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CONSENSUS STATEMENT

# Who and how to screen for Cushing's syndrome: the position statement of the Italian Society of Endocrinology

**Filippo Ceccato[1,2] · Massimo Terzolo[3] · Federico Gatto[4,5] · Salvatore Cannavò[6,7] · Antonio Bianchi[8,9] · Andrea M. Isidori[10,11] · Annamaria Colao[12,13] · Diego Ferone[4,5] · Gianluca Aimaretti[14] · Francesca Pecori Giraldi[15] · Giorgio Arnaldi[16,17] · Carla Scaroni[1,2]**

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## Abstract

**Introduction** This Position Statement presents the recommendations of the Italian Society of Endocrinology (SIE) to identify patients with Cushing's syndrome in specific clinical settings.

We set two overarching research questions:

1. Which subjects should be screened ("who" should be screened)?
2. Which is the most appropriate first-line test ("how" to screen) for screening?

All suggestions/recommendations are evidence-based and directed at endocrinologists and other physicians who deal with patients who have presumptive Cushing's syndrome.

**Methods** The recommendations, developed by a SIE committee, were formulated based on eight short-reviews commissioned to experts of the SIE Pituitary and Adrenal Clubs. These short reviews, published in a special issue of the Journal of Endocrinological Investigation, reported a comprehensive review of the literature, and each of them answered the two research questions (who and how to screen for hypercortisolism) in a specific population: (1) patients with type 2 diabetes mellitus or obesity; (2) patients with arterial hypertension; (3) children and adolescents; (4) women with hyperandrogenism and/or menstrual irregularities; (5) patients with osteoporosis and/or fractures; (6) patients with mood disorders; (7) patients with adrenal and pituitary incidentaloma; (8) patients with unusual infections or thrombotic events. Finally, 24 recommendations were formulated, based on the quality of available evidence.

**Conclusions** The evidence-based Position Statement provides clear and pragmatic advice regarding "who" and "how" to screen for Cushing's syndrome. The suggestions/recommendations are developed for all health care providers, not only endocrinologists, to raise awareness on the diagnosis of Cushing's syndrome.

**Keywords** Cushing's syndrome · Non-neoplastic hypercortisolism · Dexamethasone suppression test · Late-night salivary cortisol · Urinary free cortisol · Mild autonomous cortisol secretion

**Abbreviations**

| | |
|---|---|
| 11β-HSD | 11β-hydroxysteroid dehydrogenase |
| AI | Adrenal incidentaloma |
| BMD | Bone mineral density |
| CBG | Cortisol binding globulin |
| CS | Cushing's syndrome |
| CD | Cushing's disease |
| DST | Dexamethasone suppression test |
| DXA | Dual-energy X-ray absorptiometry |
| EAS | Ectopic ACTH secretion |
| HPA | Hypothalamic-pituitary-adrenal |
| LNSC | Late-night salivary cortisol |
| MACS | Mild autonomous cortisol secretion |
| NNH | Non-neoplastic hypercortisolism |
| PCOS | Polycystic ovary syndrome |
| PI | Pituitary incidentaloma |
| SIE | Italian Society of Endocrinology |
| T2DM | Type 2 diabetes mellitus |

Extended author information available on the last page of the article

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| | |
|---|---|
| TBS | Trabecular bone score |
| UFC | Urinary free cortisol |

## Summary of recommendations

**Overarching recommendation** *In case of suspected CS we recommend to carefully collect the clinical history, including past or current therapy (especially glucocorticoids), and performing an accurate clinical assessment to detect signs and symptoms of endogenous hypercortisolism.*

1.1 **Screening for Cushing's syndrome in patients with type 2 diabetes mellitus or overweight/obesity**.

**R 1.1.1** We recommend screening for CS in patients with T2DM that is difficult to control or have additional comorbidities that suggest an increased risk of hidden hypercortisolism (strong, ⊕⊕⊕⊕).
**R 1.1.2** We recommend screening for hypercortisolism with 1-mg DST in outpatients with T2DM (strong, ⊕⊕⊕⊕).
**R 1.1.3** We recommend screening for CS in overweight or obese subjects if excess fat shows a truncal distribution and other signs suggestive for CS are present (moderate, ⊕⊕⊕◯).
**R 1.1.4** We recommend the 1-mg DST to screen for CS in patients with truncal overweight or obesity (strong, ⊕⊕⊕⊕).

1.2 **Screening for Cushing's syndrome in patients with hypertension**.

**R 1.2.1** We recommend screening for CS in patients with high likelihood of secondary hypertension, defined by at least one of the following: young age (<40 years old), resistant or difficult-to-treat hypertension, concomitant AI, concurrent signs/symptoms that suggest hypercortisolism (moderate, ⊕⊕⊕◯).
**R 1.2.2** We suggest screening for CS in hypertensive patients with features that increase the likelihood of CS such as hypokalemia, absent nocturnal dipping, rapidly worsening hypertension (low, ⊕⊕◯◯).
**R 1.2.3** We suggest LNSC as the first screening test in patients with hypertension (low, ⊕⊕◯◯).
**R 1.2.4** In hypertensive patients with AI, we recommend considering the 50 nmol/L threshold after 1-mg DST to exclude MACS and CS (strong, ⊕⊕⊕⊕).

1.3 **Screening for Cushing's syndrome in children and adolescents**.

**R 1.3.1** We recommend increased awareness among pediatricians to recognize CS in children and adolescents presenting with growth failure, with or without weight gain, and peculiar facial changes (moderate, ⊕⊕⊕◯).
**R 1.3.2** We recommend screening for endogenous hypercortisolism in the paediatric population using one of the three first-line tests (moderate, ⊕⊕⊕◯).

1.4 **Screening for Cushing's syndrome in women with hyperandrogenism and/or menstrual irregularities**.

**R 1.4.1** We recommend screening for endogenous hypercortisolism in women with menstrual irregularities and/or hirsutism if there are other signs and/or symptoms that suggest CS (moderate, ⊕⊕⊕○).
**R 1.4.2** We suggest the 1 mg-DST or UFC in women with menstrual irregularities and/or hirsutism and high clinical suspicion (moderate, ⊕⊕◯◯).

1.5 **Screening for Cushing's syndrome in patients with bone fragility**, **osteoporosis and/or fractures**.

**R 1.5.1** We recommend to rule out CS in patients in whom the etiology of bone fragility or fractures is not readily apparent, especially if they are unusual for age and sex (moderate ⊕⊕⊕◯) (moderate ⊕⊕⊕◯).
**R 1.5.2** We suggest, in patients with unusual fractures and signs or symptoms of CS, additional tools, such as morphometry and TBS assessment during DXA evaluation, to collect more evidence for bone damage due to hypercortisolism (low, ⊕⊕◯◯).
**R 1.5.3** There is no preferred screening test to screen for CS in patients with bone derangements; the 1 mg-DST is the most frequently used test (low, ⊕⊕◯◯).

1.6 **Screening for Cushing's syndrome in patients with mood disorders**.

**R 1.6.1** Mood disorders may be associated with functional activation of the HPA axis: first-line screening tests may yield false positive results in patients with NNH (Strong, ⊕⊕⊕⊕).
**R 1.6.2** The definite distinction between NNH and CS may require long-term follow-up. In patients with low apparent likelihood of overt hypercortisolism and mildly impaired tests, we recommend re-assessment after remission or improvement of the primary condition (moderate, ⊕⊕⊕◯).
**R 1.6.3** We recommend referral to a specialized Endocrine Centre and the use of second-line tests if the differential diagnosis between CS and NNH is still in doubt after first-line screening tests (moderate, ⊕⊕⊕◯).

1.7 **Screening for Cushing's syndrome in patients with adrenal or pituitary incidentaloma**.

**R 1.7.1** We recommend that patients newly diagnosed with AI undergo biochemical evaluation to rule out MACS (strong, ⊕⊕⊕⊕).
**R 1.7.2** We recommend screening for hypercortisolism in AI with the 1-mg DST as initial test, using the cortisol cutoff at 50 nmol/L to rule out both CS and MACS. In case of unsuppressed cortisol after 1-mg DST, CS must be confirmed with increased LNSC or UFC levels (strong, ⊕⊕⊕⊕).
**R 1.7.3** We suggest screening for CS with the 1-mg DST in patients with pituitary incidentalomas and features suggestive for hypercortisolism (low, ⊕⊕◯◯).

1.8 **Screening for Cushing's syndrome in patients with unusual infections or thrombotic events**.

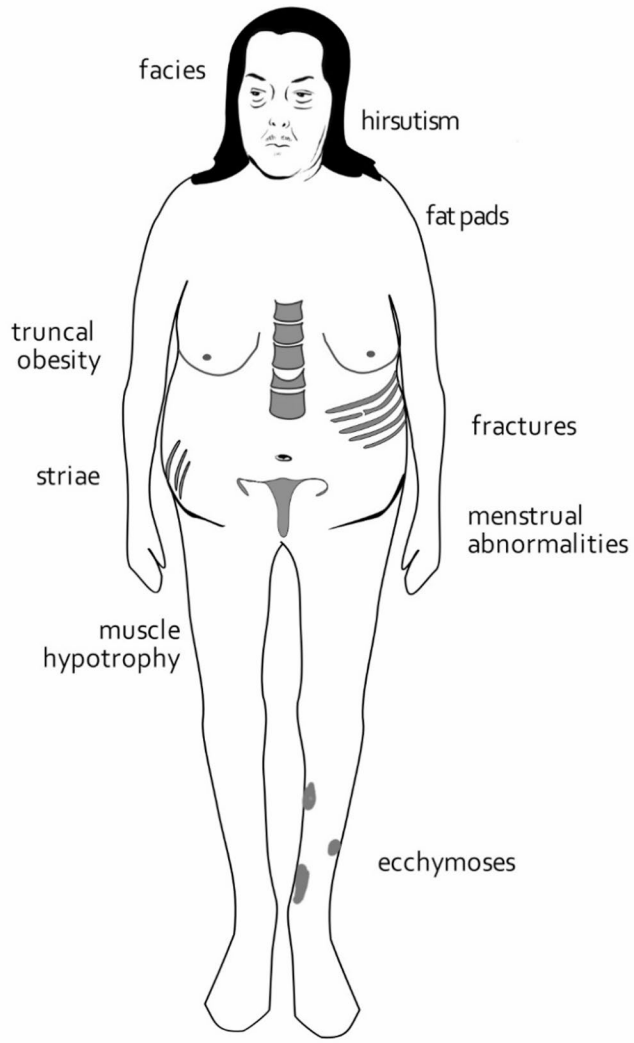


**Fig. 1** Main clinical aspects that suggest Cushing's syndrome

**R 1.8.1** We suggest that patients presenting with opportunistic infections without a known underlying cause for immunosuppression be evaluated for signs and symptoms of cortisol excess. Both severe systemic infections, as well as chronic or recurring mild cutaneous infections, should be considered suspicious for CS (very low, ⊕◯◯◯).
**R 1.8.2** We suggest that hormonal work-up for CS be carried out after the acute phase has resolved in patients with opportunistic infections, if feasible (very low, ⊕◯◯◯).
**R 1.8.3** We suggest that idiopathic thrombosis should raise suspicion for cortisol excess and lead to targeted clinical evaluation (very low, ⊕◯◯◯).

## Cushing's syndrome: definition, epidemiology, and clinical presentation

Cushing's syndrome (CS) is a clinical condition that comprises a myriad of comorbidities, signs, and symptoms due to prolonged activation of the glucocorticoid receptor, resulting from chronic exposure to high levels of glucocorticoids [1, 2]. Iatrogenic CS is the most common aetiology, due to glucocorticoids administered via any route [3]. Conversely, endogenous CS is a rare disease [4] and can be secondary to excessive ACTH secretion from a pituitary or ectopic tumour, or to independent adrenal cortisol secretion, ranging from overt CS to mild autonomous cortisol secretion (MACS) [4, 5].

The estimated prevalence of CS is about 40–79 cases per million individuals and the incidence ranges from 0.7 to 8 cases per million individuals per year [2, 6, 7]. CS is usually diagnosed in early adulthood, from 30 to 50 years of age with female prevalence especially for Cushing's disease (CD).

Morbidity and mortality are increased in patients with CS [2, 4], and diagnostic delay is common (median 2 years [8]). Therefore, suspicion of CS is essential for timely diagnosis and treatment to prevent future complications. Clinical presentation differs between subjects and no sign or symptom is unique to hypercortisolism. Some clinical features (purple striae larger than one cm, easy bruising, facial plethora, proximal muscular weakness, depicted in Fig. 1) are more specific for CS than others; although these signs are rare, their presence increases remarkably the likelihood of CS [9]. On the other hand, other cortisol-related comorbidities, e.g. diabetes, hypertension, osteoporosis, are extremely common in the general population and have a low discriminatory value; thus, establishing who should be screened is a challenging decision. Further, CS due to adrenal, pituitary o ectopic lesions (i.e., neoplastic or overt CS) must be differentiated from functional, non-neoplastic hypercortisolism (NNH) that may occur in conditions such as diabetes,

psychiatric disorders, hirsutism or obesity [10, 11]. In the past, terms such as "pseudo-Cushing's state" or "functional hypercortisolism" have been used to describe the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis without an underlying neoplasia [10]. In this Position Statement, we will use the term NNH, as recommended in the Pituitary Society guidelines [11].

According to guidelines and consensus statements [11, 12], patients with multiple, progressive clinical features suggestive for CS or worsening of these signs, especially if unusual for their age, should be screened with one of the first-line screening tests: morning serum cortisol after 1-mg dexamethasone suppression test (DST, overnight in most series), 24-hour urinary free cortisol (UFC) or late-night salivary cortisol (LNSC). A meta-analysis confirmed that all diagnostic tests for CS are highly sensitive [13], and several papers have been published in high-risk populations tested with one or more screening procedure.

The aim of this Position Statement is to provide an updated and easy-to-handle guide for CS screening in some populations, through the answer to two common, although difficult, questions: "who" is the patient that requires screening for CS and "how" to choose the best screening procedure among the three available tests. This Position Statement was developed for all physicians who deal with patients with suspected hypercortisolism.

## Methods

### Position statement working group

This Position Statement was committed by the Executive Committee (ExCo) of the Italian Society of Endocrinology (SIE) [14]. As chairs of the Position Statement, FC and CS were appointed, whereas the other members of the working group are the coordinators of the SIE Pituitary Club (FG, AB), of the SIE Adrenal club (GA, MT), as well as additional selected members of the SIE (GA, AC, DF, AMI, SC, FPG).

The working group identified the research questions ("who" and "how" to screen for endogenous CS) and eight clinical conditions at high-risk for CS or in which diagnosis is more challenging: patients with T2DM or obesity; arterial hypertension; children and adolescents; hyperandrogenism and/or menstrual irregularities; osteoporosis and/or fractures; mood disorders; adrenal or pituitary incidentaloma; unusual infections or thrombotic events.

Additional members of the SIE Pituitary and Adrenal Clubs were invited to write a Short Review, one for each clinical condition, in order to answer the two research questions. A special issue of the Journal of Endocrinological Investigation published the Short Reviews.

The working group had several on-line meetings between September 2022 and April 2025, and the recommendations were discussed with the authors of each Short Review (see Fig. 2). Endogenous hypercortisolism is a spectrum of disease, ranging from MACS to CS: the aim of this position statement was to provide a guidance for the diagnosis of CS, therefore the term hypercortisolism in the manuscript refers to overt CS (otherwise specified). Consensus was reached upon discussion; minority positions were reported in the "Reasoning" section after "Recommendations". Updated versions of the manuscript were circulated to all authors for final approval.

### Summary of methods, clinical question and literature search

The current Position Statement followed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) principles. GRADE is a systematic approach for synthesizing evidence and grading of recommendations. The main advantage of the system is to ensure transparency at all stages of the development process and provide readers with all the information required to understand the rationale of the recommendations/suggestions [15].

The first methodological step was to define the clinical question(s) to be addressed in the Position Statement, i.e., "who" and "how" to screen for endogenous hypercortisolism. These two clinical questions were the subject of each of the eight Short Reviews and were formulated in such a way that the Reviews can directly inform on the recommendations by the working group.

The recommendations are worded as "recommended" (strong recommendation) or "suggest" (weak recommendation). The meaning of a strong recommendation can be stated as follows: reasonably informed persons (clinicians, politicians and patients) would perform the management in accordance with the recommendation. For a weak recommendation (a suggestion) most persons would still act in accordance with the guideline, but a substantial number would not. The quality rating of evidence behind the recommendations was performed by the working group and was classified as very low (⊕◯◯◯, expert opinion supported by one or few small uncontrolled studies), low (⊕⊕◯◯, supported by large series of small uncontrolled studies), moderate (⊕⊕⊕◯, supported by one or few large uncontrolled studies or meta-analyses), strong (⊕⊕⊕⊕, supported by controlled studies or large series of large uncontrolled studies with sufficiently long follow-up) [16].

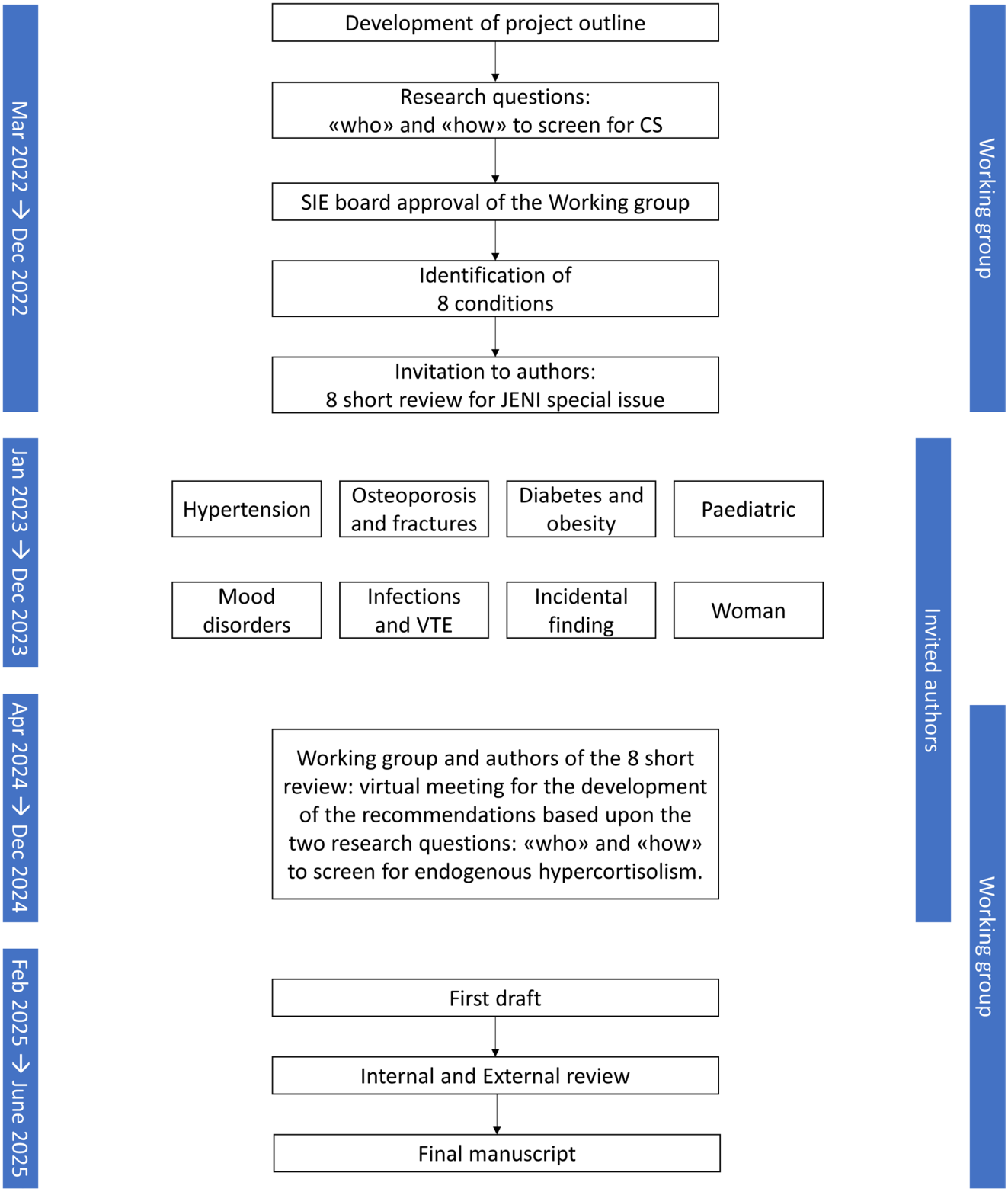


**Fig. 2** Position statement's development

## Cortisol measurement: laboratory issues and challenges

An effective screening would most likely reduce the diagnostic delay, which is one of the unmet needs in the management of CS [17]. From a methodological standpoint, screening procedures are expected to yield high sensitivity for detecting rare diseases such as CS, whereas high specificity would be required for confirmatory tests. Measurement of cortisol, be it in urine, saliva or serum represents the basis for screening for CS. Detection in different matrices carries specific collection and measurement issues; results for a given cortisol assay may differ across platforms and clinicians must be aware of this heterogeneity.

The use of exogenous glucocorticoids should be excluded prior to any testing procedure [11, 12]. Patients are not always aware of this interference and should be specifically questioned, not only on the use of oral steroids, but also creams, ointments, eye drops, inhalants, and intramuscular injections containing glucocorticoids. Moreover, patients should be carefully instructed by physicians or nurses and provided with written guidance for testing procedures.

The proposed first-line screening tests, aimed to establish the integrity of the HPA axis, are:

- LNSC: normal cortisol circadian rhythm is altered in patients with CS, and LNSC assesses the nocturnal nadir.
- Serum cortisol after low dose dexamethasone (DST) evaluates the sensitivity to glucocorticoid negative feedback. In healthy individuals, 1 mg dexamethasone administered at night inhibits ACTH secretion, resulting in reduced cortisol levels the following morning.
- 24 h UFC: measures bioavailable cortisol secreted over 24 h.

Cut-offs for UFC and LNSC should be established at each laboratory as they differ considerably between methods and assays [18].

In patients with high degree of suspicion for CS and a positive screening test result, confirmatory testing with one of the other tests should be performed. If hypercortisolism is confirmed by concordant results, NNH has to be excluded, prior to proceeding with the differential diagnosis of CS [19]. Imaging procedures [11, 12] should be carried out only after etiological subtyping, due to the possible detection of incidental pituitary and adrenal lesion. In patients with negative tests, the diagnosis of CS may be set aside. Screening tests should be repeated after 3–6 months if cyclical CS is suspected [20], or in case of sign/symptom progression.

### Remarks for LNSC

Circadian changes in cortisol secretion are easily detected in saliva: the nadir of cortisol levels occurs around midnight, and the transfer of cortisol from plasma to saliva is independent of flow rates [18]. Approximately 5% of serum cortisol is unbound -thus rapidly transferred to saliva and urine- the remainder is bound to cortisol binding globulin (CBG) with high affinity (approximately 80%) and to albumin with low affinity (approximately 15%) [18].

The use of LNSC has increased in the past decades, due to its high diagnostic accuracy, simple and noninvasive collection, and cost-effectiveness [13]. Multiple salivary cortisol collections provide a more sensitive method of detecting cyclic CS than UFC [21]. Late night saliva sampling should be carried out at usual bedtimes, rather than a fixed hour, in order to achieve unstressed levels; this decreases false positive results due to forced waking-up or staying awake beyond usual bedtimes [22].

Contamination with total cortisol from blood from teeth brushing, use of smoking, licorice, or oral hydrocortisone can limit the diagnostic accuracy of LNSC.

Salivary cortisol is suitable for patients with renal impairment (creatinine clearance < 60 mL/min) or polyuria, while LNSC is not reliable in night-shift workers.

### Remarks for UFC

Significant amounts of free cortisol appear in the urine when serum cortisol exceeds plasma protein binding capacity. Patients must be instructed to discard the first morning urine voiding, and to collect urine for the next 24 h, including the following morning. Two 24-h urine collections should be carried out to account for intra-patient variability as up to 50% difference in UFC levels can occur between different collections; this may prove relevant to correctly diagnose CS in case of mild cortisol excess; coefficient of variations were similar among two and three collections [23]. The advantages of UFC are the determination of the integrated 24-hour cortisol secretion independent from CBG levels and dexamethasone metabolism.

Sex, body mass index (BMI), age, sodium intake, urinary volume and impaired renal function (especially if < 60 mL/min) should be considered during interpretation of UFC measurements [11].

Mass spectrometry methods should be used to reduce false positive results due to cross-reactivity with synthetic steroids. There is an ongoing debate on the pros and cons of immunoassay versus chromatographic and mass spectrometry techniques: the former is available worldwide, low-cost, and easy to perform but susceptible to errors due to cross-reactivity with cortisol metabolites or exogenous

glucocorticoids. Chromatography and mass spectrometry offer greater specificity but are expensive and available only in a limited number of academic or highly specialized centers.

### Remarks for overnight 1-mg DST

The assessment of cortisol negative feedback is a simple dynamic test, resulting in the highest sensitivity but the lowest specificity, especially when the lower cut-off, i.e., serum cortisol < 50 nmol/L is used instead of the previously recommended cutoff at < 138 nmol/L [13] after 1 mg DST. Moreover, according to recent guidelines, the < 50 nmol/L threshold is also used to identify MACS [5]. Given the prevalence of AI, the chance of CS in individuals with unsuppressed serum cortisol after 1-mg DST is far lower than that of MACS. In clinical practice, 1-mg DST appears a reasonable choice given the limited availability of salivary cortisol and issues related to UFC measurements.

The 1-mg DST test must be interpreted taking into account concomitant medications, as some drugs, used in conditions suspicious for CS, may affect its diagnostic accuracy [24]. Measured serum cortisol reflects total cortisol, therefore increased CBG concentrations due to oral oestrogen treatment, pregnancy, or chronic active hepatitis, can lead to false positive results. On the other hand, 1-mg DST relies on sensitivity to glucocorticoid negative feedback and is linked to circulating dexamethasone levels after its oral intake. Dexamethasone levels can be lower than expected in patients with increased gut transit time (as in celiac disease or chronic diarrhea), due to drug malabsorption. Concomitant treatment with CYP3A4 inducers or inhibitors (Table 1, also available online at https://drug-interactions.medicine.iu.edu/ [25]) may also affect results of 1-mg DST. CYP3A4 inducers may increase dexamethasone metabolism and reduce its levels. leading to false positive results, i.e., unsuppressed serum cortisol. Conversely, CYP3A4 inhibitors may reduce dexamethasone metabolism and increase circulating dexamethasone, thereby inappropriately suppressing cortisol i.e., false negative results. The measurement of both dexamethasone and cortisol levels may reduce the risk of erroneous interpretations due to incorrect assumption of dexamethasone tablets [26].

**Table 1** Drugs that interfere with dexamethasone metabolism

| CYP 3A4 induction | CYP 3A4 inhibition |
|---|---|
| Anti-epileptics: Phenobarbital, Phenytoin, Primidone, Carbamazepine, Ethosuximide, Clonazepam, Tiagabine | Antidepressants SNRI, serotonin noradrenaline reuptake inhibitors; SSRI, selective serotonin reuptake inhibitors, atypical antipsychotics): Fluoxetine, Sertraline, Paroxetine, Trazodone, Citalopram, Bupropion, Venlafaxine, Olanzapine, Quetiapine |
| Medicinal plants: Saint John's wort (Hypericum perforatum) | |
| Antibiotics: Rifampin, Rifapentine | |
| Antihypertensives calcium channel blockers, angiotensin receptor antagonists, β-adrenoceptor blockers: Diltiazem, Verapamil, Irbesartan, Losartan, Propranolol | |
| Lipid-lowering drugs: Atorvastatin, Simvastatin | |
| Antihistamine: Cimetidine | |

## Rationale for recommendations

### Screening for Cushing's syndrome in patients with type 2 diabetes mellitus or overweight/obesity

**R 1.1.1 We recommend screening for CS in patients with T2DM that is difficult to control or have additional comorbidities that suggest an increased risk of hidden hypercortisolism (strong, ⊕⊕⊕⊕).**

*Reasoning*

Impaired glucose homeostasis, often leading to diabetes mellitus, is a common complication of CS: 20% to 45% of patients with CS have T2DM, and 10% to 30% have increased fasting glucose or impaired glucose tolerance. Thus, the overall prevalence of altered glucose metabolism in CS is close to 70% [27]. Disorders such as diabetes mellitus, obesity, arterial hypertension, dyslipidaemia are common in the general population and poorly discriminatory. Therefore, the evaluation of clinical features typical of hypercortisolism may be predictive of CS and potentially more important than any screening test alone.

The reported prevalence of CS among patients with T2DM, including some large studies with over 800 patients, ranged from nil to 3%; while prevalence of MACS was 5–9% among patients with T2DM [28]. A meta-analysis performed some 10 years ago reported a pooled prevalence of hypercortisolism (including MACS) equal to 3.4% among patients with T2DM; prevalence fell to 1.4% if different criteria and imaging were required for diagnosis of CS [29]. A more recent meta-analysis reported positive first-line tests in patients with T2DM, especially those on insulin therapy, with hypertension requiring at least 2 drugs or with diabetic micro- and macrovascular complications [30]. A high prevalence of hypercortisolism (23.8%) has been recently reported in difficult-to-control T2DM defined as HbA1c 7.5−11.5% and taking ≥ 3 antidiabetic drugs, or insulin and other drugs, taking ≥ 2 antidiabetic drugs and at least one micro or macrovascular complications, or combined with at least 2 antihypertensive medications (36.6%

in case of 3 drugs) [31]. Therefore, the skillful assessment of clinical presentation may increase the likelihood of CS at screening in patients with T2DM [28].

**R 1.1.2 We recommend screening for hypercortisolism with 1-mg DST in outpatients with T2DM (strong, ⊕⊕⊕⊕).**

*Reasoning*

Screening for hypercortisolism in patients with T2DM has been reported in 18 studies, totaling 5068 patients (range 77–993, median 186 patients) with a broad range of inclusion criteria (age, BMI, newly diagnosed, HbA1c levels, hypertension, history of comorbidities) and resulted in detection of CS from none to 9.3% of tested patients [28]. A recent study included 1057 participants with difficult-to-Control T2DM: cortisol was >50 nmol/L after 1-mg DST in 24% of patients: abdominal imaging was positive for an AI in 35% of patients [31].

The higher prevalence of hypercortisolism in some studies could also depend on the diagnostic criteria, e.g., MACS or only confirmed CS, and the screening test used. If stringent criteria to define CS and further confirmatory tests were used, prevalence of CS dropped to 0.7–1.3% [28]. The most commonly used screening test in these studies was 1-mg DST (13 studies) [28].

Satisfactory metabolic control should be achieved before testing [32], as the activation of the HPA axis may be found in poorly controlled diabetic patients per se [33], resulting in false positive results. Moreover, patients tested in outpatient setting are less likely to present stress-related increases in cortisol as commonly observed during hospitalization for poorly controlled diabetes. On the basis of these considerations, screening for hypercortisolism should be postponed after discharge. However, if glucometabolic control is not achieved despite intensive treatment, and the patient also presents resistant hypertension or other conditions that increase the likelihood of hypercortisolism, then testing for CS can be carried out nonetheless.

LNSC in patients with T2DM yielded low specificity (14%) and lower positive predictive values (22%) compared to cortisol after 1-mg DST, resulting in an overall low accuracy [34]. In fact, patients with diabetes mellitus often present impaired cortisol rhythm and the highest number of false positives at screening occurred with LNSC [28]; this was reported in series where chemiluminescence assay results were confirmed by mass spectrometry [35]. Only one study used UFC to screen for CS in patients with T2DM; thus, its role in this context cannot not be assessed. It is worth recalling that T2DM may be associated with chronic kidney disease, thus reducing the diagnostic accuracy of UFC.

**R 1.1.3 We recommend screening for CS in overweight or obese subjects if excess fat shows a truncal distribution and other signs suggestive for CS are present (moderate, ⊕⊕⊕◯).**

*Reasoning*

An excessive adiposity, as obesity is currently defined, is a common condition beyond its definition with body mass index [36], affecting half of the adult European population in 2021 38. Central obesity is very common in patients with CS and is characterized by redistribution of adipose tissue from limbs to the abdominal region, face, neck, and trunk. Generalized obesity alone may be misleading as it can lead to activation of the HPA axis per se 29. The prevalence of hypercortisolism in patients with obesity varied among the various studies as patient population and study design were different: some papers excluded patients with typical features of cortisol excess, others included MACS among causes of hypercortisolism, others evaluated only patients with confirmed CS. The pooled prevalence of CS in obese subjects from a random effect was 0.9%, considering studies that used at least two screening tests [38]. Therefore, the presence of adiposity alone is not sufficient to suspect CS, and the combination of signs and symptoms should be clinically detected in patients with obesity or in those with recent unexplained weight gain.

Although obesity and weight gain are among the chief complaints leading to screening for CS, central obesity was more common in those adults in whom CS was ruled out, indeed, the majority presented with lifelong obesity [39], in keeping with the negative correlation between slow-onset weight gain and likelihood of endogenous hypercortisolism [9].

**R 1.1.4 We recommend the 1-mg DST to screen for CS in patients with truncal overweight or obesity (strong, ⊕⊕⊕⊕).**

*Reasoning*

Clinical obesity is a chronic, systemic illness characterized by several derangements at tissue, organ or whole-body level due to excess adiposity. The risk of developing other non-communicable diseases (e.g. T2DM, hypertension, cardiovascular disease) is increased in patients with obesity [36], as well as in those with CS [2]. In patients with preclinical obesity (previously known as metabolically healthy obesity), the likelihood of hypercortisolism is low: CS should be suspected in patients with clinical obesity and other potentially cortisol-related conditions (e.g., T2DM, hypertension, osteoporosis with fractures).

Screening for hypercortisolism in patients with overweight or obesity ranged from 0 to 8.7%; it has been evaluated in 12 studies with different inclusion criteria, totaling 5314 patients (range 16–1037, median 384 patients) [28].

The biochemical work-up was very heterogeneous among these studies with LNSC (1 study), UFC (2 studies), and 1-mg DST (10 studies) used as screening tests and

different diagnostic thresholds applied. The highest diagnostic accuracy at screening for CS has been reported with cortisol after DST [28].

## Screening for Cushing's syndrome in patients with hypertension

**R 1.2.1 We recommend screening for CS in patients with high likelihood of secondary hypertension, defined by at least one of the following: young age (<40 years old), resistant or difficult-to-treat hypertension, concomitant AI, concurrent signs/symptoms that suggest hypercortisolism (moderate, ⊕⊕⊕◯).**

**R 1.2.2 We suggest screening for CS in hypertensive patients with features that increase the likelihood of CS such as hypokalaemia, absent nocturnal dipping, rapidly worsening hypertension (low, ⊕⊕◯◯).**

*Reasoning*

Hypertension is one of the most common features in patients with CS at diagnosis (~ 80%, even higher in EAS), as reported in the ERCUSYN registry [8]. Hypertension represents an early feature of hypercortisolism and may persist after achieving disease remission [41, 42]. The pathophysiology of CS-related hypertension is multifactorial [43], the cornerstone being the mineralocorticoid effect of excess cortisol. In normal conditions, the 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme inactivates cortisol to cortisone in mineralocorticoid-sensitive tissues. In severe hypercortisolism, excess cortisol saturates 11β-HSD2 activity resulting in spillover mineralocorticoid effect, with sodium retention, increased plasma volume and potassium excretion [44]. Excess cortisol also up-regulates angiotensin II-receptors type 1, modulates the vascular response to catecholamines, and induces metabolic complications (diabetes, obesity, sleep apnea) that further contribute to pathogenesis of hypertension [45].

Absence of nocturnal blood pressure dipping is observed in more than 50% of patients with CS, mirroring the disrupted cortisol circadian rhythm. CS should be excluded in patients with non-dipper 24-h ambulatory blood pressure profile and with other clinical features specific for hypercortisolism [46].

The prevalence of CS in patients with hypertension has been reported in 8 studies totaling 11,504 patients (series size 80–4429) and ranged from 0 to 7.7%, according to selection criteria [46]. Of note, older studies reporting on over 5.000 individuals used morning serum cortisol for diagnosis -currently not listed among screening tests- and prevalence of CS was considerably lower (0.1%–0.5%) [47, 48]. The highest prevalence was reported during screening in selected populations: young, i.e., < 40 years old, hypertensives (6.2% of 80 patients) [49] or individuals with an adrenal lesion (7.7% in 104 patients) [50].

Hypertension, diabetes and obesity are common in the population: thus, indiscriminate screening for CS is not justified [9]. However, timing, progression and severity of each, as well as the combination with other signs or symptoms of cortisol excess, increases the likelihood of endogenous hypercortisolism and should lead to screening for CS.

The prevalence of secondary hypertension is higher in young patients without a family history of hypertension, with early onset of elevated blood pressure levels, worsening of previously well-controlled hypertension, or difficult-to-treat hypertension [51]. There are no specific studies targeting the age threshold for secondary hypertension; in clinical practice, hypertension arising in individuals younger than 40 years of age commonly leads to testing for underlying causes. Hypertension is defined as resistant to treatment when appropriate lifestyle measures and treatment with optimal or best tolerated doses of three or more drugs (a thiazide or thiazide-like diuretic, a renin-angiotensin system blocker and a calcium channel blocker) fail to lower office BP to < 140/90 mmHg according to European guidelines [52]. Moreover, inadequate dosing and poor adherence to prescribed treatments should be excluded before screening [53].

**R 1.2.3 We suggest LNSC as the first screening test in patients with hypertension (low, ⊕⊕◯◯).**

*Reasoning*

1-mg DST was the most studied screening test in the setting of hypertensive patients: it was used in 1204 subjects, with different selection criteria (e.g., only hypertension [54], hypertension and young age [49] or hypertension and AI [50]) and assessing different post-test cortisol thresholds (from 50 to 138 nmol/L). Screening with 1-mg DST was carried out in individuals with resistant hypertension: 112 out of 423 patients presented unsuppressed cortisol after 1-mg DST (>50 nmol/L) and among them 34 had impaired LNSC, measured twice with a commercial radioimmunoassay [55]. According to the latest Consensus statements, at least two increased LNSC collections are required to proceed towards diagnosis of CS [11]. In the case of an adrenal mass, the 1-mg DST test is preferred [46].

UFC has not been evaluated as screening test in hypertensive subjects and the frequent occurrence of some degree of impaired renal function in hypertensive patients could be an additional confounding factor. One advantage of UFC is that cortisol production is unaffected by binding globulins (especially CBG) and dexamethasone increased or reduced metabolism. However, it is important to note that UFC can exhibit large variability [23]. We recommend UFC only in patients with normal kidney function as free cortisol excretion may be significantly impaired in moderate to severe

renal impairment (creatinine clearance < 60 mL/min [56]). On the other hand, substantial urinary output may provide false positive results [57]: diuresis above 3 L/day should lead to careful interpretation of UFC levels, even though the effect of water diuresis is most evident in cortisone and less in cortisol [58]. Other screening tests, such as LNSC, should be preferred in patients with hypertension-related chronic kidney disease. Finally, considering also that some antihypertensive drugs may affect dexamethasone metabolism, LNSC is likely the most discriminatory test and may be preferred in hypertensive subjects, except in patients with AI.

Metabolic syndrome is a clustering of conditions that increases the risk of cardiovascular disease [40], and many patients requiring CS screening could present with hyperglycemia, hypertension and obesity. According to the previous section regarding patients with T2DM or obesity, there is not a preferred screening test: LNSC and 1-mg DST should be considered in clinical practice, because the evidence of UFC is limited.

**R 1.2.4 In hypertensive patients with AI, we recommend considering the 50 nmol/L threshold after 1-mg DST to exclude MACS and CS (strong, ⊕⊕⊕⊕).**

*Reasoning*

A cortisol value < 50 nmol/L after 1-mg DST is adequate to rule out overt hypercortisolism in all patients and to exclude MACS in those with AI [5, 11].

Several common antihypertensive drugs, e.g. calcium channel blockers, may interfere with dexamethasone metabolism [59], leading to reduced specificity of serum cortisol after 1-mg DST [24]. Indeed, there is considerable evidence that calcium channel blockers, in particular diltiazem and verapamil are potent inhibitors of CYP3A4 and -to a lesser extent- amlodipine and nifedipine are weak inhibitors [59], reassumed in Table 1. If false positive results are suspected, we suggest other strategies to rule out CS such as retesting with 1-mg DST and concurrent measurement of dexamethasone (if available) [26], employ additional tests (e.g., LNSC), or withdrawal of the interfering drug if clinically feasible/appropriate.

## Screening for Cushing's syndrome in children and adolescents

**R 1.3.1 We recommend increased awareness among paediatricians to recognize CS in children and adolescents presenting with growth failure, with or without weight gain, and peculiar facial changes (moderate, ⊕⊕⊕◯).**

*Reasoning*

10% of endogenous CS occurs in pediatric patients; diagnosis is often challenging due to its insidious onset, the absence of early relevant signs, and rarity. Diagnostic delay from clinical onset can be nearly 3 years [60, 61].

The combination of weight gain and growth failure is pathognomonic; in fact, the inhibitory action of glucocorticoids on growth plate cartilage leads to the most evident pediatric clinical manifestation: decreased height velocity or growth arrest. Of note, growth is preserved in children with concomitant hyperandrogenism due to adrenal carcinoma. Growth arrest is reported in 40–100% of patients with CS and should be considered a red flag for hypercortisolism; height below 0 SDS and BMI above + 1.5 SDS can suggest CS, as opposed to simple obesity which often presents with tall stature [60]. Other than weight gain (prevalence 80–100%), common signs in childhood and adolescence include facial swelling (such as plethora or moon face, 50–100%), headaches, large striae rubrae, acanthosis nigricans, dorsal cervical or supraclavicular fat pads, and osteopenia with an increased fracture risk [60]. Obesity is less frequent in older adolescent females [62].

CD is the most common form of CS in pediatric patients, accounting for approximately 75–80%, with 15–20% ascribed to adrenal CS, primarily adrenal tumours in children under 8 years of age. CD in younger children with a relevant family history suggests genetic causes, i.e., *AIP*, *MEN1*, *CDKN1B*, or, more rarely, *PRKAR1A*, *USP8*, *RET* or *DICER1* [60, 63].

Incidence of adrenocortical tumours peaks in the first decade of life, with a median age at diagnosis 3–4 years; they may be isolated or in context of Li-Fraumeni or Beckwith-Wiedemann syndrome and are almost always secretory, with either virilization alone or, in approximately 80% of patients, also CS. Primary pigmented nodular adrenocortical disease is a rare congenital disorder that typically presents in late adolescence and is associated with multiple endocrine conditions (Carney's complex) mostly due to mutations in *PRKAR1A* [58]. McCune-Albright syndrome associated with bilateral adrenocortical hyperplasia is the leading cause of CS in infants [60].

The differential diagnosis of CS in children and adolescents should also consider familial partial lipodystrophy, particularly Dunnigan variety lipodystrophy: fat accumulation in the face and neck can be remarkable in this disease [64].

**R 1.3.2 We recommend screening for endogenous hypercortisolism in the pediatric population using one of the three first-line tests (moderate, ⊕⊕⊕◯).**

*Reasoning*

UFC remains a widely used screening test for pediatric CS, as it is non-invasive and can be collected at home, although the 24-h collection can be challenging for younger patients. In the pediatric population, UFC should be corrected for body surface area: values greater than 70 μg/m$^2$/day yield acceptable diagnostic accuracy (sensitivity > 88% and specificity > 90%) [60]. Mild forms of hypercortisolism may

result in false-negative results, and false-positive results can arise from NNH due to physical or emotional stress, severe obesity or depression. Given the extreme rarity of CS in children, the positive predictive value of UFC is inevitably low. UFC alone is not always considered an ideal screening tool and should be used together with other screening tests to improve the detection of CS.

Data on the sensitivity and specificity of LNSC in the pediatric population are limited but appear comparable to late-night serum cortisol, with values ranging from 93 to 100% to 95–100%, respectively [60]. Of note, salivary collection requires specific devices in young children [65]. Endocrinologists should also be aware that newborns exhibit a flat circadian cortisol curve and that the morning-to-evening ratio develops between 6 and 9 months of age [66]. Therefore, screening for CS in children under 1 year of age should not rely on assessment of circadian rhythm.

The low-dose DST is typically performed in two different forms: the 1 mg "overnight" (or Nugent) and the two-day 2 mg (or low-dose Liddle test, LDDST); In patients weighing less than 40 kg, the dose of dexamethasone should be adjusted (25 µg/kg). The LDDST has shown good sensitivity (over 90%) in pediatric CS patients; there is limited data on the sensitivity and specificity of the overnight 1-mg DST [60].

## Screening for Cushing's syndrome in women with hyperandrogenism and/or menstrual irregularities

**R 1.4.1 We recommend screening for endogenous hypercortisolism in women with menstrual irregularities and/or hirsutism if there are other signs and/or symptoms that suggest CS (moderate, ⊕⊕⊕◯).**

Women with CS frequently present menstrual irregularities, acne, hirsutism, and polycystic ovaries [67, 68]; indeed, these symptoms lead to endocrine referral in 50% of CD and 25% of adrenal CS [69]. On the other hand, the prevalence of CS in unselected patients with clinical hyperandrogenism and/or menses alterations and PCOS-like features is very low and does not justify screening. The likelihood of CS is increased and screening recommended in women presenting signs and/or symptoms of cortisol excess or unusual clinical picture for their age in addition to features of hyperandrogenism. In this context, given that menstrual irregularities frequently occur in obese women (see R. 1.1.3), rapid weight gain and increased waist circumference rather than increased BMI per se should raise suspicion for CS [70]. Of note, in patients with CS and moderately elevated cortisol levels –not sufficient to lead to hypogonadotropic hypogonadism as in full-blown CS-, the clinical presentation may overlap with polycystic ovaries [71]. The correct diagnosis is, however, crucial given the frequent use of oral estrogens to treat hyperandrogenism, which may prove extremely deleterious in misdiagnosed CS, a condition with high risk of thrombotic events [72].

**R 1.4.2 We suggest the 1 mg-DST or UFC in women with menstrual irregularities and/or hirsutism and high clinical suspicion (moderate, ⊕⊕◯◯).**

The diagnostic tests for endogenous CS in patients with clinical hyperandrogenism and/or menstrual irregularities have been assessed in 10 studies, reporting 2281 women (median 124 patients, range 28 to 873). The most frequently reported screening tests were cortisol after DST (either 1-mg or 2 mg/2 day, four studies, 515 patients), UFC (two studies, 437 patients), or their combination; only one study considered LNSC [70].

None of the screening tests achieved a solid diagnostic accuracy due to the high number of false-positive results among patients with PCOS: 25–50% presented mild UFC elevation, 5–15% reduced cortisol suppression after DST and 20% impaired cortisol rhythm [70]. Indeed, NNH can characterize women with menstrual alterations [33]. Accurate medical history is highly recommended, since serum cortisol levels can be increased by oral contraceptives or spironolactone, often administered to patients with menstrual irregularities or hirsutism: a 6-week washout is suggested before planning DST, as this is sufficient to obtain reliable results after oral contraceptive discontinuation [73]. When oral oestrogen withdrawal is unfeasible, free cortisol (LNSC or UFC) is a reasonable choice.

## Screening for Cushing's syndrome in patients with bone fragility, osteoporosis and/or fractures

**R 1.5.1 We recommend to rule out CS in patients in whom the etiology of bone fragility or fractures is not readily apparent, especially if they are unusual for age and sex (moderate ⊕⊕⊕◯).**

*Reasoning*

A rapid increase of fracture risk is observed in glucocorticoid excess, linked to impaired microarchitectural properties of bone rather than simply reduced bone density. Indeed, fragility fractures may occur even with mild reduction or even normal bone mineral density (BMD) [74]. In patients with CS, the prevalence of fragility fractures is highly variable, up to 50% in some cohorts [75]. Vertebral fractures are the most common, with an increased prevalence and severity of fractures in male patients [76]. Of note, vertebral fractures may be asymptomatic and overlooked. Hip, wrist and humeral non-traumatic fractures are less frequent [77]. The detrimental impact of cortisol excess on skeletal health results from several derangements, such as hypogonadism, reduced GH and IGF-1 secretion and activity, and impaired vitamin D metabolism [77]. The loss of skeletal muscle

mass and strength, i.e., sarcopenia, in particular proximal myopathy [78], represents an additional fracture risk factor in patients with CS.

Although glucocorticoid excess is clearly associated with an increased occurrence of fractures, the converse, i.e., how many patients with osteoporosis or fragility fractures present hypercortisolism, is not yet known. Indeed, whether screening for CS is justified in these individuals has not been established [77]. Of note, some patients with endogenous CS show osteoporosis and fractures as the only clinical feature [79].

In clinical practice, imaging (DXA scan or X-ray) is necessary to identify skeletal disease, as fragility fractures are not always clinically evident in patients with CS, as well as in the general population. Bone markers such as osteocalcin, notably suppressed in hypercortisolism, may complement bone evaluation and aid in patient selection [77].

Conditions which should raise the suspicion for CS in the bone clinic are reduced BMD compared to age-matched controls (i.e., young adults with Z-score <- 2 SD), rapid decline in BMD at repeat evaluation, patients with a suboptimal densitometric response to bone protective therapy. In addition, fragility fractures in young (< 50 years) men or pre-menopausal women, eugonadal subjects, or with inappropriately normal BMD warrant special attention. Obviously, the presence of features such as hypertension, visceral obesity, diabetes, sarcopenia, purple striae or moon face should lead to evaluation for hypercortisolism [77].

**R 1.5.2 We suggest, in patients with unusual fractures and signs or symptoms of CS, additional tools, such as morphometry and TBS assessment during DXA evaluation, to collect more evidence for bone damage due to hypercortisolism (low, ⊕⊕◯◯).**

*Reasoning*

DXA is the mainstay of assessment for patients with bone disease or fractures. In patients with suspected CS, the study of bone quality with trabecular bone score (TBS, a DXA-derived parameter) is an additional, useful element, as glucocorticoids are known to affect bone quality rather than quantity [80]. TBS has been used in patients with active CS [77, 81], but its role as a screening test has not yet been established. Morphometric vertebral assessment, indicated in patients during chronic glucocorticoid treatment, can be carried out during DXA scan and can be also useful to identify patients with CS, given that trabecular bone is most affected by cortisol excess. In addition, it may lead to detection of asymptomatic vertebral fractures [77]. Sophisticated measures of bone quality, such as HR-pQCT, are not yet widely available and, thus, not as useful for CS screening [82].

The standard evaluation at the bone clinic is unlikely to detect candidates for hypercortisolism screening: glucocorticoid excess increases the risk of fragility fractures in patients with normal BMD or osteopenia at DXA and vertebral fractures, the primary bone involvement in CS, may be asymptomatic.

**R 1.5.3 There is no preferred screening test to screen for CS in patients with bone derangements; the 1 mg-DST is the most frequently used test (low, ⊕⊕◯◯).**

*Reasoning*

Screening for CS in patients attending the bone center appears justified in selected cases. If NNH or other conditions of impaired cortisol rhythm, e.g., poorly controlled diabetes, mood disorders, are excluded, then measurement of LNSC is a reasonable choice. If urine collection is required to measure calcium excretion, UFC can be added to streamline screening. Unsuppressed cortisol after 1-mg DST (see "Remarks on 1-mg DST") may reveal MACS, which is far more frequent than overt CS [83].

## Screening for Cushing's syndrome in patients with mood disorders

**R 1.6.1 Mood disorders may be associated with functional activation of the HPA axis: first-line screening tests may yield false positive results in patients with NNH (Strong, ⊕⊕⊕⊕).**

*Reasoning*

Mood disorders are common features in patients with hypercortisolism; conversely, cortisol levels are high in patients with impaired mental health. CS is most frequently associated with major depression, generalized anxiety, panic and bipolar disorders, and even psychosis [84]. However, no study has yet reported on HPA axis derangements across the spectrum of mood disorders, therefore the incidence of overt CS in this high-risk condition is unknown.

Major depression occurs in up to 80% of patients with CS and has been described as one of the first clinical manifestations in some 25% of patients [85]. A depressive episode typically lasts 1–2 days in CS, but intermittent phases are common, with frequent and irregular exacerbations, that may reflect fluctuations of cortisol secretion [86]. Other mood disorders encountered in patients with CS are generalized anxiety (66% of patients), panic disorders (37%), and bipolar disorders (30%) [87].

In 11 studies reporting 265 patients with depression (range 3–50 patients), UFC was used most frequently followed by serum cortisol after 1-mg DST and LNSC (respectively in 11, 9 and 1 study); median prevalence of impaired responses was 66% [87]. Of note, false positive results at screening in mood disorders are due to NNH, which is caused by several mechanisms, from accentuated post-awakening cortisol rise, increased evening cortisol pulses, to impaired CRH secretion and cortisol metabolism [87]. Mild UFC elevations,

i.e., less than 3 times of upper limit of the normal range, are common and cortisol rhythm is impaired in 50% of these patients [88]. Further, dexamethasone metabolism and, thus, the accuracy of serum cortisol after 1-mg DST [24], may be affected by drugs prescribed for mood disorders: carbamazepine and primidone are known CYP3A4 inducers and fluoxetine and paroxetine CYP3A4 inhibitors [57, 89]. Ethanol too increases CYP3A4 activity and content [90]: if both mood disorder and alcoholism occur, the likelihood of NNH is increased.

**R 1.6.2 The definite distinction between NNH and CS may require long-term follow-up. In patients with low apparent likelihood of overt hypercortisolism and mildly impaired tests, we recommend re-assessment after remission or improvement of the primary condition (moderate, ⊕⊕⊕◯).**

*Reasoning*

In patients with NNH secondary to mood disorders, clinical features are more important than biochemical data. In fact, in mild cases without features of overt CS, the recovery of HPA axis after treatment of the psychiatric condition is probably the best method to confirm NNH. In recent studies, no patient with NNH showed clinical progression towards overt hypercortisolism during follow-up [91], and normalization of cortisol secretion was observed in 70% of NNH patients after appropriate treatment [91, 92]. Conversely, lack of improvement of depressive symptoms with antidepressant or anxiolytic treatments supports the diagnosis of CS.

A repeated CS screening after remission or initial improvement of the primary condition is a reasonable choice, considering that it may be difficult to achieve complete recovery in case of major depressive, eating or bipolar disorders, alcoholism and other conditions of NNH.

**R 1.6.3 We recommend referral to a specialized Endocrine Centre and the use of second-line tests if the differential diagnosis between CS and NNH is still in doubt after first-line screening tests (moderate, ⊕⊕⊕◯).**

*Reasoning*

The desmopressin test, the CRH test and the dexamethasone-suppressed CRH test are able to differentiate NNH from endogenous hypercortisolism, in particular pituitary CS [93]. The first may represent an easy and less expensive alternative, considering the current shortage of CRH in several countries [94, 95].

Since cortisol excess in NNH is usually mild, the diagnosis of endogenous CS must be established with certainty before any treatment, surgical or medical, is carried out.

## Screening for Cushing's syndrome in patients with adrenal or pituitary incidentaloma

**R 1.7.1 We recommend that patients newly diagnosed with AI undergo biochemical evaluation to rule out MACS (strong, ⊕⊕⊕⊕).**

*Reasoning*

AI is an adrenal mass (diameter ≥ 1 cm) incidentally detected during radiological studies performed for conditions unrelated to adrenal diseases. AI have become a frequent finding over the past decades, due to the widespread use of cross-sectional imaging [96]. The prevalence of AI increases with age, with peak incidence between the fifth and the seventh decade, without gender preference [97]. In individuals over 70, prevalence approaches 10% [97].

Most AI are benign and nonfunctioning (70–80%). Imaging, especially with lipid content at unenhanced CT, and clinical history are used to rule out malignancy at first detection. Adrenal function should be assessed as hormonally active AI occur in 11 and 25% of cases [97]. Cortisol excess is the most frequent hormonal alteration in AI, leading to increased cardiometabolic risk and higher all-cause mortality [98].

The prevalence of MACS, defined as ACTH-independent overproduction of glucocorticoids, without a clear Cushingoid phenotype, varies according to thresholds used at biochemical assessment, most commonly, cortisol after 1-mg DST. In a recent large study, the 50 nmol/L threshold was associated with 33.5% prevalence of MACS among AI [97]. The clinical burden of metabolic and cardiovascular comorbidities, e.g., severe hypertension, greater incidence of T2DM with need for insulin therapy, as well as the risk of vertebral fragility fractures is often more severe in patients with MACS compared to non-functioning adrenal masses [99, 100].

**R 1.7.2 We recommend screening for hypercortisolism in AI with the 1-mg DST as initial test, using the cortisol cutoff at 50 nmol/L to rule out both CS and MACS. In case of unsuppressed cortisol after 1-mg DST, CS must be confirmed with increased LNSC or UFC levels (strong, ⊕⊕⊕⊕).**

*Reasoning*

If cortisol after 1-mg DST is adequately suppressed, i.e., below 50 nmol/L [5], hypercortisolism can reasonably be excluded. However, if the patient's clinical presentation (Fig. 1) suggests CS or if symptoms worsen over the following months, re-evaluation is recommended. In order to diagnose CS after a positive 1-mg DST result, at least one of the other first-line screening tests (UFC or LNSC) must be significantly higher than the upper limit of normality [101].

UFC or LNSC have limited ability to identify MACS: mild adrenal overproduction rarely exceeds serum protein

binding capacity and leads to elevated UFC levels and cortisol circadian rhythm is often preserved in patients with MACS [97].

If overt CS has been excluded on clinical grounds, post-dexamethasone cortisol values above 50 nmol/L identify patients with MACS: a population showing increased all-cause mortality and cortisol-related comorbidities. While the risk of developing overt CS is low [5], an increased risk of new-onset adrenal hypercortisolism during follow-up has been reported in patients with large unilateral AI (≥ 28 mm) or bilateral incidentalomas [97]. In patients with non-functioning AI, worsening of cortisol-related comorbidities, e.g., new-onset, requiring additional therapy, justifies re-assessment with 1-mg DST.

**R 1.7.3 We suggest screening for CS with the 1-mg DST in patients with pituitary incidentalomas and features suggestive for hypercortisolism. (low, ⊕⊕◯◯).**

*Reasoning*

Pituitary incidentalomas (PI) are lesions in the pituitary gland incidentally discovered during brain imaging performed for unrelated reasons (e.g., headache, head trauma, neurological complaints). They are relatively common radiologic findings, with a higher prevalence in older adults and slight preponderance in women [97]. The prevalence of PI is 15–21/100.000 in the general population. They are usually benign lesions, even though they may require careful evaluation and management to determine the growth potential and impact on sellar structures. The etiology of PI varies with to age: most often adenomas in adults, Rathke's cleft cysts in children.

ACTH-dependent hypercortisolism was found in a subset of PI, emphasizing the need for thorough evaluation and monitoring [97]. Two series detected unsuppressed serum cortisol after 1-mg DST in patients with PI without clear signs of CS. Follow-up evaluations with UFC, LNSC and dynamic testing revealed ACTH-secreting pituitary adenomas in ~ 5% [102, 103]. According to the recent Pituitary Society guidelines on PI, screening for CD in patients without clinical features or suggestive comorbidities is not recommended, and should be performed if clinically warranted [104].

## Screening for Cushing's syndrome in patients with unusual infections or thrombotic events

**R 1.8.1 We suggest that patients presenting with opportunistic infections without a known underlying cause for immunosuppression be evaluated for signs and symptoms of cortisol excess. Both severe systemic infections, as well as chronic or recurring mild cutaneous infections, should be considered suspicious for CS (very low, ⊕◯◯◯).**

*Reasoning*

Chronic corticosteroid excess impairs the immune system with reduced response to opportunistic infections. The prevalence of severe infections ranges from 20% in CD to 50% in severe cortisol excess, such as EAS and cortisol-secreting adrenal carcinomas. Neoplastic disease per se is an additional risk factor for opportunistic infections [72]. Overall, patients with CS present a 5-fold higher risk for infections [105], and infections or sepsis represent the second cause of death among these patients [106, 107].

In patients with severe hypercortisolism, i.e., UFC > 10-fold the upper limit of the normal range, the severity of infections, as well as the rapid onset of the disease and preponderance of catabolic effects may mask typical features of CS [73].

*Aspergillus fumigatus*, *Cryptococcus neoformans*, *Pneumocystis jirovecii*, and *Nocardia asteroides* are the main species of opportunistic pathogens reported in patients with CS. Opportunistic infections usually appear treatment-resistant, superimposed to other infections, widespread or at uncommon sites [72].

**R 1.8.2 We suggest that hormonal work-up for CS be carried out after the acute phase has resolved in patients with opportunistic infections, if feasible (very low, ⊕◯◯◯).**

*Reasoning*

Cortisol levels will inevitably be increased in critically ill patients, as part of the physiological response to stress [72], and only a marked increase in cortisol may be suggestive of endogenous hypercortisolism. Additional issues arise from assay interference due to exogenous corticosteroid treatment [72] thus, falsely elevated UFC levels can be secondary to HPA axis activation or to the measurement of synthetic glucocorticoids with immunometric assays or non-extracted urine [108]. Moreover, some antibiotics and antiviral drugs are CYP3A inducers [109], thereby accelerating dexamethasone clearance and affecting 1-mg DST results. In some cases, tests for hypercortisolism should be repeated after recovery from infection.

If CS has been confirmed in severely ill patients with infectious diseases, treatment of hypercortisolism should be initiated promptly and the differential diagnosis postponed.

**R 1.8.3 We suggest that idiopathic thrombosis should raise suspicion for cortisol excess and lead to targeted clinical evaluation (very low, ⊕◯◯◯).**

*Reasoning*

Hypercoagulability is a well-known phenomenon in CS, the most consistent alterations being an increase in clotting factors, in particular factor VIII and von Willebrand (vWF), enhanced thrombin generation, reduced fibrinolysis and compensatory elevation of anticoagulants and fibrinolysis activators, such as protein C and plasminogen activator [72].

Biochemical testing in patients with CS revealed shortened aPTT, rapid clot formation, and prolonged clot lysis time [110].

Venous thromboembolism has been reported in 4–10% of patients with active CS [111], with even higher rates in patients with ectopic or adrenal cancer, given the known association between thromboembolism and neoplasia. Arterial events, such as myocardial infarction and stroke, represent the first cause of death in patients with CS [112].

Unfortunately, no data are available on the prevalence of CS among patients with venous or arterial thromboembolism. However, the combination of clinical signs and symptoms of hypercortisolism in idiopathic thrombosis should prompt screening for CS.

## Future directions and recommended research

In clinical practice, CS is so rare and patients with conditions at risk for CS are so common, that screening in unselected patients is neither feasible nor cost-effective [113]. As a matter of fact, arterial hypertension or overweight, bone fractures, glucose metabolism impairment as well as mood disorders are on the whole encountered in more than half of adults. Improvements in screening procedures tailored to each clinical presentation are needed but may still be a long way off. It follows that awareness for CS has to increase among physicians who deal with conditions possibly due to CS. The challenge for the clinician is thus to identify those infrequent patients -among all those with common disorders that overlap features of CS- who present a phenotype worthy of being screened for endogenous hypercortisolism (Table 2). Screening is not cost-effective, nonetheless CS is a complex disorder, with expensive treatments (being it surgery or medical therapy [114]) and with long-term complications even after remission [115]: an early diagnosis probably would be able to reduce the individual cost, and tailored pharmacoeconomic evaluation is warranted. One of the purposes of this Position Statement is to increase the awareness on CS among specialist physicians as well as general practitioners.

Reliable hormone measurements with assay-specific reference ranges are crucial to identify patients with CS among those with conditions suggestive for CS but not due to endogenous hypercortisolism. The distinction between mild forms of CS and NNH is even more reliant on assay accuracy. The choice between mass spectrometry and immunoassay calls for a pragmatic balance between high specificity, availability and cost.

The Consensus of the Pituitary Society for the diagnosis of CS indicated UFC or LNSC as first choices, with multiple LNSC possibly easier to collect [11], nonetheless the use of salivary cortisol should be further investigated in high-risk conditions, as pituitary incidentaloma. DST could also be an option if LNSC is not feasible; indeed, the access to LNSC assays may be constrained by availability and reimbursement. In the context of patients with conditions suspicious for hypercortisolism but without overt CS, UFC seems less reliable, although it remains the test with the most extensive evidence in the diagnostic approach to CS.

One major limitation of salivary cortisol measurement is the heterogeneity among assay methodologies and lack of reimbursement by the public or private health care system in several European countries. Efforts to extend its use should be considered by policy makers and health stakeholders.

Overall, we believe that serum cortisol after 1-mg DST is a solid screening test, available worldwide and not dependent on sophisticated assays. The role of concomitant dexamethasone measurement deserves to be clarified and -should it prove decisive for screening- its use made available and promoted.

The heterogeneity of inclusion criteria regarding high-risk conditions and the different definition of hypercortisolism (often combining MACS and CS) affects the possibility to calculate a pre-test probability of CS. Actually, clinical evaluation is paramount to identify patients to be screened and, indeed, several authors proposed the use of clinical scores, based upon clinical features alone [116] or in combination with first-line tests [117] but sensitivity and specificity differed across countries [118]. Further studies, with modern integrated, Bayesian based, multi-parametric approaches, i.e., "-omics" [9], using both clinical data, first-line screening tests, and assessment of novel biomarkers may devise new, combined diagnostic tools.

### Box: summary of recommendations

Clinical history, current therapy, as well as signs and symptoms of hypercortisolism must be assessed carefully before any endocrine testing. The working group recommends screening for Cushing's syndrome (CS) in the following groups:

1. Patients with type 2 diabetes mellitus, overweight or obesity with truncal distribution, adrenal or pituitary incidentaloma, patients with unusual infections or thrombotic events, and women with menstrual irregularities and/or hirsutism, who present additional comorbidities or signs/symptoms that suggest CS;
2. Young hypertensive patients, patients with resistant or difficult-to-treat hypertension, particularly if associated with hypokalemia and absent nocturnal dipping, those

**Table 2** Summary of key clinical features in the high-risk conditions or with challenging diagnosis of hypercortisolism, and suggested first-line screening test

| High-risk condition | Key clinical features | 1-mg DST | LNSC | UFC |
|---|---|---|---|---|
| Type 2 diabetes mellitus | Patients with concomitant arterial hypertension (requiring at least 2 drugs), insulin therapy, diabetic micro- and macrovascular complications, difficult-to-control patients (strong, ⊕⊕⊕⊕) A satisfactory metabolic control before testing is suggested, because NNH may be found in active decompensated diabetic patients | Recommended in an outpatient setting (strong, ⊕⊕⊕⊕) | Not suggested (high number of false positives) | Limited evidence, consider chronic kidney disease |
| Overweight or obesity | Central fat distribution combined with other clinical signs suggestive of hypercortisolism (moderate, ⊕⊕⊕◯) | Recommended (moderate, ⊕⊕⊕◯) | Limited evidence | Limited evidence |
| Arterial Hypertension | Young patients, resistant or difficult-to-treat hypertension, adrenal incidentaloma, subjects with a non-dipper 24-h ambulatory blood pressure profile, hypokalemia (moderate, ⊕⊕⊕◯) | Second choice, consider dexamethasone metabolism and hypertensive drugs First-line test only in adrenal incidentaloma | Suggested (low, ⊕⊕◯◯) | Limited evidence, consider chronic kidney disease |
| Children and adolescents | Children or adolescents with growth failure, with or without weight gain, and facial changes should be screened for CS (moderate, ⊕⊕⊕◯) | Recommended either 1 mg and the two-day 2 mg DST, dexamethasone dose 25 µg/kg in patients < 40 kg (moderate, ⊕⊕⊕◯) | High diagnostic accuracy but not recommended in newborns, and specific devices are required in young children | Recommended, cut-off of 70 µg/$m^2$/day (moderate, ⊕⊕⊕◯) |
| Hyperandrogenism and/or menstrual irregularities | First-line tests if there are other signs and/or symptoms at the clinical examination that could suggest CS. Consider NNH and that oral contraceptives or spironolactone may cause false-positive results (moderate, ⊕⊕⊕◯) | Recommended (moderate, ⊕⊕⊕◯). Medications can affect dexamethasone metabolism, and oral contraceptives increase serum cortisol | Limited evidence | Second choice, especially if oral contraceptives or drugs that impair dexamethasone metabolism cannot be discontinued |
| Bone fragility, osteoporosis and/or fractures | Bone quality is more affected than bone quantity. Thoracic and lumbar vertebral fractures in patients with normal or mild BMD reduction, densitometric diagnosis of osteoporosis in case of reduced BMD compared to age-matched controls, BMD declines more rapidly than expected, suboptimal densitometric response to bone protective therapy, fragility fractures < 50 years old men and pre-menopausal females, eugonadal subjects (moderate ⊕⊕⊕◯) | Most reported in literature (low, ⊕⊕◯◯) | Appears the best candidate, limited evidence (low, ⊕⊕◯◯) | Limited evidence |
| Mood disorders | Consider that NNH is a functional activation of the HPA axis (Strong, ⊕⊕⊕⊕) Longitudinal follow-up can be used to differentiate NNH from CS: short-term re-assessment of the patient after the recovery/improvement of mood disorder Lack of improvement with standard treatments can support the diagnosis of CS. Consider referral in a Tertiary Endocrine Center and the use of second-line tests (moderate, ⊕⊕⊕◯) | Most reported in literature, risk of false positive results due to NNH (Strong, ⊕⊕⊕⊕) Medications can affect dexamethasone metabolism. | Limited evidence, risk of false positive results due to NNH | Most reported in literature, risk of false positive results due to NNH (Strong, ⊕⊕⊕⊕) |
| Adrenal incidentaloma | Cortisol secretion and cortisol-related comorbidities characterizes MACS and CS: all patients with adrenal incidentaloma must be studied for cortisol excess (Strong, ⊕⊕⊕⊕) | Recommended, serum cortisol < 50 nmol/L can rule out MACS and overt CS (Strong, ⊕⊕⊕⊕) | Limited role to define MACS, must be considered to further proceed toward CS diagnosis in case of unsuppressed serum cortisol after 1-mg DST | Limited role to define MACS, must be considered to further proceed toward CS diagnosis in case of unsuppressed serum cortisol after 1-mg DST |
| Pituitary incidentaloma | CS screening should be offered to patients with pituitary incidentaloma and additional clinical signs and symptoms of cortisol excess (low, ⊕⊕◯◯) | Most reported in literature | Limited evidence | Limited evidence |

**Table 2** (continued)

| High-risk condition | Key clinical features | 1-mg DST | LNSC | UFC |
|---|---|---|---|---|
| Unusual infections | Opportunistic, severe systemic, chronic or recurring mild cutaneous infections should be considered suspicious for CS (low, ⊕⊕◯◯). Consider the combination of severe hypercortisolism and malignancy-related immunosuppression. Screening tests should to be repeated after the remission of the infection (very low, ⊕◯◯◯) | Limited, drug-induced false positive results | Limited evidence, drug-induced false positive results | Limited evidence, drug-induced false positive results |
| Thrombotic events | The combination of clinical signs and symptoms of hypercortisolism in idiopathic thrombosis should prompt screening for CS (very low, ⊕◯◯◯) | Limited evidence | Limited evidence | Limited evidence |

with rapidly worsening hypertension and in those with adrenal incidentaloma;
3. Children and adolescents with growth failure associated with facial changes, irrespective of weight gain;
4. Patients with osteoporosis or fractures without readily apparent causes, especially in eugonadal subjects.

The 1-mg dexamethasone suppression test is recommended for screening patients with type 2 diabetes mellitus, with truncal obesity, or with adrenal incidentaloma. It is suggested also for women with hyperandrogenism and/or menstrual irregularities. Late-night salivary cortisol is recommended as screening test in patients with hypertension, and can be considered in those with osteoporosis or fractures. In case of functional (or non-neoplastic) activation of the hypothalamic-pituitary-adrenal axis, first-line screening tests may be impaired. Therefore, we suggest reassessment of the patient after recovery/resolution of the primary condition. All three first-line screening tests may also be used in children and adolescents.

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## Declarations

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

## References

1. Boscaro M, Arnaldi G (2009) Approach to the patient with possible Cushing's syndrome. J Clin Endocrinol Metab 94(9):3121–3131. https://doi.org/10.1210/jc.2009-0612
2. Gadelha M, Gatto F, Wildemberg LE, Fleseriu M (2023) Cushing's syndrome. Lancet 402(10418):2237–2252. https://doi.org/10.1016/S0140-6736(23)01961-X
3. Laugesen K, Jørgensen JOL, Petersen I, Sørensen HT (2019) Fifteen-year nationwide trends in systemic glucocorticoid drug use in Denmark. Eur J Endocrinol 181(3):267–273. https://doi.org/10.1530/EJE-19-0305
4. Reincke M, Fleseriu M, Cushing Syndrome (2023) JAMA 330(2):170. https://doi.org/10.1001/jama.2023.11305
5. Fassnacht M, Tsagarakis S, Terzolo M et al (2023) European Society of Endocrinology clinical practice guidelines on the management of adrenal incidentalomas, in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol 189(1):G1–G42. https://doi.org/10.1093/ejendo/lvad066
6. Wengander S, Trimpou P, Papakokkinou E, Ragnarsson O (2019) The incidence of endogenous Cushing's syndrome in the modern era. Clin Endocrinol (Oxf) 91(2):263–270. https://doi.org/10.1111/cen.14014
7. Broder MS, Neary MP, Chang E, Cherepanov D, Ludlam WH (2015) Incidence of Cushing's syndrome and Cushing's disease in commercially-insured patients <65 years old in the united States. Pituitary 18(3):283–289. https://doi.org/10.1007/s11102-014-0569-6
8. Valassi E, Santos A, Yaneva M et al (2011) The European Registry on Cushing's syndrome: 2-year experience. Baseline demographic and clinical characteristics. Eur J Endocrinol 165(3):383–392. https://doi.org/10.1530/EJE-11-0272
9. Ceccato F, Bavaresco A, Ragazzi E et al (2024) Clinical and biochemical data for the diagnosis of endogenous hypercortisolism: the "Cushingomic" approach. J Clin Endocrinol Metab. https://doi.org/10.1210/clinem/dgae517
10. Scaroni C, Mondin A, Ceccato F (2022) How to rule out non-neoplastic hypercortisolemia (previously known as pseudo-cushing). Pituitary 25(5):701–704. https://doi.org/10.1007/s11102-022-01222-2
11. Fleseriu M, Auchus R, Bancos I et al (2021) Consensus on diagnosis and management of Cushing's disease: a guideline update. The Lancet Diabetes & Endocrinology 9(12):847–875. https://doi.org/10.1016/S2213-8587(21)00235-7
12. Nieman LK, Biller BMK, Findling JW et al (2008) The diagnosis of Cushing's syndrome: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 93(5):1526–1540. https://doi.org/10.1210/jc.2008-0125
13. Galm BP, Qiao N, Klibanski A, Biller BMK, Tritos NA (2020) Accuracy of laboratory tests for the diagnosis of Cushing syndrome. J Clin Endocrinol Metab 105(6):1–14. https://doi.org/10.1210/clinem/dgaa105
14. Ceccato F, Terzolo M, Scaroni C (2025) Who and how to screen for endogenous hypercortisolism in a high-risk population: a special issue of the journal of endocrinological investigations. J Endocrinol Invest 48(Suppl 1):1–2. https://doi.org/10.1007/s40618-024-02449-5

15. Guyatt G, Oxman AD, Akl EA et al (2011) GRADE guidelines: 1. introduction—GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 64(4):383–394. https://doi.org/10.1016/j.jclinepi.2010.04.026
16. Guyatt GH, Oxman AD, Vist GE et al (2008) GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336(7650):924–926. https://doi.org/10.1136/bmj.39489.470347.AD
17. Pivonello R, Scaroni C, Polistena B, Migliore A, Giustina A (2023) Unmet needs on the current medical management of cushing's syndrome: results from a Delphi panel of Italian endocrinologists. J Endocrinol Invest 46(9):1923–1934. https://doi.org/10.1007/s40618-023-02058-8
18. Petersenn S (2021) Biochemical diagnosis of Cushing's disease: screening and confirmatory testing. Best Pract Res Clin Endocrinol Metab 35(1):101519. https://doi.org/10.1016/j.beem.2021.101519
19. Tizianel I, Barbot M, Ceccato F (2024) Subtyping of cushing's syndrome: a step ahead. Exp Clin Endocrinol Diabetes 132(12):659–669. https://doi.org/10.1055/a-2299-5065
20. Nowak E, Vogel F, Albani A et al (2023) Diagnostic challenges in cyclic Cushing's syndrome: a systematic review. Lancet Diabetes Endocrinol. https://doi.org/10.1016/S2213-8587(23)00150-X
21. Jahandideh D, Swearingen B, Nachtigall LB, Klibanski A, Biller BMK, Tritos NA (2018) Characterization of cyclic cushing's disease using late night salivary cortisol testing. Clin Endocrinol (Oxf) 89(3):336–345. https://doi.org/10.1111/cen.13758
22. Raff H, Phillips JM (2019) Bedtime salivary cortisol and cortisone by LC-MS/MS in healthy adult subjects: evaluation of sampling time. J Endocr Soc 3(8):1631–1640. https://doi.org/10.1210/js.2019-00186
23. Petersenn S, Newell-Price J, Findling JW et al (2014) High variability in baseline urinary free cortisol values in patients with Cushing's disease. Clin Endocrinol (Oxf) 80(2):261–269. https://doi.org/10.1111/cen.12259
24. Valassi E, Swearingen B, Lee H et al (2009) Concomitant medication use can confound interpretation of the combined dexamethasone-corticotropin releasing hormone test in cushing's syndrome. J Clin Endocrinol Metab 94(12):4851–4859. https://doi.org/10.1210/jc.2009-1500
25. Flockhart DA, Thacker D, McDonald C, Desta Z (2025) the Flockhart Cytochrome P450 Drug-Drug Interaction Table. Division of Clinical Pharmacology, Indiana University School of Medicine (Updated 2021). Accessed January 4. https://drug-interactions.medicine.iu.edu/
26. Ceccato F, Artusi C, Barbot M et al (2020) Dexamethasone measurement during low-dose suppression test for suspected hypercortisolism: threshold development with and validation. J Endocrinol Invest 43(8):1105–1113. https://doi.org/10.1007/s40618-020-01197-6
27. Scaroni C, Zilio M, Foti M, Boscaro M (2017) Glucose metabolism abnormalities in Cushing syndrome: from molecular basis to clinical management. Endocr Rev 38(3):189–219. https://doi.org/10.1210/er.2016-1105
28. Guarnotta V, Giordano C, Reimondo G (2024) Who and how to screen for endogenous hypercortisolism in type 2 diabetes mellitus or obesity. J Endocrinol Invest. https://doi.org/10.1007/s40618-024-02455-7
29. Steffensen C, Pereira AM, Dekkers OM, Jørgensen JOL (2016) Diagnosis of endocrine disease: prevalence of hypercortisolism in type 2 diabetes patients: a systematic review and meta-analysis. Eur J Endocrinol 175(6):R247–R253. https://doi.org/10.1530/EJE-16-0434
30. Aresta C, Soranna D, Giovanelli L et al (2021) When to suspect hidden hypercortisolism in type 2 diabetes: a meta-analysis. Endocr Pract 27(12):1216–1224. https://doi.org/10.1016/j.eprac.2021.07.014
31. Buse JB, Kahn SE, Aroda VR et al (2025) Prevalence of hypercortisolism in Difficult-to-Control type 2 diabetes. Diabetes Care (Published Online April 18). https://doi.org/10.2337/dc24-2841
32. Reimondo G, Pia A, Allasino B et al (2007) Screening of Cushing's syndrome in adult patients with newly diagnosed diabetes mellitus. Clin Endocrinol (Oxf) 67(2):225–229. https://doi.org/10.1111/j.1365-2265.2007.02865.x
33. Scaroni C, Albiger NM, Palmieri S et al (2020) Approach to patients with pseudo-Cushing's states. Endocr Connect 9(1):R1–R13. https://doi.org/10.1530/EC-19-0435
34. Steffensen C, Thomsen HH, Dekkers OM, Christiansen JS, Rungby J, Jørgensen JOL (2016) Low positive predictive value of midnight salivary cortisol measurement to detect hypercortisolism in type 2 diabetes. Clin Endocrinol (Oxf) 85(2):202–206. https://doi.org/10.1111/cen.13071
35. Ceccato F, Marcelli G, Martino M et al (2019) The diagnostic accuracy of increased late night salivary cortisol for Cushing's syndrome: a real-life prospective study. J Endocrinol Invest 42(3):327–335. https://doi.org/10.1007/s40618-018-0921-1
36. Rubino F, Cummings DE, Eckel RH et al (2025) Definition and diagnostic criteria of clinical obesity. The Lancet Diabetes & Endocrinology 13(3):221–262. https://doi.org/10.1016/S2213-8587(24)00316-4
37. Ng M, Gakidou E, Lo J et al (2025) Global, regional, and National prevalence of adult overweight and obesity, 1990–2021, with forecasts to 2050: a forecasting study for the global burden of disease study 2021. Lancet 405(10481):813–838. https://doi.org/10.1016/S0140-6736(25)00355-1
38. van Hulsteijn LT, Pasquali R, Casanueva F et al (2020) Prevalence of endocrine disorders in obese patients: systematic review and meta-analysis. Eur J Endocrinol 182(1):11–21. https://doi.org/10.1530/EJE-19-0666
39. Braun LT, Vogel F, Zopp S et al (2022) Whom should we screen for Cushing syndrome? The Endocrine Society Practice Guideline Recommendations 2008 revisited. J Clin Endocrinol Metab 107(9):e3723–e3730. https://doi.org/10.1210/clinem/dgac379
40. Neeland IJ, Lim S, Tchernof A et al (2024) Metabolic syndrome. Nat Rev Dis Primers 10(1):77. https://doi.org/10.1038/s41572-024-00563-5
41. Pivonello R, De Martino MC, De Leo M et al (2007) Cushing's syndrome: aftermath of the cure. Arq Bras Endocrinol Metabol 51(8):1381–1391. https://doi.org/10.1590/s0004-27302007000800025
42. Jha S, Sinaii N, McGlotten RN, Nieman LK (2020) Remission of hypertension after surgical cure of Cushing's syndrome. Clin Endocrinol (Oxf) 92(2):124–130. https://doi.org/10.1111/cen.14129
43. Barbot M, Ceccato F, Scaroni C (2019) The pathophysiology and treatment of hypertension in patients with Cushing's syndrome. Front Endocrinol (Lausanne) 10:321. https://doi.org/10.3389/fendo.2019.00321
44. Ceccato F, Trementino L, Barbot M et al (2017) Diagnostic accuracy of increased urinary cortisol/cortisone ratio to differentiate ACTH-dependent cushing's syndrome. Clin Endocrinol (Oxf) 87(5):500–507. https://doi.org/10.1111/cen.13391
45. Martino MCD, Canu L, Bonaventura I, Vitiello C, Sparano C, Cozzolino A (2025) Hypertension and cushing's syndrome: Hunt for the red flag. J Endocrinol Invest (Published Online March 18). https://doi.org/10.1007/s40618-024-02453-9
46. Martino MCD, Canu L, Bonaventura I, Vitiello C, Sparano C, Cozzolino A (2025) Hypertension and cushing's syndrome: Hunt for the red flag. J Endocrinol Invest Published Online March. https://doi.org/10.1007/s40618-024-02453-9

47. Anderson GH, Blakeman N, Streeten DHP (1994) The effect of age on prevalence of secondary forms of hypertension in 4429 consecutively referred patients. J Hypertens 12(5):609. https://doi.org/10.1097/00004872-199405000-00015
48. Danielson M, Dammström B (1981) The prevalence of secondary and curable hypertension. Acta Med Scand 209(1–6):451–455. https://doi.org/10.1111/j.0954-6820.1981.tb11628.x
49. Trifanescu R, Carsote M, Caragheorgheopol A et al (2013) Screening for secondary endocrine hypertension in young patients. Maedica (Buchar) 8(2):108–115. http://www.ncbi.nlm.nih.gov/pubmed/24371473
50. Aoe M, Okada A, Usui T, Manaka K, Nangaku M, Makita N (2020) Comparison between the clinical characteristics of patients with adrenal incidentalomas and those with hypertension-associated adrenal tumors in a single center in Japan. Endocr J 67(6):645–654. https://doi.org/10.1507/endocrj.EJ19-0262
51. Sudano I, Suter P, Beuschlein F (2023) Secondary hypertension as a cause of treatment resistance. Blood Press. https://doi.org/10.1080/08037051.2023.2224898
52. Mancia G, Kreutz R, Brunström M et al (2023) 2023 ESH guidelines for the management of arterial hypertension the task force for the management of arterial hypertension of the European society of hypertension. J Hypertens 41(12):1874–2071. https://doi.org/10.1097/HJH.0000000000003480
53. Bruno RM, Taddei S, Borghi C et al (2020) Italian society of arterial hypertension (SIIA) position paper on the role of renal denervation in the management of the Difficult-to-Treat hypertensive patient. High Blood Press Cardiovasc Prev 27(2):109–117. https://doi.org/10.1007/s40292-020-00367-0
54. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T (2004) Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. Hypertens Res 27(3):193–202. https://doi.org/10.1291/hypres.27.193
55. Martins LC, Conceição FL, Muxfeldt ES, Salles GF (2012) Prevalence and associated factors of subclinical hypercortisolism in patients with resistant hypertension. J Hypertens 30(5):967–973. https://doi.org/10.1097/HJH.0b013e3283521484
56. Chan KCA, Lit LCW, Law ELK et al (2004) Diminished urinary free cortisol excretion in patients with moderate and severe renal impairment. Clin Chem 50(4):757–759. https://doi.org/10.1373/clinchem.2003.029934
57. Petersenn S (2022) Overnight 1 mg dexamethasone suppression test and 24 h urine free cortisol—accuracy and pitfalls when screening for cushing's syndrome. Pituitary 25(5):693–697. https://doi.org/10.1007/s11102-022-01249-5
58. Fenske M (2004) How much urinary free cortisol is really cortisol during water diuresis in healthy individuals? Clin Chem 50(6):1102–1104. https://doi.org/10.1373/clinchem.2004.032243
59. Fravel MA, Ernst M (2021) Drug interactions with antihypertensives. Curr Hypertens Rep 23(3):14. https://doi.org/10.1007/s11906-021-01131-y
60. Chioma L, Patti G, Cappa M, Maghnie M (2024) Cushing syndrome in paediatric population: who and how to screen. J Endocrinol Invest. https://doi.org/10.1007/s40618-024-02452-w
61. Savage MO, Ferrigno R (2024) Paediatric Cushing's disease: long-term outcome and predictors of recurrence. Front Endocrinol (Lausanne). https://doi.org/10.3389/fendo.2024.1345174
62. Padilla C, Stratakis CA, Tatsi C (2024) The phenotype of the pediatric patient with Cushing syndrome but without obesity. Eur J Endocrinol 191(4):399–406. https://doi.org/10.1093/ejendo/lvae114
63. Martinerie L, Bouligand J, North MO, Bertherat J, Assié G, Espiard S (2024) Consensus statement by the French society of endocrinology (SFE) and French society of pediatric endocrinology & diabetology (SFEDP) for the diagnosis of cushing's syndrome: genetics of cushing's syndrome. Ann Endocrinol (Paris) 85(4):284–293. https://doi.org/10.1016/j.ando.2024.01.005
64. Fourman LT, Lima JG, Simha V et al (2024) A rapid action plan to improve diagnosis and management of lipodystrophy syndromes. Front Endocrinol (Lausanne). https://doi.org/10.3389/fendo.2024.1383318
65. Tryphonopoulos PD, Letourneau N, Azar R (2014) Approaches to salivary cortisol collection and analysis in infants. Biol Res Nurs 16(4):398–408. https://doi.org/10.1177/1099800413507128
66. Kervezee L, Romijn M, van de Weijer KNG et al (2024) Development of 24-hour rhythms in cortisol secretion across infancy: a systematic review and meta-analysis of individual participant data. J Clin Endocrinol Metab. https://doi.org/10.1210/clinem/dgae590
67. Arnaldi G, Martino M (2019) Androgens in cushing's syndrome. Front Horm Res 53:77–91. https://doi.org/10.1159/000494904
68. Kaltsas GA, Korbonits M, Isidori AM et al (2000) How common are polycystic ovaries and the polycystic ovarian syndrome in women with cushing's syndrome? Clin Endocrinol (Oxf) 53(4):493–500. https://doi.org/10.1046/j.1365-2265.2000.01117.x
69. Akirov A, Dery L, Fleseriu M et al (2023) Cushing's syndrome in women: age-related differences in etiology and clinical picture. Pituitary 26(1):144–151. https://doi.org/10.1007/s11102-022-01292-2
70. Ferraù F, Alessi Y, Nista F, Roux A, Ferone D, Arvat E (2025) Who and how to screen for endogenous hypercortisolism among young women presenting with clinical hyperandrogenism and/or menstrual abnormalities. J Endocrinol Invest. https://doi.org/10.1007/s40618-025-02537-0
71. Penezić Z, Žarković M, Vujović S et al (2005) Gonadotropin pulsatility in cushing's syndrome compared with polycystic ovary syndrome. Gynecol Endocrinol 20(3):150–154. https://doi.org/10.1080/09513590400027190
72. Barbot M, Lazzara M, Mazzeo P, Pecori Giraldi F (2024) Unusual infections and thrombotic events in Cushing's syndrome. J Endocrinol Invest. https://doi.org/10.1007/s40618-024-02454-8
73. Vastbinder M, Kuindersma M, Mulder AH, Schuijt MP, Mudde AH (2016) The influence of oral contraceptives on overnight 1 mg dexamethasone suppression test. Neth J Med 74(4):158–161. http://www.ncbi.nlm.nih.gov/pubmed/27185774
74. Mazziotti G, Frara S, Giustina A (2018) Pituitary diseases and bone. Endocr Rev 39(4):440–488. https://doi.org/10.1210/er.2018-00005
75. Canalis E, Mazziotti G, Giustina A, Bilezikian JP (2007) Glucocorticoid-induced osteoporosis: pathophysiology and therapy. Osteoporos Int 18(10):1319–1328. https://doi.org/10.1007/s00198-007-0394-0
76. Zilio M, Barbot M, Ceccato F et al (2014) Diagnosis and complications of Cushing's disease: gender-related differences. Clin Endocrinol (Oxf). https://doi.org/10.1111/cen.12299
77. Giordano R, Parasiliti Caprino M, Loli P, Giustina A (2024) Screening for endogenous hypercortisolism in patients with osteoporosis and fractures: why, when and how. J Endocrinol Invest. https://doi.org/10.1007/s40618-024-02450-y
78. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BMK, Colao A (2016) Complications of cushing's syndrome: state of the Art. Lancet Diabetes Endocrinol 4(7):611–629. https://doi.org/10.1016/S2213-8587(16)00086-3
79. Trementino L, Appolloni G, Ceccoli L et al (2014) Bone complications in patients with cushing's syndrome: looking for clinical, biochemical, and genetic determinants. Osteoporos Int 25(3):913–921. https://doi.org/10.1007/s00198-013-2520-5
80. Belaya ZE, Hans D, Rozhinskaya LY et al (2015) The risk factors for fractures and trabecular bone-score value in patients with

endogenous cushing's syndrome. Arch Osteoporos 10(1):1–9. https://doi.org/10.1007/s11657-015-0244-1
81. Ferraù F, Giovinazzo S, Alessi Y et al (2023) Trabecular bone score, bone marrow fat and vertebral fractures in Cushing syndrome. Endocrine 80(2):441–447. https://doi.org/10.1007/s12020-023-03318-6
82. Uygur MM, Frara S, di Filippo L, Giustina A (2023) New tools for bone health assessment in secreting pituitary adenomas. Trends Endocrinol Metab 34(4):231–242. https://doi.org/10.1016/j.tem.2023.01.006
83. Zavatta G, Di Dalmazi G (2024) Mild autonomous cortisol secretion (MACS) – related osteoporosis. Exp Clin Endocrinol Diabetes 132(12):712–722. https://doi.org/10.1055/a-2329-5787
84. Pivonello R, Simeoli C, De Martino MC et al (2015) Neuropsychiatric disorders in cushing's syndrome. Front Neurosci. https://doi.org/10.3389/fnins.2015.00129
85. Santos A, Resmini E, Pascual JC, Crespo I, Webb SM (2017) Psychiatric symptoms in patients with cushing's syndrome: prevalence, diagnosis and management. Drugs 77(8):829–842. https://doi.org/10.1007/s40265-017-0735-z
86. Starkman MN (2013) Neuropsychiatric findings in Cushing syndrome and exogenous glucocorticoid administration. Endocrinol Metab Clin North Am 42(3):477–488. https://doi.org/10.1016/j.ecl.2013.05.010
87. Ferrante E, Simeoli C, Mantovani G, Pivonello R (2024) Who and how to screen for endogenous hypercortisolism in patients with mood disorders. J Endocrinol Invest. https://doi.org/10.1007/s40618-024-02457-5
88. Keller J, Flores B, Gomez RG et al (2006) Cortisol circadian rhythm alterations in psychotic major depression. Biol Psychiatry 60(3):275–281. https://doi.org/10.1016/j.biopsych.2005.10.014
89. Ueland GÅ, Methlie P, Kellmann R et al (2017) Simultaneous assay of cortisol and dexamethasone improved diagnostic accuracy of the dexamethasone suppression test. Eur J Endocrinol 176(6):705–713. https://doi.org/10.1530/EJE-17-0078
90. Feierman DE, Melinkov Z, Nanji AA (2003) Induction of CYP3A by ethanol in multiple in vitro and in vivo models. Alcohol Clin Exp Res 27(6):981–988. https://doi.org/10.1097/01.ALC.0000071738.53337.F4
91. Alwani RA, Schmit Jongbloed LW, de Jong FH, van der Lely AJ, de Herder WW, Feelders RA (2014) Differentiating between cushing's disease and pseudo-Cushing's syndrome: comparison of four tests. Eur J Endocrinol 170(4):477. https://doi.org/10.1530/EJE-13-0702
92. Yanovski JA, Cutler GB, Chrousos GP, Nieman LK (1993) Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. A new test to distinguish cushing's syndrome from pseudo-Cushing's States. JAMA 269(17):2232–2238. http://www.ncbi.nlm.nih.gov/pubmed/8386285
93. Mondin A, Barbot M, Voltan G et al (2023) Second-line tests in the differential diagnosis of neoplastic and non-neoplastic hypercortisolism: a systematic review and meta-analysis. J Endocrinol Invest 46(10):1947–1959. https://doi.org/10.1007/s40618-023-02099-z
94. Ceccato F, Di Dalmazi G (2023) Shortage of hCRH for the diagnosis of endogenous CS: the end of an era or the beginning of a new journey? J Endocrinol Invest 46(10):2189–2191. https://doi.org/10.1007/s40618-023-02113-4
95. Colao A, Scaroni C, Mezösi E et al (2024) Diagnostic work-up of ACTH-dependent cushing's syndrome in the context of CRH shortage: recommendation of a task force from the European society of endocrinology. Eur J Endocrinol 191(1):R32–R35. https://doi.org/10.1093/ejendo/lvae073
96. Ceccato F, Barbot M, Scaroni C, Boscaro M (2021) Frequently asked questions and answers (if any) in patients with adrenal incidentaloma. J Endocrinol Invest 44(12):2749–2763. https://doi.org/10.1007/s40618-021-01615-3
97. Coscia K, Verrienti M, Di Dalmazi G, Zatelli MC (2024) Who and how to screen for endogenous hypercortisolism in adrenal and pituitary incidentaloma. J Endocrinol Invest. https://doi.org/10.1007/s40618-024-02456-6
98. Deutschbein T, Reimondo G, Di Dalmazi G et al (2022) Age-dependent and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: an international, retrospective, cohort study. Lancet Diabetes Endocrinol 10(7):499–508. https://doi.org/10.1016/S2213-8587(22)00100-0
99. Zavatta G, Vicennati V, Altieri P et al (2023) Mild autonomous cortisol secretion in adrenal incidentalomas and risk of fragility fractures: a large cross-sectional study. Eur J Endocrinol 188(4):343–352. https://doi.org/10.1093/ejendo/lvad038
100. Pelsma ICM, Fassnacht M, Tsagarakis S et al (2023) Comorbidities in mild autonomous cortisol secretion and the effect of treatment: systematic review and meta-analysis. Eur J Endocrinol 189(4):S88–S101. https://doi.org/10.1093/ejendo/lvad134
101. Ceccato F, Antonelli G, Frigo AC (2017) First-line screening tests for Cushing's syndrome in patients with adrenal incidentaloma: the role of urinary free cortisol measured by LC-MS/MS. J Endocrinol Invest. https://doi.org/10.1007/s40618-017-0644-8
102. Toini A, Dolci A, Ferrante E et al (2015) Screening for ACTH-dependent hypercortisolism in patients affected with pituitary incidentaloma. Eur J Endocrinol 172(4):363–369. https://doi.org/10.1530/EJE-14-0599
103. Tamada D, Kitamura T, Otsuki M, Oshino S, Saitoh Y, Shimomura I (2016) Clinical significance of screening for subclinical cushing's disease in patients with pituitary tumors. Endocr J 63(1):47–52. https://doi.org/10.1507/endocrj.EJ15-0446
104. Fleseriu M, Gurnell M, McCormack A (2025) Pituitary incidentaloma: a Pituitary Society international consensus guideline statement. Nat Rev Endocrinol. https://doi.org/10.1038/s41574-025-01134-8
105. Yaneva M, Kalinov K, Zacharieva S (2013) Mortality in cushing's syndrome: data from 386 patients from a single tertiary referral center. Eur J Endocrinol 169(5):621–627. https://doi.org/10.1530/EJE-13-0320
106. Dekkers OM, Horváth-Puhó E, Jørgensen JOL et al (2013) Multisystem morbidity and mortality in Cushing's syndrome: a cohort study. J Clin Endocrinol Metab 98(6):2277–2284. https://doi.org/10.1210/jc.2012-3582
107. Valassi E, Tabarin A, Brue T et al (2019) High mortality within 90 days of diagnosis in patients with Cushing's syndrome: results from the ERCUSYN Registry. Eur J Endocrinol 181(5):461–472. https://doi.org/10.1530/EJE-19-0464
108. Pecori Giraldi F, Ambrogio AG (2015) Variability in laboratory parameters used for management of Cushing's syndrome. Endocrine 50(3):580–589. https://doi.org/10.1007/s12020-015-0676-9
109. Ferraù F, Ceccato F, Cannavò S, Scaroni C (2020) What we have to know about corticosteroids use during Sars-Cov-2 infection. J Endocrinol Invest. https://doi.org/10.1007/s40618-020-01384-5
110. Kastelan D, Dusek T, Kraljevic I et al (2009) Hypercoagulability in Cushing's syndrome: the role of specific haemostatic and fibrinolytic markers. Endocrine 36(1):70–74. https://doi.org/10.1007/s12020-009-9186-y
111. Suarez MG, Stack M, Hinojosa-Amaya JM (2020) Hypercoagulability in Cushing syndrome, prevalence of thrombotic events: a large, single-center, retrospective study. J Endocr Soc. https://doi.org/10.1210/jendso/bvz033
112. Ragnarsson O, Olsson DS, Papakokkinou E et al (2019) Overall and disease-specific mortality in patients with Cushing disease: a Swedish nationwide study. J Clin Endocrinol Metab 104(6):2375–2384. https://doi.org/10.1210/jc.2018-02524

113. Shimon I (2015) Screening for Cushing's syndrome: is it worthwhile? Pituitary 18(2):201–205. https://doi.org/10.1007/s11102-015-0634-9
114. Pivonello R, De Leo M, Cozzolino A, Colao A (2015) The treatment of Cushing's disease. Endocr Rev 36(4):385–486. https://doi.org/10.1210/er.2013-1048
115. Tizianel I, Lizzul L, Mondin A (2025) Cardiometabolic complications after Cushing's disease remission. J Endocrinol Invest. https://doi.org/10.1007/s40618-025-02572-x
116. Parasiliti-Caprino M, Bioletto F, Frigerio T (2021) A new clinical model to estimate the pre-test probability of Cushing's syndrome: the Cushing score. Front Endocrinol (Lausanne). https://doi.org/10.3389/fendo.2021.747549
117. León-Justel A, Madrazo-Atutxa A, Alvarez-Rios AI et al (2016) A probabilistic model for Cushing's syndrome screening in at-risk populations: a prospective multicenter study. J Clin Endocrinol Metab 101(10):3747–3754. https://doi.org/10.1210/jc.2016-1673
118. Braun LT, Vogel F, Rubinstein G et al (2023) Lack of sensitivity of diagnostic Cushing-scores in Germany: a multicenter validation. Eur J Endocrinol 188(1):59–66. https://doi.org/10.1093/ejendo/lvac016



## Authors and Affiliations

**Filippo Ceccato[1,2] · Massimo Terzolo[3] · Federico Gatto[4,5] · Salvatore Cannavò[6,7] · Antonio Bianchi[8,9] · Andrea M. Isidori[10,11] · Annamaria Colao[12,13] · Diego Ferone[4,5] · Gianluca Aimaretti[14] · Francesca Pecori Giraldi[15] · Giorgio Arnaldi[16,17] · Carla Scaroni[1,2]**

✉ Filippo Ceccato
filippo.ceccato@unipd.it

1 Endocrine Disease Unit, University-Hospital of Padova, Padova, Italy

2 Department of Medicine DIMED, University of Padova, Padova, Italy

3 Department of Clinical and Biological Sciences, Internal Medicine, San Luigi Hospital, University of Turin, Turin, Italy

4 Endocrinology Unit, Department of Internal Medicine and Medical Specialties, University of Genova, Genova, Italy

5 Endocrinology Unit, IRCCS Ospedale Policlinico San Martino, Genova, Italy

6 Department of Human Pathology of Adulthood and Childhood "G. Barresi", University of Messina, Messina, Italy

7 Endocrine Unit, "G. Martino" University Hospital, Messina, Italy

8 Dipartimento Di Medicina Traslazionale, Università Cattolica del Sacro Cuore, Rome, Italy

9 Dipartimento Di Medicina Interna, Endocrinologia E Diabetologia, Fondazione Policlinico Universitario A. Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy

10 Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

11 Endocrine and Andrological Regional Rare Disease Centre - Azienda Ospedaliero- Universitaria Policlinico Umberto I, Rome, Italy

12 Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Diabetologia, Andrologia e Nutrizione Università Federico II di Napoli, Naples, Italy

13 UNESCO Chair for Health Education and Sustainable Development, Federico II University of Naples, Naples, Italy

14 Department of Medical Sciences, University of Turin, Turin, Italy

15 Department of Clinical Sciences & Community Health, University of Milan, Via Commenda 19, Milano, Italy

16 Section of Endocrinology, PROMISE, University of Palermo, Palermo, Italy

17 Unità Operativa Complessa di Endocrinologia. A.O.U.P. Paolo Giaccone of Palermo, Palermo, Italy