



# Autonomous cortisol secretion in adrenal incidentalomas

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## Abstract

Adrenal incidentalomas (AI) are one of the most frequent reasons for consultation in Endocrinology, as they are present in 3–10% of the general population. Up to 20% of them may have autonomous cortisol secretion (ACS), a term that refers to AI carriers with biochemical evidence of excess cortisol, but without the “specific” clinical signs of Cushing’s syndrome. As ACS is associated with an increased risk of diabetes, obesity, high blood pressure (HBP), osteoporosis, cardiovascular events, and global mortality; its correct identification is of great importance. There are different laboratory assays to detect ACS, but all of them have some limitations. The dexamethasone suppression test is the most accepted for screening. However, there is no consensus on the cutoff point that should be used. Low levels of ACTH and DHEA-S and high urinary free cortisol are also associated with ACS, but in isolation they are of little value to establish the diagnosis. Considering its clinical implications and the lack of consensus in the diagnosis and in which is the most appropriate management of these patients, this review offers a quick reference guide of ACS, presenting an exhaustive review of the topic: its definition, epidemiology, diagnosis, clinical implications, treatment, and follow-up.

**Keywords** Adrenal incidentalomas · Hypercortisolism · Autonomous cortisol secretion · Cushing’s syndrome

## Introduction

By definition, an adrenal incidentaloma (AI) is an asymptomatic adrenal lesion detected in imaging tests not performed due to suspected adrenal disease [1]. Moreover, most reviews and recommendations require a size  $\geq 1$  cm and exclude from the definition adrenal lesions detected in the extension study of extra-adrenal tumor disease [2–5] and on the screening of patients with hereditary syndromes [6].

Generally, AI are nonfunctioning cortico-adrenal adenomas that do not require a specific treatment approach. However, the remaining 10–15% may be conditions that require some type of therapeutic intervention (e.g., adrenal

carcinoma, pheochromocytoma, or Cushing’s syndrome (CS)) [6].

A term widely used in the context of AI was “subclinical CS”. However, this terminology has become somewhat obsolete and it is currently more advisable to talk about autonomous cortisol secretion (ACS). This term aims to define AI patients with biochemical evidence of excess of cortisol, but without the “specific” clinical signs of CS (mainly the lack of catabolic characteristics such as myopathy and skin fragility).

It is important to identify these patients since ACS is associated with increased morbidity (diabetes, obesity, hypertension, osteoporosis, and cardiovascular events) and mortality [7, 8]. However, ACS is not easy to diagnose, mainly due to the lack of consensus on its definition and the fact that the detection of “specific” findings of CS is doctor-dependent.

In this article, we will review the definition, epidemiology, diagnosis, clinical implications, treatment, and prognosis of ACS. We will focus on the main tools for its diagnosis and describe the strengths and pitfalls of different diagnostic techniques that are currently being used, and the complications associated to ACS.

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## Epidemiology

### Adrenal incidentalomas

Due to the increase in the use of imaging tests and their better resolution, incidentalomas are an increasing clinical problem. They are most frequently located at thyroid level (50%), followed by the pituitary (10–20%), and in the adrenals (5%) [9].

AI are one of the most frequently attended problems in general Endocrinology consultation; as radiological studies report their presence in 3% of 50-year-old patients increasing up to 10% in the elderly [3, 4, 10, 11]. They are more frequent in women (female-to-male ratio: 1.7) and in Caucasians. Their prevalence increases in obese, diabetic, and hypertensive patients [12]. They are very rare in children and adolescents (0.3–0.4% of childhood tumors). In this scenario, there is a high risk of malignancy, hence special attention should be paid to them [13].

The concept of AI includes a wide spectrum of diseases. There is a marked variability of the underlying etiology depending on whether it is a surgical or clinical series, however, the most common cause in all of them is the non-functioning adrenal cortical adenoma (80%). The prevalence of malignancy and functionality is higher in the surgical series, since the lesions of larger size or with clinical–biochemical suspicion of functionality are generally operated (Table 1) [2, 3, 6, 10, 12, 14].

### Autonomous cortisol secretion

The biochemical hypercortisolism or ACS is present in up to 20% of AI and is the most frequent hormonal alteration in AI. Its prevalence is very variable in the different series, mostly justified by the lack of consensus on its diagnosis (different diagnostic tests and cutoff points used in the different studies) [15]. Bulow et al. [16] reported a prevalence of 2%; Libe et al. [17] 18%; and Terzolo et al. [18] 5–20% (review of different series).

It is slightly more frequent in women and in patients older than 50 years [18]. Like AI, its prevalence increases in patients with diabetes, obesity, high blood pressure (HBP) and osteoporosis. It accounts for 15–20% of cases of endogenous CS, and is present in 0.2–2% of the general adult population [19].

Adrenal lesions with ACS usually correspond to cortico-adrenal adenomas (>85% of cases). However, there are reported cases of cortisol-producing myelolipomas [20], primary macro- or micronodular hyperplasia and in also in some adrenal carcinomas, where ACS is found [21].

**Table 1** Causes of adrenal incidentaloma according to different studies (expressed in percentages)

Type	Study (reference)			Mean
Radiological studies	Kloos [12] <sup>a</sup>	Mantero [10]	Barzon [3]	Global
<i>Bening lesions</i>	36–100	94.3	97	86.4
Adenoma	36–100	90.9	80	76.6
Nonfunctioning	36–94	76	70	71.3
Cortisol producing	0–12	13.9	NR	10
Aldosteronoma	0–7	1	NR	2.3
Pheochromocytoma	0–11	3.4	NR	4.45
<i>Malignant lesions</i>	0–21	1.9	7	6.5
Adrenal carcinoma	0–6	1	5	3
Metastasis (lung, breast and kidney cancer, melanoma, and lymphoma)	0–21	0.96	2	4.5
<i>Miscelanea</i>	0–15	3.8	8	6.4
Surgical studies	Mantero [10] <sup>b</sup>	Favia [2]	Fassnacht [6] <sup>a</sup>	Global
<i>Bening lesions</i>	82	70.6	82	78.2
Adenoma	52	57.6	55	54.9
Nonfunctioning	44.8	NR	69	56
Cortisol producing	15.6	NR	10	12.8
Aldosteronoma	3.9	NR	6.0	5
Pheochromocytoma	11	2.9	10	10.5
Myelolipoma	8	1.5	8	5.8
Cyst	5	2.9	5	4.3
Ganglioneuroma	4	5.9	4	4.6
<i>Malignant lesions</i>	18	29.4	18	21.8
Adrenal carcinoma	12	22.1	11	15
Metastasis (lung, breast and kidney cancer, melanoma, and lymphoma)	2	4.4	7	4.5
<i>Miscelanea</i>	6	NR	NR	6

<sup>a</sup>Review of different series

<sup>b</sup>Patients who underwent adrenalectomy in the series; NR not reported

## Diagnosis of ACS

ACS is associated with increased morbidity and mortality, hence it is important to identify its presence. Therefore, hypercortisolism screening should be performed in all AI carriers.

Three criteria must be fulfilled to strictly speak of ACS [18]:

1. Presence of AI.
2. Absence of typical CS data. The main problem at this point is that CS is a continuous clinical spectrum, ranging from mild to clearly manifest forms, and the detection will be markedly conditioned by the clinician's experience.
3. Hormone study demonstrating ACS (independent ACTH). It is at this point where there are serious limitations, since there is no clear differentiation between

excess cortisol and normality (depends in part on the personal preference of the clinician), considerable overlap in hormonal outcomes and lack of consensus on which is the best diagnostic strategy. Therefore, some authors propose a combination of several parameters to establish the diagnostic criteria. Moreover, coexistence of false positives of the tests is frequent in patients with AI (severe obesity, poorly controlled diabetes, psychiatric illness...). The different diagnostic tools currently available are shown in Table 2.

### Dexamethasone suppression test

Currently, DST (1 mg) is the most used test in the diagnosis of ACS [6]. However, there is still no clear consensus about the cutoff point that should be used for the screening of ACS in AI [6, 7, 14, 22].

This parameter is altered in patients with AI, between 3 and 100% of cases, depending on the cutoff point and the dose of dexamethasone used; therefore, the sensitivity (44–100%) and specificity (24–100%) described are very variable [3, 4, 10, 12, 18, 23–27].

It is a simple and economical assay to perform that may be done on an outpatient setting. The standard test consists in the administration of 1 mg of dexamethasone at 23PM and the determination of plasma cortisol between 8.00–9.00 a.m. the following day.

### Cutoff points used

The guidelines of the National Institute of Health (NIH) and the American Association of Clinical Endocrinologists and Endocrine Surgeons (AAACE/AAES) agree that hypersecretion can be ruled out when DST is  $<5 \mu\text{g/dl}$  [4, 6, 25]. The sensitivity of this cutoff point is relatively low [44–58%], but it is the one that offers greater specificity (up to 100% according to some authors). The guidelines of the French Endocrine Society (FES) [28], the Italian Association of Clinical Endocrinologists (AME position statement) [14] and the European Endocrinology Society (ESE) [6] advocate a considerably lower cutoff point of  $1.8 \mu\text{g/dl}$  to rule out ACS. This cutoff point has been established based on different studies demonstrating an increase in morbidity and mortality above these serum values [29, 30]. These results offer a higher sensitivity (75–100%), but low specificity (67–72%). Some European studies chose an intermediate point of  $3 \mu\text{g/dl}$ , based on the fact that it offers the best sensitivity-specificity balance for morbidity and mortality above this cutoff point [7] (Table 3).

In general, we could say that ACS can be ruled out with DST  $<1.8 \mu\text{g/dl}$  and confirm its presence with levels  $>5 \mu\text{g/dl}$ . However, in patients with intermediate values in

the DST there is no consensus on what attitude should be taken, so, there are several aspects still unclear in this subgroup of patients (see below, Section special situations: possible ACS).

Some authors propose higher doses of dexamethasone (3 or 8 mg), but no advantages have been shown compared with the conventional dose of 1 mg [31]. Others use the 2-day low-dose test ( $0.5 \text{ mg}/6 \text{ h}$  for 2 days); but since it is a more uncomfortable test to perform, it should be reserved for confirmatory diagnosis or in the context of psychiatric illness, alcoholism or diabetes [15, 32].

### Limitations

For the correct interpretation of the results, the circumstances responsible for false positives in the test must be taken into account [33] (Table 4).

The simultaneous determination of dexamethasone in plasma can help confirm that sufficient concentrations are reached ( $>5.6 \text{ nmol/L}$  ( $0.22 \mu\text{g/dl}$ )) to interpret the test result reliably, but its cost prevents its incorporation into a regular screening test [34, 35].

### Urinary free cortisol

Its determination offers an integrated assessment of the plasma concentration of cortisol in 24 h, which is not affected by variations in CBG, as occurs under estrogen therapy or with mitotane.

### Cutoff points used

When interpreting the results of this test is very important to take into account the laboratory method used; with radioimmunoassay a figure of  $<150 \mu\text{g/day}$  is normal, while with high-performance liquid chromatography the normal values are  $40\text{--}50 \mu\text{g/day}$ . The latter is a more specific test for the detection of hypercortisolism [15, 36, 37].

**Limitations** One of the more frequent problems is the incorrect sample collection. In these circumstances the simultaneous measurement of creatinine in urine increases its performance [38]. On the other hand, the guidelines of the ESE recommend collecting a second sample if the first is normal and there is suspicion of CS (to rule out cyclic CS or mild hypercortisolism) [6, 39]. Renal function should be taken into account (risk of false negatives with estimated glomerular filtrations  $<30\text{--}45 \text{ ml/min}/1.73 \text{ m}^2$ ), and also that the patient does not make excessive water intake or take preparations containing glucocorticoids [40].

UFC concentrations are elevated in 5–20% of patients with ACS. This is because the sensitivity to detect minimal

**Table 2** Tests for ACS diagnosis (cutoff points, limitations, and advantages)

Test	Cutoff point	Limitations	Advantages
DST	>1.8–5 µg/dl (Table 3)	<ul style="list-style-type: none"> <li>High percentage of situations that alter its results (Table 4)</li> <li>Low sensitivity in cyclic hypercortisolism</li> <li>Failure to comply with patient intake</li> <li>Lack of consensus in cutoff point</li> </ul>	<ul style="list-style-type: none"> <li>Of choice in the screening of ACS</li> <li>Simplicity of administration of DXM</li> </ul>
UFC	>150 µg/24 h (RIA) >40–50 µg/24 h (HPLC)	<ul style="list-style-type: none"> <li>Collection of 24-h urine (recommendable measurement of 24 h creatinine excretion)</li> <li>Two samples are required</li> <li>Limited utility in patients with GFR &lt; 30–45 ml/min/1.73 m<sup>2</sup> or excessive water intake</li> <li>Low sensitivity in AI (5–20%)</li> </ul>	<ul style="list-style-type: none"> <li>Not affected by concentrations of CBG</li> <li>Useful for the confirmatory diagnosis of CS</li> <li>24-h urine cortisol metabolites: more closely related to metabolic complications and are an early marker of hypercortisolism</li> </ul>
ACTH	<10 ng/ml = ACS; 10–20 ng/ml = indeterminate; >20 ng/ml = ACTH dependent CS	<ul style="list-style-type: none"> <li>Limited sensitivity in patients with mild hypercortisolism (frequent in ACS)</li> <li>High lability of the sample</li> <li>Low inter-laboratory reproducibility</li> </ul>	<ul style="list-style-type: none"> <li>Differential diagnosis of ACTH dependent and independent CS</li> </ul>
23PM SC	>7.5 mcg/dl = hypercortisolism	<ul style="list-style-type: none"> <li>Need for hospitalization (high cost)</li> <li>Different cut points according to patient conditions (awake/sleep)</li> <li>Careful interpretation in patients with erratic sleep schedules or employed in night-shift work</li> </ul>	<ul style="list-style-type: none"> <li>High sensitivity and specificity (around 95%)</li> <li>CS confirmatory diagnosis</li> <li>Early marker of autonomy and excess of cortisol</li> <li>Limited value in cyclic hypercortisolism.</li> </ul>
23PM salC	>145 ng/dl (ELISA and LC-MS/MS)	<ul style="list-style-type: none"> <li>Lack of consensus about the cutoff point</li> <li>Need for compliance with pre-extraction conditions</li> <li>Low sensitivity in ACS (not recommended for screening)</li> <li>Higher levels in cigarette smokers</li> <li>Careful interpretation in patients with erratic sleep schedules or employed in night-shift work</li> </ul>	<ul style="list-style-type: none"> <li>Possibility of performing outpatiently (economic)</li> <li>It is not affected by changes in the concentration of CBG</li> <li>Useful in cyclic CS</li> <li>Early marker of Hypercortisolism</li> <li>23 pm salCSA = Greater sensitivity than salC in ACS</li> </ul>
DHEAS	<40 mcg/dl = high specificity for ACS	<ul style="list-style-type: none"> <li>Lack of consensus about the diagnostic value of low DHEAS</li> <li>Variability in the cut point</li> <li>Physiological decline with age</li> </ul>	<ul style="list-style-type: none"> <li>Easy to perform (outpatient setting)</li> </ul>

DST dexamethasone suppression test, ACS autonomous cortisol secretion, DXM dexamethasone, UFC urinary free cortisol, RIA radioimmunoanalysis, HPLC high-performance liquid chromatography, GFR glomerular filtration rate, CS Cushing Syndrome, AI adrenal incidentaloma, CBG cortisol binding globulin, 23 pm SC 23 pm serum cortisol, 23 pm salC 23 pm salivary cortisol, ELISA enzyme-linked immunosorbent assay, LC-MS/MS tandem mass spectrometry, 23 pm salCSA pm salivary cortisone

**Table 3** Diagnostic efficacy of the 1 mg dexamethasone suppression test

Cutoff point	SE (%)	SP (%)	μG/DL	ESE (6)	FES (28)	AME (14)	NIH (4)	AACE/AAES (25)
1.8 μg/dl	75–100	67–72	>1.8	Possible ACS	Possible ACS	Possible ACS	No ACS	No ACS
3 μg/dl	63	75	>3	Possible ACS	Possible ACS	Possible ACS	No ACS	No ACS
5 μg/dl	44–58	83–100	>5	ACS	ACS	ACS	Possible ACS	Possible ACS

SE sensitivity, SP specificity, ESE European Endocrinology Society, FES French Endocrine Society, NIH National Institute of Health, AACE/AAES American Association of Clinical Endocrinologists and Endocrine Surgeons

**Table 4** Situations that alter the Dexamethasone suppression test [33]

Causes of pseudocushing	Main mechanism	Recommended test for CS screening <sup>a</sup>
Frequent Cushingoid phenotype: (A) Pregnancy (B) Depression (C) Alcoholism (D) Morbid obesity (E) Poorly controlled DM	(A) Increase CBG and placenta's CRH (B–E) Hyperactivation of the HPA axis through neural pathways with input to the paraventricular nuclei of the hypothalamus	(A) UFC (B–E) DST 2 mg/day (2 days), dexamethasone-CRH and desmopresin test
Infrequent Cushingoid Phenotype: (A) Physical stress (hospitalization, surgery, and pain) (B) Anorexia nervosa (C) Intense physical exercise (D) Hypothalamic amenorrhea	(A–D) Hyperactivation of the HPA axis through neural pathways with input to the paraventricular nuclei of the hypothalamus	(A–D) DST 2 mg/day (2 days), dexamethasone-CRH and desmopresin test
Drugs that interfere with the results		
Accelerate the metabolism of dexamethasone: (a) Phenobarbital, (b) Phenytoin (c) Carbamazepimine (d) Primidone (e) Rifapentine (f) Ethosuximide (g) Pioglitazone	Induction of CYP 3A4: induce hepatic enzymatic clearance of dexamethasone, thereby reducing plasma dexamethasone concentrations	UFC and 23 pm serum cortisol
Decrease the metabolism of dexamethasone: (a) Apreiptan (b) Itraconazole (c) Ritonavir (d) Fluoxetine (e) Diltiazem (f) Cimetidine	Inhibition of CYP 3A4: inhibit hepatic enzymatic clearance of dexamethasone, thereby increasing plasma dexamethasone concentrations	UFC and 23 pm serum cortisol

CS Cushing Syndrome, DST dexamethasone suppression test, CBG corticosteroid-binding globulin, UFC urinary free cortisol, HPA hypothalamic–pituitary–adrenal axis

<sup>a</sup>In adrenal incidentalomas, DST may be the test of choice despite the limitations in these situations

elevations of cortisol is low, and therefore has a limited applicability as a screening test in this context.

### Urinary cortisol metabolites

An alternative method for the diagnosis of ACS is the measurement of 24-h urinary cortisol metabolites by liquid tandem mass spectrometry (LC–MS/MS). Recent studies suggest that they present a better correlation with metabolic complications and are an earlier marker of hypercortisolism compared to other parameters [41].

### Cutoff points used and limitations

Given that it is an experimental marker, the cutoff points that should be used for each metabolite are unknown. Its main

limitations are the lack of the availability of the test, the need for 24-h urine collection and the lack of validation until the current date.

### ACTH

The determination of ACTH in plasma is essential to know if CS is ACTH-dependent or independent.

### Cutoff points used

Once hypercortisolism is confirmed, an ACTH level below 10 ng/mL confirms that it is an adrenal CS, and a value >20 ng/mL determines that it is an ACTH dependent CS. In case of intermediate values, it could be considered to expand the study with other tests such as stimulation

test with CRH or DST for ACTH) [42]. However, their results should be interpreted with caution due to the lack of studies that validate these results.

### Limitations

In patients with ACS, the degree of hypercortisolism may not be enough to completely suppress the HPA axis, and ACTH levels may not be suppressed. The specificity of a low ACTH as an isolated parameter in ACS is approximately 50% (38–60%), so it should be used in combination with other tests to guide the diagnosis of ACS.

The processing of the sample should be carried out with special caution, given its lability, which may result in a falsely low result. In a multicenter Italian study, they found a low inter-laboratory reproducibility in the ACTH assay, only 60% of the samples with low ACTH were correctly classified [43].

### Night serum cortisol

It assesses the normality of the circadian rhythm of cortisol. In general, its performance in sensitivity and specificity is high, around 95%. Serum cortisol at 23 h should be used as a second line test since it requires patient hospitalization. However, it has a better correlation with several clinical conditions associated with hypercortisolism (mainly with cardiovascular risk) compared to other tests, and it is an early marker of autonomy and excess of cortisol [44].

### Cutoff points used

Cutoff normal value depends on the extraction conditions and the range of normality of each center; but generally all cortisol values at 23 h  $>7.5 \mu\text{g/dl}$  are considered pathological [45].

### Limitations

The main limitation lies in the need of hospitalization for its proper collection and the possibility of false negatives in patients with cyclic hypercortisolism (complete HPA axis integrity may exist in the quiescence phase).

### Salivary cortisol and cortisone

These tests have the advantage that they are not affected by the concentrations of CBG, are economic and can be done at an outpatient setting.

Several recent studies have found that normal nocturnal salivary cortisol does not rule out ACS in patients with AI, therefore it should not be used as a screening test [46, 47]. In the recent study of Ceccato daily salivary cortisol rhythm was maintained in AI patients, without significant differences between patients with ACS and nonfunctioning

adenomas (NFA), concluding that it was not an adequate parameter to detect ACS in these patients [48]. Several studies have found salivary cortisone a better biomarker for free cortisol than salivary cortisol [49].

### Cutoff point used

Depending on the laboratory method used, there are different ranges of normality and differences in the sensitivity and specificity of the tests [45, 50, 51]. The methods that offer the best results are ELISA and LC–MS/MS [52]. With these techniques, normal nocturnal serum cortisol levels (23–24PM) are  $<145 \text{ ng/dL}$  ( $4 \text{ nmol/L}$ ).

### Limitations

The usefulness of salivary cortisol in AI is low (sensitivity of 22–76%) given that it does not discriminate ACS from normality. In cigarette smokers, salivary cortisol levels are higher than in nonsmokers [53], and the results may be altered in patients working at night or in shifts, as is the case with 23PM-serum cortisol.

The diagnostic ability of salivary cortisone in ACS can be compared, according to some studies, to that of nocturnal serum cortisol.

### Dehydroepiandrosterone sulfate

Low serum levels of dehydroepiandrosterone sulfate (DHEAS) are one of the most frequent alterations in patients with ACS. According to the AACE/AAES guidelines [25], a low level of DHEAS supports the diagnosis of ACS. However, other authors consider that it is not a reliable parameter [54, 55] and the FES [28], Korean [56] and ESE [6] guidelines do not recommend the systematic measurement of DHEAS in patients with AI.

### Cutoff points used

The cutoff point used for the screening of ACS is variable according to the different studies. Yener found that a level  $<40 \mu\text{g/dl}$  shows a good diagnostic capacity (73.7%), being the one that offers a better balance between sensitivity (68%) and specificity (75%) [57]. Furthermore, a recent study investigating the serum steroid profiling by LC–MS/MS in AI, found that low DHEA had good accuracy in predicting ACS (sensitivity and specificity of 71 and 76% for a cutoff of  $0.93 \text{ ng/mL}$ ) [58].

### Limitations

It should be taken into account that DHEAS levels decrease physiologically with age [55]. Therefore, its specificity in

the diagnosis of ACS decreases in the elderly population. The mass spectrometry-based assays are the best available techniques for the measurement of DHEAS, thanks to multianalytical capability at high level of specificity and sensitivity [58].

## Clinical implications of ACS

In ACS, by definition there are no specific signs or symptoms of hypercortisolism, but it is documented a higher frequency of several metabolic disorders, including HBP, type 2 diabetes (T2DM), obesity, osteoporosis, and increased mortality [29, 59] (Fig. 1).

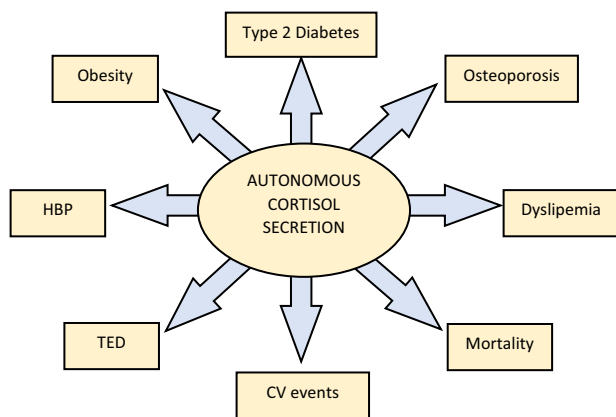
### Metabolic syndrome

There are basically two theories to explain the association between metabolic syndrome and ACS. One of them propose that it is mediated by the excess of cortisol (Terzolo) finds that triglyceride levels (considered an indirect marker of insulin resistance) were higher in patients with ACS regardless of BMI, attributing an effect mediated by cortisol [60]. On the contrary, others studies (Masserini) have found that it is mainly related to age and hyperinsulinism and in the absence of alterations in glucidic metabolism, ACS has no effect on lipid metabolism, that is, there is no direct effect of cortisol per se [61].

Lifestyle modifications are recommended, especially dietary control, physical exercise and weight control in patients with ACS and metabolic syndrome.

### Type 2 diabetes

Excess glucocorticoids alter the glucose metabolism through several complex patho-physiological mechanisms



**Fig. 1** Comorbidities possibly associated with autonomous cortisol secretion. *TED* thromboembolic disease, *HBP* high blood pressure

and are associated with various degrees of glucose intolerance, such as altered basal glycemia, carbohydrate intolerance, and overt diabetes [62].

There is a higher prevalence of T2DM in patients with ACS, estimated in the range 20–75% (variable according to the diagnostic criteria used). In this regard, because basal glycemia levels may be normal it is recommended to use oral glucose tolerance test or glycated hemoglobin for diagnosis [62]. The risk of T2DM increases proportionally with the degree of hypercortisolism, the patients' age and the size of the adenoma [62].

The prevalence of ACS in T2DM varies between 0 and 9.4% [63–65], and the risk increases in those with poor metabolic control, microvascular complications, obesity and HBP [66].

Regarding the antidiabetic therapy for glycemic control in ACS patients, the same recommendations for T2DM may be used.

### High blood pressure

Between 40 and 90% of patients have HBP. There seems to be a relationship with the duration of CS [67, 68] and a tendency toward higher BP than in patients with CS [67]. It is the alteration that improves more after surgery in patients with ACS [69].

The ESE guidelines recommend screening for HBP and T2DM in all patients with possible ACS and ACS [6].

Regarding the treatment of HBP, the ABC study group formulated a treatment algorithm that is specifically tailored for the management of hypercortisolism-related hypertension. They propose as a first-line treatment the use of any of the two blockers of the renin–angiotensin system, in light of the many evidences supporting a major alteration of this pathway in endogenous hypercortisolism, and for their cardioprotective effects [70].

### Osteoporosis

ACS can lead to an increased risk of osteoporosis and vertebral fractures [71]. ACS can be detected in nearly 5% of patients with osteoporosis and in 10% with osteoporotic vertebral fractures [71]. The prevalence of osteoporosis in ACS is higher than in NFA (48 vs. 13%) [72]. It is partially explained by a decrease in bone mineral density (BMD) and a deterioration in bone quality [71]. The role of dual X-ray absorptiometry in predicting the risk of fractures is unknown (given that there is greater deterioration of the microarchitecture than of the BMD) [73]. Most studies reported a reduction in BMD at the spine assessed by either DXA [73, 74] or quantitative computed tomography [71]. TBS has also been reported to be lower

and to predict the development of fracture in this group of patients [73]. ACS is reported to be associated with a decrease in blood osteocalcin levels, but no consistent changes in other markers or bone formation or markers of bone resorption [75].

Besides, it seems that there is an individual susceptibility to develop bone complications due to hypercortisolism. This is conditioned by different polymorphisms in the glucocorticoid receptor [76] and is a function of the activity of 11beta-hydroxysteroid dehydrogenase [77].

Guidelines and recommendations for the pharmacological treatment of glucocorticoid-induced osteoporosis [78, 79] recommend to start pharmacological treatment if the risk of fracture is moderate or high (regarding the age, BMD, menopausal status, and history of previous fracture) [75].

### Cardiovascular disease and mortality

Several studies show an increase in mortality in ACS patients, mostly from cardiovascular causes. Nevertheless, it is still unclear if the mortality rate is higher than that of the general population [80, 81]. Patients with ACS had a higher rate of cardiovascular disease and death than NFA patients [30]. Cardiovascular disease and infections are the most frequent causes of death in ACS [29]. There are several evidences that support the increase in cardiovascular risk in ACS; among them, the higher waist-to-hip ratio, higher percentage of visceral fat, larger number of atheromatous plaques, and increased intima-media thickness, which have been observed in these patients in several studies [29, 81–83].

We recommend treat intensively HBP, diabetes and all the cardiovascular risk factors in these patients (taking into account the risk/benefit balance) with the aim of reduce the global cardiovascular risk, so it should be considered the indication of statins, antihyperglycemics and antihypertensive drugs in ACS patients.

### Other associations

Some authors have found an association with thromboembolic disease [84], autoimmune thyroid disease [85], and psychiatric diseases, among others.

### Follow-up evolution

The follow-up recommendations in patients with AI are designed to detect malignant and functioning tumors (mainly ACS [3]). Some studies report that the risk of tumor hyperfunctionality reaches a peak at 3–4 years of follow-up, although there is still no clear consensus about the time of follow-up necessary prior to discharge [86].

### Risk of ACS in NFA

The risk that a patient with a negative functionality study progresses toward ACS is variable according to the different series ranging from 6.6 to 31% according to the different criteria used to diagnose ACS and the follow-up period. A higher risk of progression to ACS has been observed in adrenal lesions >2.5–3 cm and bilateral lesions [86, 87].

Until data will be available from large prospective studies, a reasonable approach may be to repeat the DST annually at least for 5 years in NFA (and consider also ACTH and UFC levels in patients with possible ACS). Similarly, we recommend that a careful monitoring of comorbidities potentially related to hypercortisolism should be performed annually.

### Risk of overt CS in ACS

The risk of evolution from ACS to CS is very low (<1% according to multiple studies [3, 27, 80, 86]). Some series [86, 88] reported that a size >3 cm and unilateral uptake of iodocholesterol were considered predictive factors of overt CS.

In patients with ACS an annual clinical reassessment for cortisol excess and comorbidities potentially related to cortisol excess should be performed [6].

### Treatment

The indication for surgical resection of an AI needs to be discussed by a multidisciplinary group once the recommended morphological and biochemical workup is completed. It is important to consider the patient's age, life expectancy, comorbidities, etc. The laparoscopic approach is the first line approach for the great majority of AI, once carcinoma has been ruled out.

Patients with ACS and possible ACS are at risk for transient insufficiency of the HPA axis after surgery. This will be prevented with intra and postoperative glucocorticoid supplementation treatment, preferably by hydrocortisone [6].

Several smaller studies have shown improvement of glucose metabolism and body weight after surgical resection [54, 89]. Furthermore in a prospective randomized study of 45 ACS patients found that 70% of operated patients showed improved of diabetes and HBP and patients who received conservative treatment showed deterioration in the glucemic and BP control [90]. These data suggest that surgical treatment is more beneficial than conservative treatment. Previous prospective, nonrandomized studies found similar results [83, 91–93].



## Special situations

### Bilateral AI

Bilateral AI (BAI) represent about 15% of AI [26, 94]. The distribution of the etiologies of BAI differs from that of unilateral AI. The most common causes are metastasis, primary bilateral macronodular adrenal hyperplasia (BMAH) and bilateral cortical adenomas [95]. BMAH and bilateral cortical adenomas are usually linked with a cortisol excess. In BMAH the cortisol secretion is partly regulated by the expression of multiple aberrant G protein-coupled receptors [96]. They are present in up to 87% of patients [97].

The hormonal evaluation in BAI is similar to unilateral AI, but in contrast to unilateral AI, adrenal insufficiency and congenital adrenal hyperplasia (specially 21-hydroxylase deficiency) should be excluded [6]. It is known that ACS is more prevalent in BAI than in unilateral adenomas patients. For example, in the Vassiliadi retrospective study (41.5 vs. 12.2%) [98]; Vassilatou prospective study (35.1 vs. 17.9%) [99] and Pasternak in a surgical serie (21.7 vs. 6.2%) [100].

The management of these patients is the most complicated issue. Bilateral adrenalectomy may be considered if UFC levels are >3–4 times the upper limit of normal (ULN) with both adrenals having relatively symmetrical size [95]. However, many groups reported the benefits of unilateral adrenalectomy in small series of patients with BMAH and modest cortisol secretion (<2–3 times ULN): with a remission rate of hypercortisolism reaching 97% with a recurrence rate of 23% [101, 102].

### Possible ACS

Nowadays, one of the most concerning issues are the patients with intermediate values in the DST (1.8–5 mcg/dl) (possible ACS) [6]. We must bear in mind that the result of the DST should be interpreted more as a continuous variable instead of categorical (yes/no) [6].

Exist evidence that patients with DST between 1.8 and 5 mcg/dl have higher risk of T2DM, hypertension, cardiovascular disease and mortality, and this risk increased during the follow-up [6, 8, 30]. So, we should look for comorbidities potentially related to cortisol excess in these patients [6].

None of the different additional tests evaluated in different studies was convincing enough to establish the diagnosis of ACS in these patients [6]. However, in a large number of studies a combination of DST and other parameters (specially high UFC and low ACTH) are used to improve the sensitivity and specificity [17, 47, 103]. Furthermore, the FES [28] and Korean guidelines [56] and AME position statement [14] recommend that patient with DST > 1.8 mcg/dl should receive second line

investigation, with at the very minimum a determination of UFC, serum, or salivary cortisol at midnight and ACTH.

Most of the panel members of the ESE guidelines recommend to repeat the DST after 3–12 months and re-evaluate comorbidities [6]. This attitude seems reasonable, especially in patients with DST closer to 5 mcg/dl if we take into account that a significant percentage evolves to ACS or developed potentially cortisol related complications during the follow-up [8, 30].

Our recommendation in patients with DST between 1.8 and 5 mcg/dl is to extend the study with a second-line test (mainly with UFC and ACTH) if there are comorbidities potentially related to excess cortisol (T2DM, hypertension, disease cardiovascular, etc). In all other cases the DST should be repeated after 6–12 months (before or after depending on the results of the previous DST).

## Conclusion

ACS is the most common hormonal alteration in AI, present in approximately 20% of these patients. It is associated with an increased risk of T2DM, HBP, obesity, dyslipidemia, and global cardiovascular risk. Therefore, it is of great importance to correctly identify these patients.

There are different tests for the screening of hypercortisolism. However, all of them have some limitations. In the absence of a single test considered as the gold standard, it is necessary the combination of different tests to reach an adequate diagnostic capacity.

The indication of surgery in ACS patients should be based mainly in the hormonal evaluation and the comorbidities potentially mediated by cortisol.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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