REVIEW

Approach to patients with pseudo-Cushing's states

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Abstract

The distinction between pseudo-Cushing's states (PCS) and Cushing's syndrome (CS) poses a significant clinical challenge even for expert endocrinologists. A patient's clinical history can sometimes help to distinguish between them (as in the case of alcoholic individuals), but the overlap in clinical and laboratory findings makes it difficult to arrive at a definitive diagnosis. We aim to describe the most common situations that can give rise to a condition resembling overt endogenous hypercortisolism and try to answer questions that physicians often face in clinical practice. It is important to know the relative prevalence of these different situations, bearing in mind that most of the conditions generating PCS are relatively common (such as metabolic syndrome and polycystic ovary syndrome), while CS is rare in the general population. Physicians should consider CS in the presence of additional features. Appropriate treatment of underlying conditions is essential as it can reverse the hormonal abnormalities associated with PCS. Close surveillance and a thorough assessment of a patient's hormone status will ultimately orient the diagnosis and treatment options over time.

Key Words

- pseudo-Cushing's
- neuropsychiatric disorders
- PCOS
- alcoholism
- diabetes
- obesity
- eating disorders

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Introduction

Physiological activation of the hypothalamic-pituitary-adrenal (HPA) axis can be found in several situations, such as major surgery, severe illness, intensive physical exercise, and prolonged fasting leading to improve the ability of the organism to survival (1).

Some common endocrine diseases including obesity, polycystic ovary syndrome (PCOS), poorly controlled diabetes mellitus (DM), chronic alcoholism, and psychiatric disorders may also coincide with HPA axis activation (2). Under such conditions, there may be some clinical signs of hypercortisolism, which may be temporary or more persistent, giving rise to what is called pseudo-Cushing's syndrome (PCS).

The differential diagnosis of PCS and Cushing's syndrome (CS) is still a major clinical challenge even for expert endocrinologists. Study findings are inconsistent and no consensus on the correct diagnosis has been reached as yet. Standard adrenal steroid measurements often overlap in cases of PCS and CS, and several drugs affect the dynamic test results.

By describing and discussing a clinical case, this review attempts to answer some common questions that physicians may face in the clinical practice.

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Clinical case

A 26-year-old female was referred to a tertiary endocrine center for suspected endogenous hypercortisolism. She had a long-standing diagnosis of type-1 DM and a psychiatric condition characterized by depression and anxiety that was treated with fluoxetine.

She had experienced oligomenorrhea since her first menstrual cycle at 14 years of age. At 20 years of age, she was diagnosed with polycystic ovarian syndrome (PCOS) based on her oligomenorrhea, hyperandrogenism, and micropolycystic ovaries found on pelvic ultrasound. She began taking oral estro-progestin therapy and was continuing to do so at the time of our assessment. During a hospitalization for a suspected bowel obstruction, she had revealed some clinical signs suggestive of hypercortisolism (central adipose distribution, hirsutism, and round face, but no evidence of proximal myopathy or reddish purple striae). She was overweight (BMI 28 kg/m^2, with a waist circumference of 92 cm), with high blood pressure (140/90 mmHg).

Laboratory findings revealed high levels of serum cortisol at 08:00 h (29 µg/dL; n.v. 5–25), and high urinary free cortisol (UFC) (280 µg/24 h; n.v. 36–137), while ACTH levels at 08:00 h were in the normal range (26 pg/mL; n.v. 10–50). The patient's diabetes was poorly controlled (HbA1c 8%).

The suspected CS was confirmed by the lack of cortisol suppression after 1 mg dexamethasone (10 µg/dL, n.v. <1.8) and the absence of a cortisol circadian rhythm (midnight serum cortisol, MserC, 12 µg/dL; n.v. <7.5).

Which clinical features are the strongest indicators of CS?

Easy bruising, facial plethora, proximal myopathy, and cutaneous striae (reddish purple 1 cm wide) are reportedly the clinical features that best discriminate CS (3). These signs were all lacking in our patient, but we knew that most of these features have a low sensitivity. The patient had a round face and was overweight, with central adipose distribution and hirsutism, features that are commonly seen in CS patients, but of lower discriminatory value. On the other hand, these signs are indistinguishable from patients with PCOS, which is a common disease among women of reproductive age (4), whereas CS is more rare (5).

Which is the first-line approach for patients with suspected CS?

The Endocrine Society guidelines recommend using the 1 mg dexamethasone suppression test (1-mg DST), late-night salivary cortisol (LNSC; two measurements), or UFC (two measurements) for first-line screening purposes. Then, additional methods such as a longer, low-dose DST (2 mg for 48 h) and midnight serum cortisol, may be considered (3, 6).

LNSC is increasingly used nowadays (7), based on the assumption that most patients with CS lose their normal cortisol diurnal pattern and have persistently elevated cortisol levels throughout the day, whereas PCS patients retain their normal secretion rhythm, albeit on a higher setting, with a cortisol nadir at midnight (8). Although further studies are needed to confirm these findings, LNSC seems to perform better than 1-mg DST or UFC in distinguishing PCS from CS. Unlike the midnight serum cortisol measurement that requires inpatient admission, saliva collection is easy to perform in an outpatient setting. However, LNSC is not widely used in the clinical practice and the studies reported have not set a clearly defined cut-off value to be used in the diagnosis of CS. Thus, each center should determine its own cut-off value based on the method adopted for LNSC measurement.

UFC provides an integrated assessment of cortisol secretion over a 24-h period. It measures the cortisol that is not bound to corticosteroid-binding globulin (CBG), which is filtered by the kidney unchanged. A falsely low UFC can occur when creatinine clearance falls less than 60 mL/min (3). It is important that patients provide complete urine collections with appropriate total volumes (this may require an adequate patient education) and the concomitant measurement of urinary creatinine levels.

Which aspects should be considered before screening patients with suspected CS?

When performing cortisol assays, it is important to consider analytical bias. There are two ways to measure cortisol: immunoassays, for example, RIA, ELISA, or automated chemiluminescence (ECLIA) and liquid chromatography-tandem mass spectrometry (LC–MS/MS). Antibody-based immunoassays can be affected by cross-reactivity (especially cortisone, which has a structure similar to that of cortisol) and synthetic glucocorticoids (9). In fact, cortisol is converted into inactive cortisone by 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2.

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Structure-based assays like LC–MS/MS do not pose this problem and allow for various glucocorticoids and metabolites to be separated, and they are probably the most accurate methods for assessing cortisol and cortisone levels (9).

The concomitant use of commonly prescribed therapies may alter dexamethasone metabolism, interfering with its use in a suppression test. Some types of medication can interfere with the CYP3A4 enzyme system, either accelerating (i.e. phenobarbital, phenytoin, carbamazepine, pioglitazone, primidone, rifampin, rifapentine, and ethosuximide) or impairing (i.e. aprepitant, itraconazole, ritonavir, fluoxetine, diltiazem, and cimetidine) dexamethasone metabolism (3).

Measuring dexamethasone levels could help to identify any abnormal clearance. A recent study has used a rapid and sensitive LC–MS/MS assay for measuring dexamethasone levels in a cohort of normal healthy postmenopausal women. Applying a serum dexamethasone cut-off of >3.3 nmol/L was associated with a serum cortisol ≤50 nmol/L in 84/95 of the individuals (10). However, serum dexamethasone levels are not measured routinely in international clinical practice.

Oral estrogens can increase CBG levels and consequently raise total cortisol levels, again potentially generating abnormal test results (11, 12, 13). Oral estrogens should be withdrawn for at least 6 weeks before testing or retesting patients with suspected CS (3).

Our patient was taking fluoxetine, which inhibit the CYP3A4 system leading to impaired dexamethasone metabolism, and oral estrogens that can influence the result of any test.

How can patients' concomitant conditions influence the diagnosis of PCS?

Neuropsychiatric disorders

HPA axis hyperactivity is a frequent finding in patients with major depressive disorder (MDD) (14). Overall, about 20–30% of patients with MDD reveal hypercortisolemia (15, 16). Depressed male patients appear to have a more reliable cortisol hypersecretion, raising the issue of sex differences in HPA axis hyperactivity in depressed patients (16, 17) (Table 1).

The post-awakening surge in cortisol is known to be accentuated in individuals at risk of MDD (18), and about one in two patients with MDD have high evening cortisol levels indicative of disrupted diurnal cortisol rhythms (14, 19). The defect that induces HPA axis overactivity seems to be localized in the upper part of the hypothalamus, and there have been reports of increased levels of

Table 1 Most common causes of PCS and main associated abnormalities of the hypothalamus-pituitary adrenal-axis (HPA) reported.

Causes of PCS	Abnormalities of HPA axis reported
Neuropsychiatric disorders	Accentuated post-awakening surge in cortisol
	High evening cortisol levels in some patients
	Increased or decreased CRH levels
	Resistance to the feedback action of DST on HPA axis activity
	Reduced activity of cortisol-deactivating enzymes (5-α-reductase and 11β-HSD type 2)
Polycystic ovary syndrome	Mildly elevated UFC levels in ~50% of patients
	Decreased corticosteroid-binding globulin
	Increased androstenedione, total and free testosterone levels
	Reduced sex hormone-binding globulin
Obesity	Increased cortisol reactivity to physical and psychosocial stressors
	Preserved pituitary sensitivity to feedback inhibition by DST
	Increased 5 α-reductase type 1 activity in the liver
	Upregulation of cortisol output due to great expression of 11β-HSD type 1
Alcohol abuse	Stimulated CRH production with increased ACTH levels
	Higher fasting cortisol at 08.30 h
	Impaired cortisol clearance due to hepatic dysfunction
	Abnormal cortisol suppression to 1-mg DST
Eating disorders	Reduced cortisol clearance
	Changes in the affinity of cortisol to CBG
	Resistance to the action of glucocorticoids
	High CRH levels with normal ACTH
	Preserved circadian rhythm of cortisol

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corticotropin-releasing hormone (CRH) in depression, as well as a significant decrease in this hormone in atypical depression (20).

A proportion of patients with MDD also have a greater resistance to the feedback action of glucocorticoids on HPA axis activity (20, 21) and this weaker sensitivity is often restored by effective psychopharmacological therapy (22).

Data regarding the pulsatile release of cortisol and ACTH in depressed patients are ambiguous, with reports of increased (23), unchanged (24), or reduced (25) frequencies. Analyzing these conflicting findings of different studies suggests that the prevalence of abnormalities in cortisol and ACTH levels might depend partly on how they are measured and the type of depressed patients examined. Many such patients nonetheless reveal one or more abnormal test among an abnormal cortisol suppression with the 1-mg DST or higher night-time and urinary cortisol levels.

A reduced activity of the intracellular cortisol-deactivating enzymes (5-α-reductase and 11β-HSD type 2) has been demonstrated in depressed patients too, and this may raise the cortisol bio-availability within tissues (25). Certain therapies for depression may improve 5-α-reductase activity without affecting 11β-HSD type 2 activity, and this would explain why the disrupted glucocorticoid metabolism may be only partly improved by antidepressants (25).

Finally, it has been claimed that cortisol dysfunction in patients with MDD could be one of the factors involved in their higher risk of metabolic syndrome and type 2 DM (26) and their higher cardiovascular mortality (27, 28).

Polycystic ovary syndrome (PCOS)

Anovulation, oligo-amenorrhea, hirsutism, acne, insulin resistance, DM, overweight/obesity, and hypertension may be seen to a variable degree in PCOS and CS patients. There is a difference in their prevalence, however, PCOS is quite common, occurring in 6.6% of women of reproductive age (4), whereas CS is a rare condition with an incidence of 0.7–2.6 cases per million population yearly (5). The two conditions may overlap in some young women. In a retrospective review of patients of reproductive age with a confirmed diagnosis of CS, one in two were initially diagnosed with isolated PCOS (29). The patients had nearly identical clinical profiles, except that hirsutism and menstrual irregularities were more common in those initially diagnosed with PCOS. These findings are of interest because treatment for PCOS did not solve the hypercortisolism, and the oral estrogens generally used to treat PCOS may be deleterious in CS patients due to their high thrombotic risk. A delay in the diagnosis of CS can have important clinical consequences too.

On the other hand, a prospective study conducted on premenopausal women with proven CS found signs and symptoms of hyperandrogenism in all patients, menstrual irregularities in 70%, and a polycystic ovarian morphology in 46% (30). This report confirms that a PCOS phenotype is common in CS women, particularly among those with moderately high glucocorticoid levels (31). When circulating cortisol exceeds critical levels, there is an inhibitory effect on the hypothalamic control of gonadotropin release and ovarian volumes seem to be preserved.

Pall *et al.* (32) hypothesized that total testosterone (TT) levels could be lower in patients with mild CS compared to women with PCOS. They found that the optimal cut-off for TT was 1.39 nmol/L, yielding a sensitivity of 95% and a specificity of 70%. The evaluation was done with a well-validated assay, but most commercial assays lack of accuracy and precision when measuring testosterone in women. Moreover, only subjects identified with mild CS of pituitary origin were included in the study and so the criteria cannot be applied to patients with other forms of CS. Thus, larger studies are needed to confirm these data.

It may be reasonable to screen women with PCOS for hypercortisolism. The 1-mg DST has a high specificity and diagnostic accuracy. The test maintained its sensitivity using a lower cut-off, and this is important in a screening setting (33). Measuring midnight plasma cortisol levels is a highly sensitive method too, while caution is needed in interpreting mildly elevated UFC levels in women with PCOS. In both, normal weight and obese patients with PCOS, UFC may exceed the upper limit of the normal range in about 50% of the patients (34).

Diabetes mellitus, obesity and metabolic syndrome

Although limited data are available on the prevalence of CS in patients with DM, the diagnosis should be considered particularly in cases with a number of highly discriminatory signs or symptoms.

After screening a large number of patients attending diabetes clinics in an outpatient setting and in conditions of standard clinical practice, Terzolo *et al.* (35) found a frequency of previously unsuspected CS of 0.7% (in the largest series published so far). Its frequency was higher (5.1%) among patients whose diabetes and hypertension were poorly controlled despite intensive treatment. In a previous series, Catargi *et al.* (36) found a definitive CS in

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2% of overweight, type-2 DM patients referred for poor metabolic control. In both studies, a first screening was performed with the 1-mg DST.

Although these results do not support a wide-scale screening for CS in type-2 DM patients, a case-finding approach is warranted for selected patients with uncontrolled glycemia or resistant hypertension.

Obesity is an ongoing pandemic condition, the prevalence of which is continuing to rise worldwide.

The association between cortisol and obesity has been extensively studied and it appears that obesity is often, but not always, related to a hyperresponsive HPA axis (37). The studies indicate that cortisol secretion is often elevated but that circulatory concentrations are normal or low probably due to substantial changes in peripheral metabolism of cortisol (38). These changes are tissue specific, with increased inactivation of cortisol in the liver (by increased 5α-reductase type 1 activity that metabolizes cortisol to its tetrahydro derivatives) and increased regeneration of cortisol in adipose tissue (39). The increased inactivation of cortisol in the liver may be responsible for the compensatory activation of the HPA axis.

Women with abdominal obesity phenotype have an exaggerated ACTH and cortisol response to combined CRH/AVP (corticotropin-releasing hormone/arginine vasopressin) stimulation (40). Higher than normal UFC values have been found in women with abdominal obesity (41, 42). However, these data were not confirmed when anxiety and depression were excluded (40), suggesting that confounding factors are capable of altering the HPA axis in obesity.

Increased cortisol reactivity observed after both, physical and psychosocial acute stressors, seems to be the one parameter where results are consistent in obese subjects (40, 41, 43).

Subtle differences in pituitary feedback sensitivity have been found in the cortisol response to dexamethasone test. Abdominal obesity seems to be associated with poorer response using 0.5 mg of dexamethasone overnight (43). However, Pasquali *et al.* (44) demonstrated that pituitary sensitivity to dexamethasone administration is preserved in obese subjects of both sexes even at low dosages.

Abraham *et al.* (45) analyzed the associations between three parameters of activity of the HPA axis (nadir of salivary cortisol levels, UFC, and the 1 mg DST) and weight, metabolic syndrome and psychosocial stress in a large number of overweight and obese individuals with at least two features of CS. Apart from a weak correlation of salivary cortisol in men, they found no relationship between waist circumference and any cortisol parameter. Their findings do not support a strong link between systemic cortisol and obesity or metabolic syndrome.

Many of the inconsistencies found in the different studies may be associated to various cortisol measurements methodology and different study designs.

Particular attention has been posed to the activity of the 11β-HSD type 1 localized in different tissues. This is a microsomal enzyme which converts inactive cortisone to active cortisol, thus regulating intracellular cortisol access to glucocorticoid receptor. Although data are inconsistent, there is a suggestion that local glucocorticoids action may be amplified by upregulated activity of 11β-HSD type 1 in visceral and hepatic tissue of obese subjects (46, 47). Omental fat can generate active cortisol from inactive cortisone via 11β-HSD type 1, amplify glucocorticoid receptor activation, and promote pre-adipocyte differentiation and adipocyte hypertrophy, inducing adiposity (46). The increased expression of 11β-HSD type 1 in omental adipose tissue seems to support its involvement in severe obesity (48).

However, a number of respective studies have been performed in animal models and in humans with controversial results (49) probably associated to the differentially expression and activity of 11β-HSD type 1 in different species and tissues.

Several inhibitors of 11-βHSD type 1 have consequently been tested in animals, obtaining an improvement in glucose control and lipid profile (47, 50).

Even though being controversially discussed, the role of 11β-HSD type 1 in the pathogenesis of the metabolic syndrome must be further considered.

Which other situations can be associated with PCS?

Alcohol abuse

The first report on patients with symptoms mimicking CS caused by alcoholism appeared at the end of the 1970s (51). Studies conducted in mice demonstrated that alcohol can stimulate CRH production via the hypothalamic paraventricular nucleus, with consequent HPA axis activation and increased levels of ACTH (52). Induction of the enzyme 11-βHSD type 1 was demonstrated in patients with alcoholic liver disease, coinciding with an increased cortisol production or an impaired cortisol clearance due to hepatic dysfunction.

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Although the severe classical phenotype is not difficult to diagnose, chronic alcoholism can induce a phenotype that largely overlaps with CS. Several case reports have provided details emerging from the physical examination of patients with symptoms compatible with CS, which eventually proved to be the result of alcohol addiction: 87% had a moon face, 81% had muscle weakness or tiredness, 75% had truncal obesity, 69% had hypertension, and 12% had cutaneous striae (53).

Coiro *et al.* (54) measured cortisol in ten women with alcohol-induced PCS and found a higher fasting cortisol at 08.30 h than in controls. Frias *et al.* (55) examined adolescents during episodes of acute alcohol intoxication, finding elevated cortisol levels, which was more pronounced in females. Studying the presence of cortisol in the hair, Stalder *et al.* (56) detected higher levels in individuals who had recently stopped drinking alcohol than in either controls or individuals who had abstained for a longer period. When heavy drinkers were compared with light drinkers, the former had higher salivary cortisol levels on awakening and 30 min afterwards (57). An insufficient cortisol suppression in the 1-mg DST was also reported in several studies (53).

Interestingly, case reports invariably indicated that alcohol withdrawal led to the disappearance of patients' symptoms and biochemical disruptions (58).

A detailed clinical history is crucially important in patients with CS, and alcoholic patients should undergo a repeat of their clinical and biochemical work-up after they have stopped drinking alcohol for at least 1 month (53).

Eating disorders

Anorexia nervosa is a psychiatric disorder characterized by an extreme restriction of food intake despite being underweight, with a refusal to maintain body weight above the minimum threshold of normality. It can severely alter the patient's eating behavior and is associated with mortality rates of up to 22%. Patients, usually women, have endocrine abnormalities such as amenorrhea, an impaired body temperature regulation, and hypercortisolism, probably linked to chronic fasting-related stress. HPA axis dysregulation may persist even after weight recovery, however, suggesting that it may be involved in the pathogenesis of the disease itself (59).

Hypercortisolism has been amply documented in young women with anorexia nervosa. Many mechanisms have been hypothesized, including a reduced cortisol clearance, changes in the affinity of cortisol for CBG, or in the concentration of glucocorticoid receptor. In particular, resistance to the action of glucocorticoids may explain not only the hypercortisolism but also the lack of clinical signs of cortisol excess in such underweight women.

The mechanisms behind hypercortisolism in anorexia nervosa appear to differ from those associated with MDD, with CRH having a more important role in stimulating the HPA axis in the case of anorexia. The powerful anorectic effect of CRH could contribute to the severe weight loss observed in this disorder (60): the levels of CRH are higher than normal, while those of ACTH are normal, and the latter has a reduced response to stimulation with CRH, whereas adrenal cortisol production increases after stimulation with ACTH. Despite the changes in the HPA axis, the circadian rhythm of cortisol generally seems to be preserved in anorexia patients, although conflicting data emerged from some studies (61).

The functional cortisol excess seen in anorexia and bulimia can have numerous negative effects. Some data point to its role in promoting the psychological and cognitive changes observed in patients with anorexia. A relationship has also emerged between the lack of cortisol response to 1-mg DST suppression and the presence and severity of depressive symptoms associated with eating disorders.

Some authors have also underscored the possible role of cortisol in the onset of attention deficit, especially in anorexia. Nutritional deficiencies and hypercortisolism may also promote bone loss in such patients, and the influence of hypercortisolism on other pituitary axes (the gonadotropic, growth hormone, and thyroid) may be responsible for oligomenorrhea-amenorrhea and a reduced TSH secretion despite low levels of T3 and T4. Finally, although the hypercortisolism associated with anorexia nervosa may not lead to a build-up of fat tissue due to the lack of substrates, it can affect the disproportionate accumulation of central adiposity after recovery from illness.

Getting back to our patient

As regards her diagnosis, our patient had several conditions (a neuropsychiatric disorder, overweight, and PCOS) that can be associated with PCS, and most of them could be secondary to a genuine endogenous hypercortisolism. Another aspect to emphasize concerns the treatments she was taking (oral contraceptives and antidepressants) that may have interfered with her hormone assessment (i.e. serum cortisol midnight and in the 1-mg DST). When she was retested again after discontinuing these drugs,

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she still had mildly increased UFC levels (170 µg/24 h; n.v. 36–137), with normal serum cortisol levels 2 days after a 2-mg DST (1.2 µg/L (n.v. <1.8)). LNSC was measured twice using the RIA method, producing different results (2.0 and 3.6 nmol/L n.v. 0.5–2.6).

With these results, mild CS still could not be ruled out.

What more can be done to exclude or confirm CS?

Second-line tests

Midnight serum cortisol (MserC)

A single MserC measurement using a cut-off of 207 nmol/L (7.5 µg/dL) was reportedly able to discriminate CS from PCS with a 96% sensitivity and 100% specificity (62). This was confirmed in further studies (63, 64) using higher cut-offs of 242 nmol/L; 8.8 µg/dL in wakeful patients (51) and <256 nmol/L (9.3 µg/dL) in all patients with PCS (65). In one study (63), a midnight:morning cortisol ratio of 0.67 also seemed to discriminate well between cases of CS and PCS (Table 2), but this test involves hospitalizing the patient and may produce false-negative results in patients with mild or quiescent CS.

Dexamethasone-suppressed corticotropin-releasing hormone stimulation test (*Dex*–CRH)

This combined test involves 2 days of dexamethasone suppression followed by CRH stimulation. It was first introduced in 1993, based on the observation that patients with PCS are thought to have a decreased ACTH response to exogenous CRH stimulation, while the ability to suppress cortisol after exogenous administration of dexamethasone persists (65). This test has shown a variable performance in different studies, however. The first study reported an excellent discrimination between CS and PCS patients when serum cortisol concentrations measured 15 min after administering CRH were higher than 38 nmol/L (1.4 µg/dL) (65). But these findings were not confirmed in later studies (64, 66) in which the test's weaker diagnostic performance led to the proposal of new cutoffs for cortisol 15 min after administering CRH. When plasma ACTH was analyzed in the same population, the best diagnostic performance was obtained with an ACTH concentration higher than 3.5 pmol/L (16 pg/mL) 15 min after administering CRH (64). Different thresholds for ACTH and cortisol response proposed in other studies reportedly achieved different sensitivity and specificity levels (67) (Table 2).

Differences in the reliability of the Dex–CRH test emerging from the above-mentioned studies may be due to the use of different test protocols, ovine as opposed to human CRH preparations, doses of 1 µg/kg or 100 µg, different cortisol and ACTH assays (especially as regards measurements in the low range), and patients' characteristics (degree of hypercortisolism, adrenal vs pituitary CS).

Low-dose dexamethasone suppression test (LDDST)

This test which involved 2 days of dexamethasone suppression was found to perform reasonably well. In two previous studies, the diagnostic accuracy of the Dex–CRH test did not differ significantly from that of the standard LDDST when cortisol cut-offs of 50 nmol/L (1.8 µg/dL) (66) or 55 nmol/L (2.0 µg/dL) (64) were used, though another study did not confirm as much (63).

CRH stimulation test

This test is generally used to differentiate between ACTH-dependent CS forms (68). Its low diagnostic accuracy when used alone to discriminate between patients with Cushing's disease (CD) and those with PCS has prevented its use for this purpose. Arnaldi *et al.* (69) recently examined whether applying novel criteria would enable the test to distinguish CD from PCS and from controls (CT). The authors suggested two separate combinations of parameters, each capable of diagnosing CD: (i.) basal serum cortisol >331 nmol/L (12 µg/dL) and peak plasma ACTH >12 pmol/L (54 pg/mL) or (ii.) peak serum cortisol >580 nmol/L (21 µg/dL) and peak plasma ACTH >10 pmol/L (45 pg/mL). These combinations yielded a greater diagnostic accuracy.

Desmopressin stimulation test (DDAVP)

DDAVP usually elicits a marked rise in plasma ACTH and serum cortisol in most CD patients, but not in cases of PCS or healthy individuals (70, 71).

It was previously claimed that a rise in plasma ACTH of at least 6 pmol/L within 30 min after DDAVP injection (Δ-ACTH) was the most effective diagnostic criterion for distinguishing CD from PCS patients (70).

Rollin *et al.* (72) found that a peak ACTH concentration of 15.8 pmol/L or an absolute increment of ACTH of 8.1 pmol/L over baseline could be used to correctly diagnose 93 or 92% of the patients, respectively.

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Table 2 Summary of published studies on the use of second-line tests in the differential diagnosis of pseudo-Cushing's states and Cushing's syndrome.

First author, year (reference)	Patients, *n*°	Second-line test	Protocols	Assays	Cut-offs for interpretation	SE (%)	SP (%)	Remarks
Papanicolaou DA, 1998 (62)	PCS 23 CS 240 (CD 198, EAS 27, ACS 15)	Midnight serum cortisol	Serum was obtained at 12:00 h using an indwelling venous catheter placed at 22:00. Patients were fasting from 21:00 h.	RIA – Fluorescence polarization immunoassay	Cortisol 207 nmol/L (7.5 µg/dL)	96	100	Hospital admission is required. In a subset of CD patients, the cortisol diurnal rhythm is preserved.
Alwani RA, 2014 (63)	PCS 20 CD 53	Midnight serum cortisol	Serum was obtained at 12:00 h in wakeful patients, using an indwelling venous catheter placed one day in advance.	Chemiluminescence immunoassay	Cortisol 243 nmol/L (8.8 µg/dL)	98	95	The midnight serum cortisol test may be falsely negative in patients with mild or quiescent CD.
Gatta B, 2007 (64)	PCS 14 CD 17 (mild)	Midnight serum cortisol	Serum was obtained at 12:00 husing an indwelling venous catheter placed at least 6 h before blood sampling. All patients had at least one night of habituation to the hospital environment.	RIA – Chemiluminescence immunoassay	Cortisol 256 nmol/L (9.3 µg/dL)	100	100	
Alwani RA, 2014 (63)	PCS 20 CD 53	Cortisol midnight:morning ratio	Serum was obtained at 09:00 h and 12:00 h in wakeful patients, using an indwelling venous catheter placed one day in advance.	Chemiluminescence immunoassay	Ratio 0.67	87	100	
Yanovski JA, 1993 (65)	PCS 19 CS 39 (CD 35, ACS 2, EAS 2)	DST-CRH test	Serial blood samples for ACTH and cortisol were obtained at time −15′, −10′, −5′, 0′, 5′, 15′, 30′, 45′, 60′ after administering 1 µg/kg of oCRH as an i.v. bolus at 08:00 and within 2 h of administering the last 0.5 mg Dexa in the 2 mg/day suppression test (0.5 mg Dexa every 6 h for 48 h starting at 12:00).	RIA	Cortisol at 15′ 38 nmol/L (1.4 µg/dL)	100	100	Expensive and cumbersome. Hospital admission is required. Lack of standardization of protocols and assays. Inter-individual variation in Dexa metabolism. Normal pituitary corticotrophs may retain a degree of responsiveness to pharmacological doses of exogenous CRH while suppressed with Dexa.
Martin NM, 2006 (66)	PCS 3 CS 12 (CD 8, ACS 4) CS-E 16	DST-CRH test	Patients received 0.5 mg Dexa orally every 6 h for 48 h, starting at 09:00. 48 h after the first Dexa dose a blood sample for serum cortisol was taken and a ninth dose of 0.5 mg Dexa was given. 2 h after the ninth dose, a blood sample was taken and an i.v. bolus of 100 µg hCRH was done. A final blood sample was collected 15′ after hCRH injection.	Chemiluminescence immunoassay	Cortisol at 15′ 50 nmol/L (1.8 µg/dL)	100	88	
Gatta B, 2007 (64)	PCS 14 CD 17 (mild)	DST-CRH test	Serial blood samples for ACTH and cortisol were obtained at time −15′, −10′, −5′, 0′, 5′, 15′, 30′, 45′, 60′ after administration of 100 µg hCRH as an i.v. bolus at 08:00 and within 2 h from the administration of the last 0.5 mg Dexa tablet in the 2 mg/day suppression test (0.5 mg Dexa every 6 h for 48 h starting at 12:00). oCRH was used in 6 CD patients.	RIA – Chemiluminescence immunoassay	Cortisol at 15′ 110 nmol/L (4 µg/dL) ACTH at 15′ 3.5 pmol/L (16 pg/mL)	100 100	86 85	
Erickson D, 2007 (67)	PCS 30 CD 21	DST-CRH test	Subjects initiated 0.5 mg Dexa orally every 6 h for 8 doses (first dose at 12:00). oCRH in a dose of 1 µg/kg (maximum 100 µg) was administered i.v. 2 h after completion of the last dose of Dexa.	Chemiluminescence immunoassay	Cortisol at 15′ 70 nmol/L (2.5 µg/dL) ACTH at 15′ 5.9 pmol/L (27 pg/mL)	90 95	90 97	
Alwani RA, 2014 (63)	PCS 20 CD 53	DST-CRH test	Patients received 0.5 mg Dexa orally every 6 h for 48 h, starting at 12:00. This was followed by i.v. administration of 1 µg/kg hCRH 2 h after the last Dexa tablet. Serum cortisol and ACTH were measured at time −15′, −1′, 5′, 15′, 30′, 45′, 60′ after CRH administration.	Chemiluminescence immunoassay	Cortisol at 15′ 87 nmol/L (3.2 µg/dL)	94	100	
Pecori-Giraldi F, 2007 (70)	PCS 23 CS 32 (CD 29, ACS 3)	DST-CRH test	Serial blood samples for ACTH and cortisol were obtained at time −30′, 0′, 15′, 30′, 45′, 60′, 90′ and 120′ after administration of 100 µg oCRH injected as an i.v. bolus within 2 h (08:00) of administering the last 0.5 mg Dexa tablet in the 2 mg/day suppression test (0.5 mg Dexa every 6 h for 48 h starting at 12:00).	Immunometric assay	Cortisol at 15′ 38 nmol/L (1.4 µg/dL)	100	62.5	

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Martin NM, 2006 (66)	PCS 3 CS 12 (CD 8, ACS 4) CS-E 16	LDDST	0.5 mg Dexa orally, every 6 h for 48 h, starting at 09:00.	Chemiluminescence immunoassay	Cortisol 50 nmol/L (1.8 μg/dL)	100	88	Inter-individual variation in Dexa metabolism.
Gatta B, 2007 (64)	PCS 14 CD 17 (mild)	LDDST	0.5 mg Dexa orally, every 6 h for 48 h starting at 12:00.	RIA – Chemiluminescence immunoassay	Cortisol 55 nmol/L (2.0 μg/dL)	94	86	
Alwani RA, 2014 (63)	20 PCS 53 CD	LDDST	0.5 mg Dexa orally, every 6 h for 48 h, starting at 12:00.	Chemiluminescence immunoassay	Cortisol 50 nmol/L (1.8 μg/dL)	94	84	
Yanovski JA, 1993 (65)	PCS 19 CS 39 (CD 35, ACS 2, EAS 2)	CRH stimulation test	Serial blood samples for ACTH and cortisol were obtained at time −15′, −10′, −5′, 0′, 5′, 15′, 30′, 45′, 60′ after administering 1 μg/kg oCRH as an i.v. bolus at 08:00.	RIA	Sum of post-CRH cortisol levels >3450 nmol/L (125 μg/dL)	64	100	Lack of standardization of protocols and assays. Excellent diagnostic performance with combined ACTH and cortisol analysis. Test interpretation based entirely on absolute cut-off values, rather than on their increment (deeply dependent on assay methodology).
Arnaldi G, 2009 (69)	PCS 26 CS 58 (CD 51, EAS 7) CT 31	CRH stimulation test	An i.v. bolus of 100 μg hCRH was injected over 30 s at 08:30. Blood samples were collected at time −15′, 0′, 15′, 30′, 45′, 60′, 90′ and 120′. Basal serum cortisol and basal plasma ACTH were the means of the two baseline values (−15 and 0 min) before CRH administration.	Chemiluminescent immunometric assay	Basal serum cortisol >331 nmol/L (12 μg/dL) and peak plasma ACTH >12 pmol/L (54 pg/mL) Peak serum cortisol >580 nmol/L (21 μg/dL) and peak plasma ACTH >10 pmol/L (45 pg/mL)	91.3 94.8	98.2 91.2	
Tirabassi G, 2011 (74)	PCS 18 CD 30 CT 12	CRH stimulation test	An i.v. bolus of 100 μg hCRH was injected over 30 s at 08:30. Blood samples were collected at time −15′, 0′, 15′, 30′, 45′, 60′, 90′ and 120′. Basal serum cortisol and basal plasma ACTH were the means of the two baseline values (−15 and 0 min) before CRH administration.	Chemiluminescent immunometric assay	Basal serum cortisol >331 nmol/L (12 μg/dL) and peak plasma ACTH >12 pmol/L (54 pg/mL) Peak serum cortisol >580 nmol/L (21 μg/dL) and peak plasma ACTH >10 pmol/L (45 pg/mL)	96.6 90	100 83.3	
Moro M, 2000 (71)	PCS 30 CD 76 SO 36 CT 31 PCS 30 CD 20 (mild)	DDAVP	Serial blood samples for ACTH and cortisol were obtained from an indwelling venous catheter at time −30′, 0′, 15′, 30′, 45′, 60′, 90′ and 120′ after administering 10 μg DDAVP i.v.	Two-site IRMA	Δ-ACTH ≥6 pmol/L within 30′ after DDAVP injection	86.8 90	90.7 96.7	Less expensive than CRH stimulation test. Excellent diagnostic performance with combined ACTH and cortisol analysis. Useful in cases of mild hypercortisolism given its independence from UFC, 1-mg DST and midnight serum cortisol. Lack of standardization of ACTH and cortisol assays.
Pecori-Giraldi F, 2007 (70)	PCS 23 CS 32 (CD 29, ACS 3)	DDAVP	Serial blood samples were obtained from an indwelling venous catheter 30′ before the test, at 0′ and 10′, 20′, 30′, 45′, 60′, 90′ and 120′ after administration of 10 μg DDAVP i.v.	Immunometric assay	Δ-ACTH ≥6 pmol/L within 30′ after DDAVP injection	81.5	90	
Rollin G, 2015 (72)	CD 68 PCS 75	DDAVP	Serial blood samples for ACTH and cortisol measurements were obtained from an indwelling venous catheter 15′ before the test, at 0′, 15′, 30′, 45′ and 60′ after administration of 10 μg DDAVP iv	Chemiluminescent immunometric assay	Peak ACTH of 15.8 pmol/L or Δ-ACTH ≥8.1 pmol/L 15′–30′ following DDAVP injection	90.8 88	94.6 96.4	
Tirabassi G, 2010 (73)	PCS 28 CD 52 CT 31	DDAVP	Serial blood samples were obtained from an indwelling venous catheter at time −15′, 0′ 10′, 20′, 30′, 45′, 60′, 90′ and 120′ after administering 10 μg DDAVP i.v. Basal serum cortisol and basal plasma ACTH were the means of the two baseline values (−15 and 0 min) before DDAVP administration.	Chemiluminescent immunometric assay	Δ-ACTH >6 pmol/L within 30′ after DDAVP injection Basal serum cortisol >331 nmol/L (12 μg/dL) and Δ-ACTH >4 pmol/L (18 pg/mL)	75 90.3	89.8 91.5	
Tirabassi G, 2011 (74)	PCS 18 CD 30 CT 12	DDAVP	Serial blood samples were obtained from an indwelling venous catheter at time −15′, 0′ 10′, 20′, 30′, 45′, 60′, 90′ and 120′ after administering 10 μg DDAVP i.v. Basal serum cortisol and basal plasma ACTH were the means of the two baseline values (−15 and 0 min) before DDAVP administration.	Chemiluminescent immunometric assay	Basal serum cortisol >331 nmol/L (12 μg/dL) and Δ-ACTH >4 pmol/L (18 pg/mL)	96.6	100	

ACS, adrenal Cushing's syndrome; CD, Cushing's disease; CS, Cushing's syndrome; CS-E, Cushing's syndrome excluded; CT, controls; Dexa, dexamethasone; Dexa-CRH test, dexamethasone-suppressed corticotropin-releasing hormone test; EAS, ectopic ACTH syndrome; hCRH, human-sequence CRH; LDDST, low-dose dexamethasone suppression test; oCRH, ovine-sequence CRH; PCS, pseudo-Cushing's state; SE, sensitivity; SO, simple obesity; SP, specificity; UFC, urinary free cortisol.

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To improve the diagnostic performance of the DDAVP test, Tirabassi *et al.* (73) proposed a new combination of parameters consisting of simultaneous basal serum cortisol and Δ-ACTH levels: they found that a diagnosis of CD could be excluded for patients who were positive for one or neither of these parameters.

Combined assessment of second-line tests

Only a few studies have compared multiple tests in the same study population. Alwani *et al.* (63) found that the Dex–CRH test and LNSC or MserC measurements achieved a high diagnostic accuracy in distinguishing between true CD and PCS. The results of the different tests were concordant in most patients, and combining the tests did not improve the diagnostic yield. The authors suggested using LNSC as the first choice, with each diagnostic center validating the diagnostic threshold. Other authors (64) recommended using the LDDST and MserC measurement at the start due to a low diagnostic performance of the Dex–CRH.

Tirabassi *et al.* (74) reported that the stimulation with human CRH and DDAVP tests showed an excellent diagnostic performance (sensitivity 96.6%, specificity 100% for both). These tests showed a better agreement than those resulting from all other possible combinations of the other tests considered (UFC, 1-mg DST and serum cortisol circadian rhythm) in the same population of patients with CD or PCS. Using new interpretation criteria based on the simultaneous analysis of ACTH and cortisol (69, 73), the authors suggested using the human CRH or the DDAVP test, with no particular preference for either, and using both tests only in particular cases.

Back to our patient

The CRH test was performed after pre-treatment with 2 mg dexamethasone for 48 h (cortisol 0.9 µg/dL to 1 µg/dL at 15 min) followed by a DDAVP test (baseline cortisol: 19 µg/dL response: 21 µg/dL; ACTH raised from 17 to 20 pg/mL). The result of both tests was suggestive of PCS. Thus, the persistence of a mildly increased UFC level was judged to be due to inaccuracy of the assay method available.

The patient's follow-up, implementing lifestyle modifications for weight loss, showed a mild regression of the clinical signs of hypercortisolism, thereby reinforcing the diagnosis of PCS (in a case of real CS, they would have been expected to progress).

Summary and a suggested approach

Clinical situations involving HPA axis activation giving rise to a so-called PCS remain a major diagnostic challenge for endocrinologists.

Patients may present with obesity, DM, depression, and PCOS that are frequent in the general population. At diagnosis, the patients will often have already consulted several specialists (gynecologists, psychiatrists, and internists) and may have been given different treatments that could interfere with their laboratory test results or confer an additional risk for patients with an occult CS.

An accurate clinical examination is needed to identify any features that might help to discriminate cases of CS, but such signs might not be apparent in mild hypercortisolism, making its diagnosis more difficult.

An accurate treatment of any condition that may lead to a suspected PCS is important because it can reverse the related hormone abnormalities. We encourage physicians to consider CS particularly if the patient has more than one feature of the disorder or a poor control of the single situation despite maximal therapy.

The choice of the optimal screening test in a high-risk population relies on an expert understanding of the diagnostic performance of the various tests in different clinical settings. Although LC–MS/MS is probably the most accurate method for assessing UFC levels, have not achieved widespread use in routine clinical practice, and steroid cross-reactivity remains an issue when immunoassays are used to measure serum and urinary cortisol levels. LNSC needs to be further validated for use in this setting, demonstrating its accuracy in the differential diagnosis of PCS and CS.

Regarding the Dex–CRH test and/or the easier and less expensive DDAVP test, there is still debate on the optimal cut-off values for each of these tests in different clinical settings (obesity, MDD, and alcoholism), and none of them has become the gold standard for differentiating CS from PCS.

When a diagnosis is unreliable, we suggest treating the associated conditions and adopting a close follow-up, because the hypercortisolism associated with CS may progress, and the condition might consequently be diagnosed at a later date.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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All authors contributed to the writing of the manuscript after a thorough literature search, dividing the work according to each single item treated, discussing the clinical case, and providing a critical feedback on the manuscript. Prof Scaroni, Prof Colao, and Prof Pivonello particularly conceived the original idea and contributed to the final version of the manuscript.

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