Notably, the ethnicity-related difference persisted in BMI-matched Asian and European subgroups [116]. Supporting a role for androgen excess, a study investigating untreated children with non-classic CAH reported an increase in insulin resistance [95].

4.4 | Cardiovascular Disease

Women with PCOS face prolonged exposure to a constellation of cardiovascular risk factors, including obesity, insulin

resistance, abnormal glucose metabolism, dyslipidaemia and hypertension. Studies have shown that women with PCOS have an increased incidence of major cardiovascular events [5]. However, a lack of prospective longitudinal research with fully characterised cohorts means that it remains uncertain if androgen excess is a primary driver of increased cardiovascular events. The 2023 PCOS evidence-based guideline designates PCOS as a CVD risk, albeit based on low-quality evidence [33]. A recent 22-year prospective Finnish study with robust phenotyping reaffirmed this, showing higher cardiovascular risks in women with PCOS versus controls [117, 118]. The majority of metabolic studies in CAH have focused on children or adolescents; however, a Swedish cohort of 588 patients with CAH of all ages showed an increased risk of CVD (OR 2.7) compared to the reference population; in particular women with non-classic CAH were at increased risk [119]. Virilising tumours do not have data or longitudinal follow-up regarding the risk of CVD owing to their rare incidence, however, case reports support an increased prevalence of cardiovascular disease [45, 120].

5 | Clinical Evaluation of the Patient With Features of Androgen Excess

5.1 | History

When taking a clinical history from a patient presenting with features of androgen excess, it is important to document a thorough menstrual history and to clarify the presence of specific features including acne, hirsutism and alopecia. The rapidity and age of symptom onset are important discriminators and so these features along with duration and severity of symptoms should be established (Table 3). Late and rapid onset of virilising signs and symptoms are suggestive of an underlying androgen-producing tumour. In contrast, early onset of mild features of androgen excess that progress only slowly are more in keeping clinically with PCOS. Given the nature of signs and symptoms of androgen excess, which go right to the core of body image, the history should also cover the psychological impact of these on the patient.

5.1.1 | Acne

Acne is common in adolescence and young adulthood and is estimated to affect 45%–85% individuals aged 18–25 years [121, 122]. Treatment-resistant acne is often indicative of hyperandrogenism in puberty. Androgen-related acne may continue into adulthood (persistent acne), may occur for the first time in adulthood (adult-onset acne) or may re-present after resolution in adolescence (recidivant acne). Postmenopausal acne is uncommon, and where severe features of this occur, a prompt assessment of androgen levels should be undertaken [1]. Whilst it may occur in the absence of other features of androgen excess, elevated androgen levels are identified in over 50% of women with acne [123]. Details of additional precipitants that may contribute to acne should be recorded including the use of comedogenic make-up [124, 125], lifestyle stressors [126, 127] and smoking [128].

TABLE 3 | Summary of recommendations.

Clinical evaluation	
1	Women presenting with clinical features or suspicion of androgen excess should be assessed for hirsutism, acne, female pattern hair-loss and virilisation.
2	Older patient age and rapid onset and progression of symptoms should be considered as predictive factors for underlying non-PCOS pathology.
3	Assessment for syndrome-specific clinical features should be undertaken, for example, Cushing's syndrome, severe insulin resistance.
4	Women with a suspicion for neoplastic androgen excess should be urgently referred to a centre with relevant expertise.
Biochemical evaluation	
1	Testosterone, SHBG, A4, DHEAS, 17OHP, E2, LH and FSH, as well as a measure of free T, should be measured in all women presenting clinical features or suspicion of androgen excess.
2	Samples for the investigation of androgen excess should be collected in the morning following an overnight fast. <i>Where possible</i> samples should be collected in the early follicular phase.
3	Evidence of severe biochemical androgen excess (e.g., serum T > 5 nmol/l) should trigger urgent ovarian and/or adrenal imaging.
4	LC-MS/MS-based androgen measurement should be undertaken where possible due to limitations with immunoassay including cross-reactivity at low circulating androgen concentrations; in centres where immunoassay is used, a second sample should be run by LC-MS/MS if the immunoassay-based androgen measurement does not align with the clinical picture.
5	Dynamic testing (e.g., GnRH analogue test or 96hr dexamethasone suppression test) should be considered in selected cases to aid the in the localisation of the source of androgen excess (Figure 3).
6	Syndrome-specific biochemical evaluation should be undertaken in the presence of strong clinical suspicion, for example, metabolic work up for SIR to include fasting lipids, adiponectin and leptin
Imaging	
1	When there is a high index of suspicion for PCOS, transvaginal ultrasound, where appropriate, is the first-line imaging modality if it will change the diagnostic outcome (ie only one of either oligomenorrhoea or androgen excess present)
2	If clinical indications suggest non-PCOS pathology, cross-sectional imaging of the ovaries and/or adrenals should be undertaken as appropriate
3	MRI pelvis should be undertaken if an ovarian tumour is strongly suspected.
4	Urgent cross-sectional imaging (CT or MRI) should be undertaken if an adrenal pathology is suspected.

5.1.2 | Hirsutism

Given that both acne and hirsutism may result from androgen excess, it is perhaps unsurprising that up to 30% of adult women with acne also have hirsutism [129]. Hirsutism is defined as the presence of terminal hair in a male-pattern distribution. Its prevalence varies with ethnicity [130, 131] and it can result in significant psychological distress [132, 133]. The clinical history should determine the distribution of hair growth, any measures undertaken for hair removal and the frequency of undertaking such measures.

5.1.3 | Female Pattern Hair-Loss (FPHL)

Scalp hair-loss in women is relatively common, and its prevalence increases with age, affecting ~50% of women over the age of 80 years [134]. Depending on whether it occurs before or after the menopause, it may be considered as either early- or late-onset [135]. The term FPHL is now more widely adopted than 'androgenic alopecia' in recognition of the fact that factors other than androgens may contribute to its pathophysiology [134, 136]. Nevertheless, androgen excess remains an important cause of FPHL, and may cause distinct patterns of alopecia, including hair-loss with frontal accentuation [137] or central expansion of hair-loss with frontal hairline preservation [138].

5.1.4 | Menstrual History

Although PCOS is the most common cause of androgen excess, other primary causes of hyperandrogenism can also result in menstrual irregularity via PCOS-independent mechanisms, either because of the underlying disease process (e.g., hypogonadotropic hypogonadism secondary to Cushing's disease [139]) or due to disruption of the hypothalamic-pituitary-gonadal (HPG) axis by elevated androgens of any cause [140]. The menstrual cycle history should include age of menarche, frequency of menstrual cycles (oligomenorrhoea, typically defined as average menstrual cycle length > 35 days or fewer than eight cycles per year [3, 33]) and, where relevant, age of menopause and last menstrual bleed.

5.1.5 | Virilisation

Virilisation may occur in the setting of severe androgen excess and causally points towards potentially neoplastic or monogenic non-PCOS pathology. Signs of virilisation include deepening of the voice, increased muscle mass, breast atrophy, ambiguous genitalia in neonates, clitoromegaly and rapidly progressive hirsutism, but a rising haematocrit is also commonly observed on review of the laboratory record. These manifestations are extremely unusual with the conventional PCOS phenotype and should prompt an urgent evaluation for other causes [3]. Virilisation can present at birth in girls with monogenic disorders like CAH or result from rare non-PCOS pathologies such as ACC, OHT, Cushing's disease or VOTs [141]. Menopausal status and the pattern and severity of AE are crucial in the comprehensive evaluation and diagnosis of severe AE associated

with virilisation. Virilisation presenting with clitoromegaly can vary from mild to severe, graded on the Prader scale [142]. There is significant variability in clitoral size within the normal population. Clitoral enlargement is typically assessed by measuring the length of the glans or by calculating the clitoral index (length × width) [143, 144]. Lengths exceeding 10 mm or an index over 35 mm² are considered pathological. T also significantly impacts laryngeal development, leading to deepening of the voice and thyroid cartilage enlargement in females exposed to AE [145].

5.2 | Physical Examination

The purpose of the physical examination in this context is to confirm or refute the presence of virilising features (as opposed to clinical hyperandrogenism without virilisation), to provide an impression of the degree of severity and to identify features that might indicate an underlying cause. It is important to gauge patient expectations and tolerance in advance of the physical examination, especially in terms of how comfortable they are with exposing areas of their body about which they may harbour feelings of shame or embarrassment.

Features of general hyperandrogenism comprise unwanted body and/or facial hair, acne and FPHL. The modified Ferriman-Gallwey score (mFG) should be used to detect hirsutism [33], depending on ethnicity, but the underlying status may be greatly influenced by the intensity and effectiveness of cosmetic measures such as epilation and skin care. Therefore, it may be both more informative and better tolerated to show the mFG visual analogue figures to the patient and ask them to self-score based on the appearance before intervention.

Features of virilisation comprise deepening of the voice, laryngeal protuberance, more severe FPHL, clitoral hypertrophy and a generally masculine habitus, with squaring-off of the jawline, coarser facial appearance and more prominent musculature. Although hyperandrogenism in the context of a virilising condition is typically more severe, it is still possible to have relatively severe hirsutism whilst maintaining an overall feminine appearance. Examination for clitoral hypertrophy may be an aspect that a gynaecologist may feel more competent to perform; for other clinicians the patient will usually advise whether she has perceived an increase in clitoral size, sensitivity or local discomfort.

Features of metabolic syndrome that may be associated with hyperandrogenism include obesity (based on BMI and waist circumference), and acanthosis nigricans and skin tags signifying insulin resistance. Metabolic syndrome occurs in around 90% of premenopausal women with PCOS and postmenopausal women with ovarian hyperthecosis. It is ubiquitous in Cushing's syndrome and acromegaly, which are associated with their own characteristic features. The presence of acanthosis nigricans or skin tags in relatively lean patients should raise the suspicion of SIR (see above), while disproportionate muscularity of limbs with prominent veins and abnormal adipose distribution should raise the suspicion of lipodystrophy.

6 | Laboratory Work Up of Suspected Androgen Excess

6.1 | Patient Preparation

A review of historic laboratory values may show a rise in haemoglobin and haematocrit, with the development of erythrocytosis in severe cases of androgen excess. Steroid hormone concentrations are subject to diurnal variation and may fluctuate during the menstrual cycle. Concentrations typically peak in the morning at 8:00–10:00 h before falling in the afternoon and evening. Most evidence-based reference ranges are derived from fasted, early morning follicular phase samples [31, 146, 147]. Contrary to previous dogma, serum testosterone levels do not appear to fluctuate significantly during the menstrual cycle when measured by LC-MS/MS methods [31]. Conversely, luteal phase A4 levels appear to be significantly higher than those measured in the follicular phase. Although many patients with androgen excess have amenorrhoea or irregular cycles, *where possible* specimens should be collected during the follicular phase at 8:00–10:00 h following an overnight fast (Table 3).

Patients taking the combined oral contraceptive pill (COCP) will typically have reduced concentrations of circulating sex steroids due to a reduction in gonadotropin stimulation and increased SHBG concentrations. If undertaking biochemical investigations, the COCP should ideally be stopped for at least 3 months before sampling [33]. Most testosterone immunoassay methods are also prone to cross-reactivity with progestogenic components of the combined pill (e.g., norethisterone) that could over-estimate testosterone concentrations [148].

Recommendations

Samples for the investigation of androgen excess should be collected in the morning (at 8:00–10:00 h) following an overnight fast. *Where possible*, samples should be collected in the early follicular phase, although this may not be feasible when the cycle is disrupted or absent.

Where appropriate, evidence based, age-specific reference ranges should be adopted.

It is recommended that the COCP is stopped for a period of 3 months before undertaking biochemical investigations.

6.2 | Testosterone

Validated, highly accurate tandem mass spectrometry (LC-MS/MS) assays are recommended to measure serum total testosterone in women [33]. However, across the UK, immunoassays remain the most accessible analytical technique (UK NEQAS, Table S1). Most T immunoassays display a bias relative to LC-MS/MS in female samples, which may be either positive or negative depending on the manufacturer and can also be concentration-dependent. Further data on the performance of several commercially available immunoassays in the United Kingdom compared to gold standard LC-MS/MS are provided in the UK NEQAS, Figure S1. This bias has been attributed to poor

standardisation, poor sensitivity and nonspecific cross-reactivity [148–150]. Bias may also exhibit temporal variation thereby reflecting reagent and calibrator reformulation (UK NEQAS, Figure S1). Conversely, LC-MS/MS assays rely on purification and chromatography to minimise matrix effects, improve sensitivity and reduce analytical interference. The mass spectrometer adds additional specificity as detection is based on mass-to-charge ratio rather than antibody-antigen reactions. Nevertheless, LC-MS/MS has limitations and variation between methods has been attributed to differences in sample preparation and calibration [151, 152]. Thus, although measurement by LC-MS/MS remains the reference standard, this may not be viable for many UK hospital laboratories.

Recommendations

Exclude the use of exogenous testosterone, for example, in postmenopausal women.

All results should be interpreted against evidence-based reference ranges. These should be categorised as either age-specific and/or pre- and post-menopausal.

A diagnosis of biochemical hyperandrogenism should be evaluated with an index of free testosterone, particularly if total testosterone is normal in a patient with clinical androgen excess (see below).

Testosterone concentrations $> 5 \text{ nmol/L}^1$ warrant further investigation including validation with LC-MS/MS and follow-up with ovarian and/or adrenal imaging as appropriate.

Testosterone concentrations that do not otherwise align with the clinical picture should be validated with LC-MS/MS.

6.3 | Free Testosterone

In the circulation, approximately 1%–4% of endogenous T is free, with the rest specifically and avidly bound to SHBG and weakly bound to other proteins including albumin, orosomucoid and cortisol binding globulin [153]. Two major models have been proposed for the interaction of T with its target AR. Briefly, the first stipulates that only free T is biologically active. The second states that all non-SHBG bound T is bioactive. An index of free T should be provided in patients being investigated for AE [33].

6.3.1 | Sex Hormone Binding Globulin (SHBG)

All SHBG methods in the UK utilise immunoassay (UK NEQAS, Table S2). As SHBG is a large protein, most manufacturers utilise two capture antibodies in an uncompetitive ‘sandwich’ principle which produces a chemiluminescent signal proportional to the concentration of SHBG. Data suggest that SHBG assays are well standardised across most manufacturers (UK NEQAS, Figure S2). SHBG concentrations may be lower in patients with obesity, hypothyroidism, hyperandrogenism, postmenopause, T2DM or metabolic syndrome. Conversely, elevated concentrations may be

observed in patients with hyperthyroidism or cirrhosis, or in those taking the COCP or anticonvulsants.

6.3.2 | Free Androgen Index (FAI)

The FAI defines the ratio of T to SHBG [154]. The FAI has been shown to correlate with free T measured by equilibrium dialysis; however, at low SHBG concentrations (< 30 nmol/L) the FAI becomes less accurate and may overestimate free androgens [155]. Thus, caution should be applied if using an isolated raised FAI with an SHBG < 30 nmol/L to support a diagnosis of biochemical hyperandrogenism. In addition, despite the good agreement between SHBG assays, the variation observed across testosterone assays can impact the bias of the FAI (UK NEQAS, Figure S3).

6.3.3 | Calculated Free Testosterone (cFT)

cFT accounts for both the binding capacity of SHBG for testosterone and the serum albumin concentration. It exhibits superior agreement with equilibrium dialysis measurements of free testosterone over a wide range of SHBG concentrations making it more robust than FAI [155]. This has been borne out in a recent meta-analysis which determined cFT to exhibit slightly superior diagnostic sensitivity than the FAI [33].

Recommendations

Evaluate an index of free testosterone on females being investigated for hyperandrogenaemia, particularly if total testosterone is normal in a patient with clinical androgen excess.

If using the FAI, results should be interpreted with caution if the patient has an SHBG concentration < 30 nmol/L.

All results should be interpreted against evidence-based reference ranges. These should be categorised as either age-specific and/or pre- and post-menopausal.

6.4 | Androstenedione (A4)

A4 is an active androgen with less potency for the AR than T. In females, both ovarian theca cells and the adrenal zona reticularis contribute approximately equally to circulating androstenedione (A4) concentrations. In suspected PCOS, measurement of A4 can be considered in patients with a total T or index of free testosterone result within their respective reference ranges [33]. Elevated concentrations may be observed in AE conditions including PCOS, CAH and ACC. Across the UK, only a limited number of laboratories measure A4; most of these use LC-MS/MS for analysis with the rest using immunoassay (UK NEQAS, Table S3). In general, A4 immunoassays display a positive bias when compared to LC-MS/MS (UK NEQAS, Figure S4). This has been attributed to non-specific antibody-antigen interactions and poor standardisation [156, 157]. However, like T, LC-MS/MS A4 assays are prone to variation due to differences in sample preparation and calibration [152]. A4 levels may also vary significantly across the menstrual cycle.

Recommendations:

A4 should be measured in all patients being investigated for suspected androgen excess.

A4 should be measured using a well validated LC-MS/MS method, where possible

If using immunoassay for analysis, consider validation using an LC-MS/MS method where the biochemical findings do not align with the clinical picture.

All results should be interpreted against evidence-based reference ranges. These should be categorised as either age-specific and/or pre- and post-menopausal.

Due to the absence of robust data at present and in view of significant variability in laboratory platforms for measuring A4 in the United Kingdom and Ireland, at present we do not recommend a specific A4 threshold above which investigations for non-PCOS pathology should be triggered.

6.5 | Dehydroepiandrosterone Sulfate (DHEAS)

DHEAS is produced by the conjugation of sulfate to dehydroepiandrosterone (DHEA) mediated by the enzyme sulfotransferase 2A1 (SULT2A1). Synthesis occurs almost exclusively in the adrenal gland [158, 159]. DHEAS is measured instead of DHEA in most clinical laboratories due to challenges with analysis of the latter. Although not androgenic, DHEAS is the major adrenally derived C19 steroid. Elevated DHEAS concentrations strongly suggest an adrenal source of androgen excess and are useful in the biochemical work up of an adrenal mass [1]. DHEAS should be measured in all patients with clinical or other biochemical features of androgen excess. Across the UK, immunoassay is the most widely used methodology for DHEAS analysis (UK NEQAS, Table S4). Most manufacturers currently display a positive bias when compared to LC-MS/MS users (UK NEQAS, Figure S5), which has been attributed to issues with assay standardisation and specificity [160]. An important consideration when interpreting DHEAS results is that concentrations are not prone to diurnal variation [158], and there is no significant change throughout the menstrual cycle [31], although concentrations decline with advancing age [26, 29, 31].

Recommendations:

DHEAS measurement is an important adjunctive test when discriminating whether elevated active androgen concentrations are of adrenal or ovarian origin.

DHEAS should be measured using a well-validated LC-MS/MS method, where possible.

All results should be interpreted against evidence-based reference ranges. These should be categorised as either age-specific and/or pre- and post-menopausal.

Due to the absence of robust data at present and in view of significant variability in laboratory platforms for measuring DHEAS in the United Kingdom and Ireland, at present we do not recommend a specific DHEAS threshold above which investigations for non-PCOS pathology should be triggered.

6.6 | DHT

DHT is the product of enzymatic 5-alpha-reduction of testosterone. Although a more potent androgen than T, this action is largely expressed at the tissue level through peripheral conversion and so DHT circulates at much lower concentrations. Moreover, it is not available for routine clinical analysis in the majority of laboratories and the evidence base for its measurement in patients with androgen excess is poor [33]. Therefore, DHT is not recommended for measurement in the biochemical work-up of patients with androgen excess.

6.7 | Gonadotropins

Measurement of serum gonadotrophins may be useful in the evaluation of androgen excess particularly if menstrual cycle is affected but have greater diagnostic utility in severe androgen excess as a useful adjunct in the interpretation of severe biochemical disturbances. As there is both positive and negative feedback of gonadotropins by oestradiol, gonadotropin values should be assessed in the context of oestradiol concentrations. Suppression of gonadotropins is typically seen in both pre- and postmenopausal women with severe androgen excess due to CAH or ACC and strongly suggests non-PCOS pathology. In women with OHT, gonadotropins are typically not suppressed and even in VOTs levels are often observed in the postmenopausal range [161].

6.8 | 17-hydroxyprogesterone (17OHP)

Although not androgenic itself, 17OHP is an important intermediate in the adrenal synthesis of glucocorticoids and androgens. Its measurement can therefore be useful alongside other steroid hormones in the differential diagnosis of androgen excess. Elevated 17OHP concentrations in combination with elevated androgens and low cortisol is a typical finding in CAH due to 21OHD [48], the latter being important to exclude in the work-up of PCOS [33]. Conversely, elevated 17OHP, androgen and cortisol concentrations may be suggestive of uncontrolled adrenal overproduction as observed in ACC. Most UK laboratories measure 17OHP using LC-MS/MS (UK NEQAS, Table S5), with most users typically exhibiting good agreement (UK NEQAS, Figure S6) [162]. However, some laboratories still use immunoassay which can display both negative and positive bias relative to LC-MS/MS ([UK NEQAS, Table S5]). Importantly, 17OHP concentrations are subject to diurnal variation and are higher in the luteal phase of the cycle [31], therefore they should ideally be measured in the early follicular phase and in the morning.

Recommendations:

Measure 17OHP in the biochemical work up of all patients being investigated for androgen excess, using an LC-MS/MS assay where possible.

All results should be interpreted against evidence-based reference ranges. These should be categorised into follicular- and luteal-phases specific for pre-menopausal patients.

For results > ULN of the follicular phase but < ULN of the luteal phase, we recommend collecting a second sample after 6 weeks to confirm the result. If the result remains elevated, the patient should be referred to Endocrinology for consideration of 17OHP testing after ACTH stimulation.

A summary of assay performance for several androgenic steroids and other associated parameters is shown in UK NEQAS Figure S7.

6.9 | Urine or Plasma Steroid Metabolomics by LC-MS/MS

Urinary steroid metabolomics may have an important role in the evaluation of an adrenal mass and can also be considered in the diagnostic work up of patients with suspected CAH. However, it is expensive, turnaround times may be slow and it is not available in most centres. Nevertheless, where it is readily available, it can usually identify adrenal hyperandrogenism, including all forms of CAH and many secretory ACCs. It can also help to discriminate between benign and malignant adrenal masses.

6.10 | Metabolic Testing in the Work-up of Severe Androgen Excess

In patients with a clinical suspicion of SIR (acanthosis nigricans, abnormal adipose distribution, family history of T2DM, unusually severe fatty liver or dyslipidaemia), we recommend the following biochemical work up:

- Paired fasting insulin and glucose
- HbA1C (oral glucose tolerance test is more sensitive in PCOS)
- Fasting lipid profile
- Liver function tests
- SHBG
- Liver ultrasound
- Serum leptin and adiponectin.

7 | The Role of Dynamic Endocrine Testing and Venous Sampling in the Investigation of Severe Androgen Excess

Broadly, dynamic testing can be considered as an adjunct investigation to assist in localisation of the source of severe androgen excess (i) when the clinical and/or biochemical features are not consistent with PCOS or CAH, (ii) when imaging has not identified a clear adrenal or ovarian mass or (iii) when the biochemical phenotype is equivocal for adrenal or ovarian-derived disease.

Patients with clinical and biochemical features of severe androgen excess and with a detected adrenal or ovarian tumour on cross-sectional imaging can generally proceed to surgical intervention without delay, particularly in the case of suspected ACC.

However, incidental adnexal masses occur frequently in postmenopausal women with a prevalence in asymptomatic women of 3.3%–18% [163, 164]. Similarly, adrenal incidentalomas are also detected in at least 5% of the general population, the majority of which are benign and non-functioning. While androgen-producing adrenal tumours are typically apparent on imaging, ovarian tumours may remain radiologically occult due to their small size. Therefore, a detected incidental tumour may not be the source of androgen excess. Furthermore, the poor sensitivity of cross-sectional imaging in the detection of small ovarian tumours does not exclude neoplastic ovarian androgen excess, hence the potential importance of biochemical localisation with additional dynamic testing in selected cases before surgical intervention.

7.1 | GnRH Suppression Test

As LH is the central regulator of ovarian androgen synthesis, a GnRH suppression test can be utilised to confirm an ovarian source of androgen production in cases where this is clinically or biochemically suspected (e.g. isolated or predominant increase in serum T). GnRH analogues induce a prolonged stimulation of GnRH receptors in pituitary gonadotroph cells, leading to desensitisation and subsequent downregulation of these receptors. The net effect of this is to suppress gonadotropins, with subsequent suppression of T in cases of gonadotropin-dependent ovarian T secretion. This response is typically observed in OHT, but also commonly in VOTs [165] and in patients with monogenic SIR [76]. This test is not of diagnostic utility if gonadotropin suppression is already evident at baseline, as this finding confirms gonadotropin-independent androgen excess (observed in poorly controlled CAH, ACC and a proportion of patients with VOT).

A GnRH analogue (e.g., triptorelin 3 mg or leuprorelin 3.75 mg) is administered intramuscularly or subcutaneously with androgens measured at baseline and at 28 days after the injection [166–169]. It is important not to measure androgens sooner than 28 days after the GnRH analogue administration, as there is usually an initial agonist phase when gonadotropins (and potentially androgens) may increase before subsequent suppression after 14–28 days. Although some advocate a 2- or 3-month suppression test using longer-acting depot formulations [161] this extends the timeframe of investigation and in our experience is not usually necessary. Suppression of gonadotropins and consequently androgens by at least 50% with a GnRH analogue confirms gonadotropin-dependent ovarian hyperandrogenism [161, 170]. GnRH antagonists such as cetrorelix can also be considered for this purpose and avoid the initial surge in serum T levels that are observed with GnRH analogues.

There are, however, a few caveats and limitations to acknowledge in this situation. First, some VOTs with completely autonomous androgen production can still retain minimal gonadotropin regulation of androgen secretion and testosterone will therefore not suppress; commonly, these ovarian tumours have gonadotropin suppression at baseline and have an increased risk of malignancy. Second, very rare cases of extra-ovarian LH-dependent adrenal androgen production have been reported, for example, from an adrenal tumour [171], with shrinkage of an adrenal tumour with LH suppression also

recently described [172]. Third, this test does not reliably discriminate between ovarian hyperthecosis and VOTs with confidence [45, 165]. Finally, it is uncertain how much greater localisation sensitivity arises from dynamic testing compared with basal DHEAS levels; usually raised in adrenal hyperandrogenism and normal or low with an ovarian source [173].

In the scenario of an isolated or predominant increase in testosterone, a GnRH analogue test should be undertaken first, particularly in cases with negative or equivocal imaging. However, if there is a concomitant increase in androstenedione or DHEAS then we recommend performing the 96-h dexamethasone suppression test first before undertaking the GnRH test, as the hypothalamic-pituitary-gonadal axis may remain suppressed for 2–3 months following the administration of GnRH, making it therefore more difficult to interpret subsequent test results. Importantly, a positive response to GnRH analogue testing signposts a potential long-term treatment modality in women for whom surgery may not be an option.

7.2 | 96-h Dexamethasone Suppression Test (DST)

As adrenal androgen production is regulated by ACTH, hypothalamic-pituitary-adrenal axis suppression with dexamethasone can be used to confirm an adrenal source of androgen excess in selected cases. Androgen suppression confirms the presence of ACTH-dependent hyperandrogenism [174] and is also a clear indication of benign disease [175]. In principle, suppression of DHEAS after dexamethasone implies that adrenal androgen secretion remains under the control of ACTH, and therefore strongly legislates against ACTH-independent malignant disease such as adrenocortical carcinoma. As DHEAS is exclusively of adrenal gland origin, dexamethasone suppression testing is not generally needed when DHEAS is the sole androgen that is elevated. Additionally, patients with an adrenal mass and clear biochemical elevation of adrenal androgen excess do not require this test and should proceed to adrenalectomy. However, it has a diagnostic role in those patients with normal adrenal imaging with a biochemical picture suggestive of adrenal origin, for example, preferential elevation in DHEAS.

Exceptionally rarely, androgen excess from bilateral macronodular adrenal hyperplasia has been reported, either in isolation [176, 177] or with concomitant glucocorticoid excess [178–181]. For optimal androgen suppression that accounts for the 22h half-life of DHEAS [182], the test should be undertaken as a prolonged protocol by administration of dexamethasone 0.5mg every 6h for 4 days, with androgens measured at baseline and on the morning of the fifth day. This suppresses androgens with less variability compared to 24, 48 and 72 h of dexamethasone [21, 183]. Androgen suppression by over 50% of the basal value supports adrenal androgen dominance [175, 184, 185].

7.3 | Simultaneous Adrenal and Ovarian Vein Sampling

In exceptional cases, when uncertainty persists after dynamic testing and cross-sectional imaging, simultaneous ovarian and

adrenal venous catheterisation may aid in the localisation of the source of androgen excess [170]. In this interventional radiology procedure, the adrenal and ovarian veins are cannulated simultaneously; peripheral, adrenal and ovarian venous effluent sampling is undertaken to demonstrate differential gradients in androgen levels [186]. The ratios of adrenal:peripheral and ovarian:peripheral testosterone concentrations are calculated, and the right and left ratios are compared. However, there are no agreed or standardised criteria to guide the interpretation of results.

Some groups confirmed successful ovarian cannulation using an ovarian:peripheral gradient for oestradiol > 2 [187, 188]. Successful adrenal vein cannulation is confirmed using an adrenal:peripheral cortisol gradient > 2 at baseline and > 5 after ACTH stimulation [189]. A maximal ovarian:peripheral gradient or adrenal:peripheral gradient for testosterone of 2 or more is accepted to localise pathology if the contralateral value was near 1 [187]. Adrenal vein testosterone levels should be corrected by dividing them the corresponding cortisol level, to control for the differential flow rates and drainage of the right and left adrenal veins. An ovarian:peripheral gradient for testosterone of > 2.7 has been used to localise androgen-producing ovarian tumours [190, 191] in historical studies. An additional study reported that a right:left ovarian effluent testosterone ratio ≥ 1.44 identified 90% of right sided tumours (a value < 1.44 correctly identified 85% women with left-sided or bilateral lesions); a left:right ovarian effluent testosterone > 15 correctly categorised two-thirds of women with left sided tumours [188]. Rarely, some groups assessed adrenal and ovarian effluent androgens before and after stimulation with ACTH and hCG [188, 192].

In practice, postmenopausal women with suppressed androgens after GnRH analogue should undergo bilateral oophorectomy unless there is a clear unilateral lesion; in this scenario, a discussion with the patient around unilateral or bilateral oophorectomy should be undertaken. However, in premenopausal women lacking a radiologically defined unilateral lesion, simultaneous adrenal and ovarian vein sampling should be considered rather more seriously. Ovarian vein sampling in such cases may lateralise and, therefore, indicate targeted unilateral surgical oophorectomy whilst preserving fertility.

It is important to note that this procedure is technically challenging, invasive and exposes patients to radiation and contrast toxicity [193]. The four-vein catheterisation success rates can be poor [187] and therefore the procedure should be restricted to centres with expertise in the area.

8 | Imaging

The primary objective of imaging in the work up of patients with severe androgen excess is to identify structural culprit adrenal or ovarian pathology and also to differentiate between incidental and clinically relevant lesions. The imaging approach can vary depending on the level of suspicion for PCOS versus non-PCOS pathology. When there is a high index of suspicion for PCOS, particularly in premenopausal women with irregular menstrual cycles and insidious onset of clinical features of

hyperandrogenism, transvaginal ultrasound, if acceptable to the patient, is the most accurate as the first-line radiological investigation. The transvaginal ultrasound should particularly be undertaken by a professional with expertise in ovarian ultrasonography. The purpose of this is to screen for polycystic ovarian morphology to support the suspected diagnosis, rather than to identify a suspected neoplasma. However, from a diagnostic perspective this may be unnecessary in those patients with typical features of androgen excess and oligomenorrhoea [33]. If clinical indications suggest non-PCOS pathology such as rapidly progressive severe clinical androgen excess or severe biochemical disturbances (e.g., serum T $> 5\text{nmol/L}$), cross-sectional imaging of the ovaries and/or adrenals should be undertaken as appropriate. In patients with severe biochemical androgen excess, we recommend both ovarian and adrenal imaging irrespective of the biochemical pattern.

8.1 | Ovarian Imaging

When ovarian pathology is primarily suspected (e.g., isolated or predominant testosterone excess), transvaginal ultrasonography with colour Doppler is readily accessible, and should be considered as first-line imaging [33]. Bilateral increased ovarian volume is typically found in women with OHT. However, it should be noted that VOTs are generally small (Leydig cell tumours $< 3\text{cm}$) and isoechoic. Therefore, magnetic resonance imaging (MRI) should be the next step if ultrasonography is negative in the setting of a high clinical suspicion for androgen-secreting tumours [194]. MRI is more sensitive and specific than ultrasound for identifying ovarian tumours with high positive and negative predictive values of 78% and 100%, respectively [195]. Assessment with contrast-enhanced MRI further increases sensitivity to 81% and specificity to 98% [196].

If MRI is unavailable or contraindicated, computed tomography (CT) can be considered as an alternate modality. However, it involves exposure to low-dose ionising radiation, which should be avoided wherever possible in premenopausal women, and has limited value in detecting and characterising ovarian tumours [194]. In a study by Yan Liu et al., the sensitivity, specificity and accuracy of the combined application of ultrasound and CT were 89, 94.7% and 91.7%, respectively, which were higher than those of either ultrasound or CT in isolation in diagnosing a pelvic mass [197].

The evidence for using 18-fluorodeoxyglucose-positron emission tomography (^{18}F FDG-PET) is limited. Successful identification of androgen-secreting tumours has been reported only in a few isolated cases [198–200]. PET/CT is not recommended as the primary tool for VOT detection due to its high false-positive rates [196]. Therefore, this should be reserved only for selected cases when ultrasound and cross-sectional imaging are equivocal or non-diagnostic.

8.2 | Adrenal Imaging

Adrenal imaging to assess for androgen excess should urgently be undertaken in premenopausal women with severe biochemical disturbance characterised by significant predominant

elevation of adrenal androgens (DHEAS and A4) and testosterone, and in all postmenopausal women with new-onset clinical and/or biochemical androgen excess [1]. The recommended first-line investigation is an urgent unenhanced CT of the adrenal glands to allow better characterisation of a potential adrenal mass [73] (Table 3).

CT imaging has a 99% sensitivity for ACC with a cut-off of over 20HU; however, its specificity is poor [201]. It can lead to increased detection of adrenal incidentalomas, particularly in older patients, necessitating multidisciplinary team involvement to guide decision-making to prevent unnecessary surgical intervention.

MRI can also be undertaken to assess for adrenal gland neoplasia; chemical-shift imaging can differentiate benign from malignant tumours by examining for signal loss on in-and-out-of-phase MRI [73]. ¹⁸FDG-PET is another imaging modality that can help differentiate benign from malignant adrenal masses [73]. This modality is highly sensitive to detecting metabolic changes but spatial resolution for anatomical localisation and specificity are poor, with a significant proportion of benign adenomas demonstrating tracer uptake.

Case studies have demonstrated the utility of iodine-131 6-beta-monomethyl-19-nor cholesterol (NP-59) scintigraphy in localising pathologic sources of ovarian and adrenal androgen excess [170, 202, 203]. In the documented cases, androgen-secreting ovarian and adrenal tumours showed unilateral uptake, OHT showed bilateral ovarian uptake, congenital adrenal hyperplasia was associated with bilateral adrenal uptake and adrenocortical cancer was associated with no uptake or loss of normal physiological uptake. There have been no reports of false-positive findings with NP-59 scintigraphy for hyperandrogenism reported in the literature. The use of NP-59 scintigraphy has disadvantages, which include subpar iodine-131 image quality, comparatively high radiation exposure, longer imaging times, radiopharmaceutical costs, limited availability of NP-59 and possible side effects from using high-dose dexamethasone as a pre-medication. An ¹⁸FDG version of NP-59 is currently under development for PET imaging and may become available for use in localising the source of androgen excess in the future [204].

Overall, comprehensive data directly comparing differing imaging approaches in the evaluation of severe androgen excess are lacking; therefore, clinical presentation in tandem with reasoned biochemical evaluation are crucial to determining which imaging modality is most appropriate.

Recommendations

When there is a high index of suspicion for PCOS, transvaginal ultrasound is the first-line imaging modality in selected patients and where culturally acceptable. Imaging is not necessary if two Rotterdam criteria are otherwise met (i.e., androgen excess and oligomenorrhoea) unless there are otherwise atypical clinical and biochemical features.

If clinical indications suggest non-PCOS pathology, cross-sectional imaging of the ovaries and/or adrenals should be undertaken as appropriate

MRI pelvis should be undertaken if an ovarian tumour is strongly suspected.

Urgent cross-sectional imaging (CT or MRI) should be undertaken if an adrenal pathology is suspected.

9 | Patient and Nursing Perspective

Symptoms of significant and severe androgen excess such as hirsutism, hair loss, acne and virilisation can have a significant negative impact on patient quality of life and mental health [205]. They can contribute to low self-esteem, increased social anxiety, stress and negative body image contributing to social withdrawal. There are social and cultural influences on patients' perceptions of their hirsutism, hair loss, acne and virilisation. Social norms concerned with beauty emphasise the relevance of female physical appearance. In many cultures, scalp hair represents beauty. Thus, scalp hair loss can increase feelings of failing to meet dominant beauty standards. Consideration also needs to be given to shifting gender norms. Symptoms such as hirsutism and virilisation may not be problematic depending on the patient's gender identity or assigned gender at birth in patients with DSD.

Patients also frequently report how symptoms such as hirsutism, hair loss, acne and virilisation are not taken seriously by clinicians, who instead commonly consider these as purely cosmetic problems, and thereby underestimate their profound psychological impact, along with the negative impact of stigma associated with gender norms. Moreover, some of the measures used may not be appropriate for some ethnicities particularly in relation to hirsutism. Treatment options frequently do not meet patient expectations due to lack of efficacy and, in some cases, women are left untreated. There is a need for a better understanding of the full burden of such symptoms and for more qualitative research on patients' subjective experiences.

The vast majority of patients presenting with androgen excess will have PCOS, and the non-PCOS pathologies outlined above are rare. In the United Kingdom, Ireland and further afield, PCOS care is highly variable. Despite being a common endocrinological disorder, many patients with PCOS report dissatisfaction with the diagnostic process, healthcare providers and lack of treatments, and they may consequently seek information from alternative sources [206]. Women with PCOS and their healthcare providers would benefit from better educational and evidence-based strategies and resources which could support patient activation for self-management. Providing effective patient and nursing professional education has the potential to improve quality of life and facilitate healthcare professionals in transforming patient care. High quality, culturally appropriate and inclusive information should be provided to all people with PCOS, and individuals should be asked about their key concerns and priorities for management to determine care.

A clear androgen excess pathway or model of care can support healthcare professionals and patients to navigate patient symptom management more effectively. Entities responsible for health professional education should ensure that information and education on androgen excess are systematically embedded

at all levels of health professional training to address knowledge gaps. Furthermore, introducing competencies based on PCOS from novice to proficient for nurses caring for patients with androgen excess would greatly improve resources for patients.

Comprehensive healthy behavioural or cognitive behavioural interventions could be considered to improve health outcomes in women and individuals with androgen excess. Barriers and facilitators to optimise engagement should be discussed, including psychological factors (mental health, eating disorders, body image, self-esteem, subfertility, quality of life), physical limitations and socioeconomic and sociocultural factors. Referral to suitably trained allied healthcare professionals needs to be considered when women with PCOS need support with optimising their nutrition e.g. culturally appropriate foods that can be tailored, and the value of broader family engagement should be considered.

Acknowledgements

The authors and Society for Endocrinology would like to thank Professor Steve Franks, Professor Terhi Piltonen, and the Association for Laboratory Medicine for their helpful feedback and comments during preparation of this guideline. This work received no specific funding. However, contributors in this work receive research funding from the following: MWOR: Health Research Board Emerging Clinician Scientist Award (ECSA-2020-001); A.A.: NIHR Clinician Scientist Award (CS-2018-18-ST2-002); S.C. received research funding from the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre; P.K.: NIHR Advanced Clinician Scientist Award (NIHR303671); R.K.S.: Wellcome Trust (WT210572).

Endnotes

¹ Caution should be exercised when applying absolute cut-offs above which investigations for neoplastic or monogenic disease should be triggered. This is due a wide variability of immunoassay platforms in individual hospitals across the United Kingdom and Ireland.

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