Investigation of patients with atypical or severe hyperandrogenaemia including androgen-secreting ovarian teratoma

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Abstract
Approximately 7% of women of reproductive age manifest polycystic ovary syndrome (PCOS) and <0.5% have other causes of hyperandrogenism including congenital adrenal hyperplasia (CAH), androgen-secreting tumour of an ovary or an adrenal gland, Cushing’s syndrome or hyperthecosis. The presence of features atypical of PCOS should prompt more extensive evaluation than that usually undertaken. Features atypical of PCOS include the onset of symptoms outside the decade of 15–25 years, rapid progression of symptoms, the development of virilization and a serum testosterone concentration in excess of twice the upper limit of the reference range. Ethnic background, family history and specific clinical findings, e.g. Cushingoid appearance, may inform a focused investigation. Otherwise, patients should have measurement of 17-hydroxyprogesterone (17-OHP) under basal conditions ideally in the early morning, and if abnormal, they should have measurement of 17-OHP one hour after the administration of synthetic ACTH, 250 μg i.v., to screen for CAH, which is present in ~2% of hyperandrogenic patients. The overnight cortisol suppression test employing 1 mg dexamethasone at midnight is a sensitive test for Cushing’s syndrome. Coronal tomographic (CT) scanning of the adrenals and transvaginal ultrasonography of the ovaries are the investigations of choice when screening for tumours in these organs. Less frequently required is catheterization and sampling from both adrenal and ovarian veins, which is a technically demanding procedure with potential complications which may provide definitive diagnostic information not available from other investigations. Illustrative case reports highlight some complexities in the investigation of hyperandrogenic patients presenting with features atypical of PCOS and include only the ninth case report of an androgen-secreting ovarian teratoma.

Introduction
Hyperandrogenism is an essential component of polycystic ovary syndrome (PCOS), which is the most common endocrine disorder to affect young women. The term PCOS was defined at a meeting of experts in Rotterdam, 2003, to require two of the three following features: i) clinical or hormonal evidence of androgen excess; ii) oligomenorrhoea or amenorrhoea; iii) ultrasonographic evidence of polycystic ovaries (1). Therefore, the term PCOS covers a wide range of related presentations from the patient with hirsutism who continues to ovulate and menstruate and has polycystic ovaries through the non-hirsute, oligomenorrhoeic woman with polycystic ovaries to the hirsute patient with hyperandrogenaemia with or without polycystic ovaries and several other combinations. Approximately 7% of women are affected by PCOS (2, 3). The diagnostic approach to the patients presenting with hirsutism should recognize that while PCOS and closely related disorders, such as idiopathic hirsutism, will be the diagnosis in >95% of patients, alternative diagnoses exist which require specific and possibly urgent treatment, and this paper addresses their investigation and diagnosis (Table 1) (4, 5).

Patients with PCOS usually present between the ages of 15–25 years, and their symptoms are slowly progressive with over 1 year usually elapsing from the time the patients first notice the appearance of hair to seeking medical advice (6, 7). PCOS is rarely associated with evidence of virilization or severe hyperandrogenaemia (Table 1). Evaluation with a detailed history and clinical examination may be adequate to make the diagnosis of PCOS (8). However, measurement of serum testosterone, total or an index of free testosterone, is useful to screen for the rare patient with cryptic severe hyperandrogenaemia, which will prompt evaluation for diagnoses alternative to PCOS. Serum testosterone concentrations
Hyperthecosis ovary including severe hyperandrogenaemia (Table 2). in the hirsute patient with features atypical for PCOS syndrome, screening for these disorders is warranted and/or in the absence of features of Cushing’s syndrome may have obvious signs of glucocorticoid excess. In rare cases, PCOS also co-presents as a manifestation of acromegaly (11, 12). However, even in the absence of a suggestive family or ethnic history and/or in the absence of features of Cushing’s syndrome, screening for these disorders is warranted in the hirsute patient with features atypical for PCOS including severe hyperandrogenaemia (Table 2).

**Screening tests**

**Cortisol suppression test**

The dexamethasone, 1 mg overnight, cortisol suppression test is a widely used screening procedure for Cushing’s syndrome. In normal subjects, serum cortisol will be suppressed to <50–100 nmol/l in blood obtained 8–9 h following the administration of 1 mg dexamethasone by mouth at midnight (16–18). The cut-off point depends on the local assay characteristics and has been the subject of recent upward revision (16). False negative results are rare. False positive results have been reported to occur in up to 30% of patients tested, and they may be associated with anxiety, obesity, sleep deprivation, excessive alcohol intake or drugs which induce rapid clearance of dexamethasone, e.g. anticonvulsants (16). The 48-h ‘low-dose’ cortisol suppression test may be used if the overnight test produces a positive or equivocal result. However, a recent meta-analysis concludes that the performance of the frequently used screening tests for Cushing’s syndrome, including the overnight and ‘low-dose’ 48-h dexamethasone suppression tests, is similar (19, 20).

**Screening for CAH due to 21-hydroxylase deficiency**

Although 21-hydroxylase deficiency is not the only form of CAH that is associated with hyperandrogenaemia and its manifestations, it is by far the most frequently encountered type, 21-hydroxylase deficiency, including those of Hispanic origin, Ashkenazi Jews and Eskimos (13, 14). Patients with Cushing’s syndrome may have obvious signs of glucocorticoid excess. In rare cases, PCOS also co-presents as a manifestation of acromegaly (15). However, even in the absence of a suggestive family or ethnic history and/or in the absence of features of Cushing’s syndrome, screening for these disorders is warranted in the hirsute patient with features atypical for PCOS including severe hyperandrogenaemia (Table 2).

### Table 1 Differential diagnosis of clinical hyperandrogenism.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Frequency (%)</th>
<th>Age of onset years</th>
<th>Time of onset to presentation</th>
<th>Menstrual disturbance</th>
<th>Virilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS and related disorders</td>
<td>&gt;95</td>
<td>15–25</td>
<td>Years</td>
<td>+/−</td>
<td>Rare</td>
</tr>
<tr>
<td>CAH</td>
<td>1–2</td>
<td>Congenital</td>
<td>Birth/adolescence/adulthood</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Adrenal tumour</td>
<td>&lt;1</td>
<td>Any time</td>
<td>Weeks–months</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Ovarian tumour</td>
<td>&lt;1</td>
<td>Any time</td>
<td>Weeks–months</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cushing’s syndrome</td>
<td>&lt;1</td>
<td>Any time</td>
<td>Months–years</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td>Hyperthecosis ovary</td>
<td>&lt;1</td>
<td>Pre- to post-menopause</td>
<td>Months–years</td>
<td>+</td>
<td>+/−</td>
</tr>
</tbody>
</table>

PCOS, polycystic ovary syndrome; CAH, congenital adrenal hyperplasia; (+), present; (−), absent; (+/−), present or absent.

which are greater than twice the upper limit of the reference range or indices of free testosterone which are over fivefold elevated suggest a diagnosis other than PCOS. While detection of polycystic ovaries by ultrasound examination is one of the criteria which may contribute to a diagnosis of PCOS according to the Rotterdam criteria, it is not essential, and indeed, the finding is non-specific since it is found in ~25% of young women (9) and may occur in other disorders included in the differential diagnosis of hirsutism, e.g. congenital adrenal hyperplasia (CAH) (10). Ovarian hyperthecosis presents similarly to PCOS, and the two diagnoses can be difficult to distinguish (11, 12). Features of hyperandrogenism in ovarian hyperthecosis persist beyond the menopause, and indeed, ovarian hyperthecosis may account for the majority of cases of post-menopausal hyperandrogenaemia. Women with hyperthecosis also have severe hirsutism and other clinical evidence of virilization not seen in PCOS such as cliteromegaly, temporal balding and deepening of the voice (11, 12).

The patient who presents with any features which are atypical of PCOS requires comprehensive evaluation. The investigations and their order will depend on the clinical setting and the finding of other abnormalities. Some ethnic groups have a high prevalence of CAH of the most frequently encountered type, 21-hydroxylase deficiency, including those of Hispanic origin, Ashkenazi Jews and Eskimos (13, 14). Patients with Cushing’s syndrome may have obvious signs of glucocorticoid excess. In rare cases, PCOS also co-presents as a manifestation of acromegaly (15). However, even in the absence of a suggestive family or ethnic history and/or in the absence of features of Cushing’s syndrome, screening for these disorders is warranted in the hirsute patient with features atypical for PCOS including severe hyperandrogenaemia (Table 2).

### Table 2 Features atypical of polycystic ovarian syndrome which should prompt comprehensive evaluation.

<table>
<thead>
<tr>
<th>Presentation outside the age of 15–25 years</th>
<th>Rapid progression</th>
<th>Presence of virilization</th>
<th>Testosterone levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 year between hirsutism being noticed and seeking medical advice</td>
<td>Presence of virilization</td>
<td>Testosterone levels</td>
<td>Total/&gt; twice upper limit of normal</td>
</tr>
</tbody>
</table>

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mild to severe hirsutism and menstrual disorders appear at various points from childhood to young adulthood. Significantly elevated early morning serum 17-hydroxyprogesterone (17-OHP), also associated with markedly elevated concentrations of serum testosterone and androstenedione, is highly characteristic of 21-hydroxylation deficiency, an autosomally recessive genetic disorder. Measurement of 17-OHP in serum 1 h after the administration of synthetic ACTH (1–24 ACTH), 250 µg i.v. or i.m., is particularly useful when access to early morning blood samples is not available. Figure 1 demonstrates the relationship of basal and ACTH-stimulated 17-OHP values in normal subjects, carriers (heterozygotes who are not affected in a clinically significant manner) and homozygotes for non-classical (mild) and classical 21-hydroxylase deficiency (22). It has been reported recently that basal serum 17-hydroprogesterone concentrations in excess of 5 nmol/l provide 100% sensitivity and 88.6% specificity in screening for 21-hydroxylase deficiency as confirmed by an ACTH-stimulated 17-OHP concentration in excess of 30 nmol/l (23). We recommend screening with an early morning baseline serum 17-OHP concentration and following up abnormal results with the ACTH stimulation test. The authors caution that each laboratory should establish local diagnostic values. Other enzymatic deficiencies accounting for CAH are very rare in most societies. CAH due to 11-hydroxylase deficiency is associated with hypertension and hypokalaemia and is characterized by elevated serum 11-deoxycortisol and 11-deoxycorticosterone concentrations (24). 11-hydroxylase deficiency has been reported with increased frequency in a Turkish population (25). CAH due to 3βol-dehydrogenase, δ4–5-isomerase is characterized by features of adrenal insufficiency, hirsutism, menstrual disorders, virilization and elevated δ5 steroids including DHEA, DHEAS. 17-hydroxypregnenolone and pregnenolone (26).

**Screening for adrenal tumour**

CT scanning and magnetic resonance imaging (MRI) scanning of the adrenal glands are sensitive tests for the presence of an adrenal adenoma or adenocarcinoma which is usually greater than 1 cm in diameter (27). Occasionally, iodochloramethane scanning, preformed under ACTH suppression using dexamethasone, 0.5 mg every 6 h for at least 5 days, may provide additional information where other imaging yields equivocal results (28). ACTH suppression is required to have only autonomous adrenal function picked up on scanning. In addition, measurement of androgens in serum after 5 days of adrenal suppression will distinguish between androgens maintained by suppressible ACTH stimulation as in the case of CAH and androgens secreted autonomously by tumours of adrenal glands or ovaries (29). Prolonged, 5-day adrenal suppression is necessary to allow time for the normal slow clearance of adrenal androgens, particularly DHEAS.

**Screening for an ovarian tumour**

Ultrasound examination is the procedure of choice when imaging ovaries in the evaluation of hyperandrogenaemia for polycystic characteristics or tumours. This is best performed using a combination of transabdominal ultrasonography and transvaginal ultrasonography (30). MRI scanning can also be used for the diagnosis of adnexal masses. However, this modality is most useful in differentiating an ovarian tumour from a uterine myoma or during pregnancy (31). Androgen-secreting ovarian tumours are frequently small solid masses with non-specific ultrasonographic or MRI appearances (32). Rarely extra-ovarian sources of androgens may also be detected by this examination (33).

**Venous sampling to detect an obscure source of hyperandrogenaemia**

In occasional hyperandrogenaemic patients who have normal results to the overnight cortisol suppression test, serum 17-OHP concentrations in the early morning or following synthetic ACTH administration and when CT and MRI scanning of the adrenals and ultrasound imaging of the ovaries have failed to detect a relevant abnormality, venous sampling may be helpful. This procedure should be undertaken by a radiologist highly experienced in the technique which is technically demanding and failure to catheterize one or more
A catheter is threaded into the femoral vein, which is then positioned if possible deep in both adrenal veins and both ovarian veins in sequence. When the catheter tip is in position, a sample of blood is obtained. At approximately the same time, a sample of blood is also obtained from a peripheral vein, e.g. arm. Measurement of the androgen or androgens present in excess, usually testosterone and/or androstenedione, in all samples will usually allow the identification of an exaggerated adrenal or ovarian vein to peripheral vein androgen concentration gradient. A greater than 1–9 gradient from peripheral vein steroid concentration to concentration in a vein immediately draining either an adrenal gland or an ovary is highly indicative of the source of androgen excess (34–36). In this way, it is usually possible to locate the source of the androgen excess. Complications are infrequent but may include bleeding at the puncture site, rupture of a deep vein and thrombosis.

**Illustrative cases**

**Case 1**

A 30-year-old woman presented with recent onset of acne, mild hirsutism and irregularity of menstruation with intervals varying between 4 and 8 weeks in the preceding 12 months. She was noted to be hypertensive. On subsequent evaluation, she was noted to be hypokalaemic with serum potassium of 3.1 mmol/l. In addition, the serum testosterone concentration was markedly elevated, 7.5 nmol/l (reference range 0.5–2.0), as were those of androstenedione, 23.1 nmol/l (reference range 3.3–9.9), and DHEAS, 24.0 nmol/l (reference range 0.9–12). The early morning serum 17-OHP concentration was 3.4 nmol/l (reference range 1–5). Following the administration of 1 mg dexamethasone at midnight, serum cortisol concentrations fell from 351 to 27 nmol/l. CT scanning of the adrenal glands revealed a 5-cm right-sided adrenal mass. The right adrenal was resected and it revealed the presence of an adrenocortical tumour which apart from the large dimensions generally appeared to be benign. Absolute certainty of benignity or malignancy is not possible in the absence of metastases in the case of adrenal tumours or indeed most endocrine tissue tumours (37). Post-operatively, the patient was normotensive and normokalaemic, and after several months, acne cleared and regular menstruation resumed. Testosterone, androstenedione and DHEAS levels returned to normal, and at a 3-year follow-up visit, all features were maintained with no evidence of recurrence on CT scanning.

**Comment** Although this patient’s presenting features were relatively mild, the late age of the first appearance of symptoms and the marked degree of hyperandrogenaemia prompted extensive evaluation, which was rewarded with the identification of an adrenal tumour. Unusual features in this patient were hypertension and hypokalaemia. The serum aldosterone and plasma renin activity and the aldosterone to renin activity ratio were normal, thus excluding primary hyperaldosteronism. It is tempting to suggest that the tumour also secreted an unusual mineralocorticoid. However, in that instance the plasma renin activity would be suppressed.

**Case 2**

A 25-year-old woman presented with a history of amenorrhea for 21 months which had its onset following 3 months of treatment with a combined oral contraceptive pill for irregular menstruation. Menarche was at 16 years with regular menstruation occurring approximately at 31-day intervals and was associated with dysmenorrhea. Mild hirsutism had been noted several years prior to presentation affecting the upper lip, inter-mammary region, lower abdomen and upper thighs. No treatment had been pursued by the patient. In addition to hirsutism, physical examination revealed cliteromegaly and anterior fusion of the labia. Investigations of steroid concentrations in serum are summarized in Table 3. The 0900 h serum 17-OHP concentration was 663 nmol/l, while serum cortisol concentration was 270 nmol/l (reference range 200–550 nmol/l). One hour following the administration of synthetic ACTH, 250 µg i.v., the serum 17-OHP value rose to 1108 nmol/l, while testosterone climbed to 13.7 nmol/l, androstenedione to 91 nmol/l and the cortisol response was to a concentration of

<table>
<thead>
<tr>
<th>Testosterone (nmol/l)</th>
<th>SHBG (nmol/l)</th>
<th>T/SHBG</th>
<th>Androstenedione (nmol/l)</th>
<th>17-OHP (nmol/l)</th>
<th>Oestrone (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference 0.6–2.0</td>
<td>27–86</td>
<td>1.0–5.2</td>
<td>2.4–7.3</td>
<td>1–5</td>
<td>50–340</td>
</tr>
<tr>
<td>Basal 9.5</td>
<td>16.9</td>
<td>16.9</td>
<td>50.4</td>
<td>663</td>
<td>550</td>
</tr>
<tr>
<td>Post-synacthen 13.7</td>
<td>91</td>
<td>91</td>
<td>1108</td>
<td>570</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone (6 weeks 0.5 mg at midnight) 0.8</td>
<td>68.8</td>
<td>1.1</td>
<td>3.5</td>
<td>10.1</td>
<td>125</td>
</tr>
</tbody>
</table>

Table 3 Basal, ACTH-stimulated and dexamethasone-treated serum steroid and sex hormone-binding globulin (SHBG) concentrations in Case 2.
Table 4 Androgen and steroid concentrations drawn during selective venous sampling of the adrenal and ovarian veins in Case 3.

<table>
<thead>
<tr>
<th></th>
<th>Left adrenal</th>
<th>Left ovary</th>
<th>Right adrenal</th>
<th>Right ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Central</td>
<td>Periph</td>
<td>C/P</td>
<td>Central</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td>22</td>
<td>8.9</td>
<td>2.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Androstenedione (nmol/l)</td>
<td>349</td>
<td>21.7</td>
<td>16.1</td>
<td>23</td>
</tr>
<tr>
<td>DHEAS (nmol/l)</td>
<td>6.3</td>
<td>5.2</td>
<td>1.2</td>
<td>5.9</td>
</tr>
<tr>
<td>17-OHP (nmol/l)</td>
<td>358</td>
<td>25</td>
<td>14.3</td>
<td>15.4</td>
</tr>
<tr>
<td>11-DOC (nmol/l)</td>
<td>307</td>
<td>18</td>
<td>17.1</td>
<td>21</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>7760</td>
<td>727</td>
<td>10.7</td>
<td>664</td>
</tr>
</tbody>
</table>

Centrally (Central) and peripherally (Periph) drawn samples. Gradients are expressed as central to peripheral serum concentrations (C/P). DHEAS, DHEA sulphate; 17-OHP, 17-hydroxyprogesterone; 11-DOC, 11-deoxycorticisol.
androstenedione and 17-OHP across the right ovary (Table 4). Transvaginal ultrasound examination was performed and showed a 2-cm, central, cystic lesion in the right ovary (Fig. 2).

Elective laparotomy and right oophorectomy were undertaken 3 years following the initial presentation. The patient had remained amenorrhoeic for this period other than occasional withdrawal bleeding in response to the administration of medroxyprogesterone acetate, 10 mg daily for 5 days. The patient elected to use cosmetic measures for the treatment of hirsutism in the intervening period. The right ovary at the time of surgery was enlarged, but a discrete lesion was not obvious on gross examination. Palpation of the left ovary and the broad ligament intra-operatively did not demonstrate any abnormality. Macroscopic examination of the right ovary following resection showed multiple cysts containing yellow and cream material. A benign ovarian teratoma, 2.2×2.0×2.1 cm, was described on histological examination to contain abundant diffuse Leydig cells (Fig. 3) in addition to mature skin, skin appendages, fat, bone and thyroid tissue. The surrounding ovarian stroma was normal and was not indicative of polycystic ovaries or hyperthecosis.

Following right oophorectomy, a significant reduction was seen in androgen concentrations with a fall in serum testosterone from 9.0 to 3.7 nmol/l and in the free testosterone index from 35.2 to 15.2, while serum androstenedione fell from 37.2 to 9.6 nmol/l. The post-operative fall in serum androgens was sustained, and 3 weeks following oophorectomy, the patient had her first spontaneous menstrual period in over 3 years. Regular menses followed this at monthly intervals. Measurement of mid-luteal phase serum progesterone yielded a value of 37.2 nmol/l indicative of ovulation. Ten months post-operatively, the patient became pregnant. Pregnancy was complicated by gestational diabetes, which was treated with insulin. Facial acne vulgaris was also noted at this time. A healthy female infant weighing 3.75 kg was delivered. The patient made a good post partum recovery and she decided not to breastfeed. Menstruation returned 6 weeks post partum.

Comment This report demonstrates the usefulness of venous catheterization and blood sampling in identifying the source of cryptic androgen excess and the superiority of transvaginal rather than of transabdominal ultrasonographic examination of the ovaries. It also highlights a number of other relevant issues. Confounding features include the spontaneous pulsatile nature of adrenal and to a lesser extent ovarian hormonal secretion, variable degrees of dilution of hormonal secretion depending on whether or not other veins have joined proximal to the point of sampling and anatomical variations in venous drainage. The finding of a significant gradient isolated to androstenedione across the left adrenal, 16.1, was probably due to the
pulsatile nature of ACTH and adrenal steroid secretion. This is supported by the finding of serum cortisol concentration which was >10-fold higher in the venous blood draining the left adrenal than the right adrenal (Table 4).

We have reported previously of an 11-year-old girl who presented with signs of virilization and provided additional related lessons (33). In that patient, serum androgen concentrations were 3–5-fold elevated above the upper limits of reference ranges. There was a normal serum 17-OHP response to ACTH stimulation, 27.5 nmol/l, while cortisol was suppressed normally following the administration of dexamethasone. Ultrasound and MRI scanning of the pelvis and CT scan of the adrenals were all reported as normal. Bilateral ovarian sound and MRI scanning of the pelvis and CT scan of the adrenal (Table 4).

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