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Consensus

Consensus statement by the French Society of Endocrinology (SFE) and French Society of Pediatric Endocrinology & Diabetology (SFEDP) on diagnosis of Cushing's syndrome

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ABSTRACT

Cushing's syndrome is defined by prolonged exposure to glucocorticoids, leading to excess morbidity and mortality. Diagnosis of this rare pathology is difficult due to the low specificity of the clinical signs, the variable severity of the clinical presentation, and the difficulties of interpretation associated with the diagnostic methods. The present consensus paper by 38 experts of the French Society of Endocrinology and the French Society of Pediatric Endocrinology and Diabetology aimed firstly to detail the circumstances suggesting diagnosis and the biologic diagnosis tools and their interpretation for positive diagnosis and for etiologic diagnosis according to ACTH-independent and -dependent mechanisms. Secondly, situations making diagnosis complex (pregnancy, intense hypercortisolism, fluctuating Cushing's syndrome, pediatric forms and genetically determined forms) were detailed. Lastly, methods of surveillance and diagnosis of recurrence were dealt with in the final section.

1. Introduction

Cushing's syndrome results from prolonged exposure to glucocorticoids, leading to morbidity and, without treatment, to high mortality. Except in case of exposure to exogenous glucocorticoids, it is a rare pathology, with incidence of 1.2–5 per million per year [1–3] and prevalence of 39–79 per million, depending on series [1–5]. It may have various causes: pituitary or ectopic ACTH hypersecretion, or glucocorticoid hypersecretion due to adrenal lesion(s). Pituitary-dependent Cushing's syndrome (Cushing's disease) is the most frequent etiology, although studies of incidence and prevalence are few and generally limited by small samples sizes [6–8].

Cushing's syndrome mostly affects adults, with a median age of 41 years at diagnosis, and marked female predominance of 3:1 [9]. ACTH-dependent Cushing's syndrome is the most frequent form, at 71–82% [3,10–12], including 86–92% of patients with Cushing's disease. Ectopic ACTH secretion accounts for 5–14% of cases of Cushing's syndrome [12,13]. Adrenal (ACTH-independent) Cushing's syndrome is diagnosed in 18–40% of cases, mainly due to cortisol-producing adenoma (64–72%) and secreting adrenocortical carcinoma (19–23%). Micro- and macronodular hyperplasia each account for less than 2% of cases.

Clinical presentation varies, and less severe forms are increasingly frequent due to improved knowledge of the pathology and biologic diagnosis. Most national and international consensus statements date back more than 10 years. In France, the PNDS national of diagnosis and care protocol for Cushing's syndrome (https://www.has-sante.fr/upload/docs/application/pdf/2008-12/pnds_syndrome_de_cushing_version_web_051208.pdf) and, internationally, the Endocrine Society Clinical Practice Guideline for diagnosis of Cushing's syndrome [14] were both published in 2008. Given progress in biologic assays and in knowledge in the field, the French Society of Endocrinology (SFE) and the French Society of Pediatric Endocrinology and Diabetology (SFEDP) chose diagnosis of Cushing's syndrome as the subject of a consensus statement to the SFE annual congress in October 2020. The work-group based its analysis on the 2008 guidelines and the publications supporting them, with an exhaustive review of the literature from January 2008 to July 2020. Levels of evidence for each recommendation were graded as follows: ++++, high level; +++, moderate level; ++, low level; and + very low level.

2. In which clinical situations should biologic exploration be undertaken?

Cushing's syndrome is a rare pathology causing specific and rare symptoms but is also associated with other pathologies that are fairly common in the general population (Table 1).

A Spanish study, aiming to develop a predictive score for Cushing's syndrome, analyzed 353 patients thought to be at-risk on clinical grounds, and identified 26 cases (prevalence, 7.4%). Dorsocervical fat accumulation (bison hump) showed an odds ratio of 3.32 for association with Cushing's syndrome, although this was lower than the odds ratios of muscular atrophy (odds ratio 15.2) or osteoporosis (odds ratio 4.6) [15]. Frequent infection or hypokalemia, although less specific than signs of hypercatabolism, also indicate screening for hypercortisolism. Uncontrolled hypertension and/or type-2 diabetes are not strongly suggestive of Cushing's syndrome unless they are resistant to conventional treatment or concern a young subject.

Beyond the typical presentation of Cushing's syndrome, signs suggestive of hypercortisolism should be screened for in patients with certain pathologies that are frequent in the general population and which we shall deal with successively, and the question also arises of screening for Cushing's syndrome in case of less specific clinical symptoms.

2.1. Type-2 diabetes

In non-selected patients with type-2 diabetes, Cushing's syndrome is very rare, at less than 1% in most studies [16]. Several studies reported low prevalence of "occult" Cushing's syndrome in type-2 diabetes [17–26]. With the exception of one report, prevalence ranged between 0 and 3.3%, depending on sample size, age and gender, associated clinical signs (obesity, hyper- tension), duration and severity of diabetes (on Hb A1c test), and hormonal tests performed to confirm Cushing's syndrome. This low incidence, the strong likelihood of false-positives on biologic screening and the low level of hypercortisolism in the absence of clinical signs argue against systematic screening [24]. We therefore recommend abstaining from biologic exploration in the absence of suggestive clinical signs, in agreement with

Table 1
Symptoms, signs and diseases reported in hypercortisolism.

Symptoms	Signs	Diseases
More specific		
Facial swelling	Faciotruncular fat distribution	
Muscle weakness	Tendency for ecchymosis, slow healing, thin skin	
	Purple striae (especially if large,)	
	Facial erythrosis (plethora)	
	Proximal myopathy	
	Fractures (especially if spontaneous or low-energy)	
Non-specific, frequent in general population		
Weight gain	Overweight	Obesity
Fatigue	Peripheral edema	Glucose tolerance disorder, type-2 diabetes
Depression	Acne	Hypertension
Irritability	Hirsutism, alopecia	Osteoporosis
Anxiety		Hypokalemia
Superior function disorder (memory, concentration, etc.)		Phlebitis, pulmonary embolism
Pain		Urinary stones
Erectile disorder		Infections
Irregular menses		Cardiovascular complications
		Polycystic ovary syndrome

Endocrine Society and American Diabetes Association guidelines [2,14].

2.2. Obesity

A 2020 meta-analysis included 22 studies published between 1996 and 2018, with a total of 5819 patients with mean BMI between 29.8 and 51.5 kg/m^2 [27]. Prevalence of hypercortisolism ranged between 0 and 9.3%. The studies included obese patients and two other characteristics with little specificity for Cushing's syndrome, all reporting 0% prevalence of Cushing's syndrome [28,29]. Studies associating obesity and type-2 diabetes reported prevalence of 1.1%, (95% CI: 0.3–21; I2: 70%). Two other recent studies confirmed the rarity of Cushing's syndrome in the overall obese population: 0% [30] and 0.7% [31].

Hypercortisolism screening in obese patients should be guided by relevant signs, including signs of hypercatabolism (skin atrophy, osteoporosis, spontaneous ecchymosis, proximal amyotrophy, purple vergetures), which increase the likelihood of revealing Cushing's syndrome.

2.3. Hypertension

Hypertension is found in 58–85% of adults [32] and rather less frequently (47–49%) in children [33,34] with Cushing's syndrome. Conversely, Cushing's syndrome is an exceptional cause of overall blood pressure elevation, with rates of 0.5–1.5% in large American and Japanese series, but where the methodology was of poor quality [35,36]. Prevalence reached 8% in a Brazilian study of low-level Cushing's syndrome, without etiologic investigation or histologic proof [37]. Prevalence seemed higher in selected patients: 7.5% (6/80) in 12–40 year-olds referred by cardiologists for screening for secondary blood pressure elevation [38]. The French Health Authority (HAS), French, European cardiology societies and American College of Cardiology do not recommend biologic screening for Cushing's syndrome in case of hypertension without suggestive signs [39–41] (https://www.has-sante.fr/upload/docs/application/pdf/2016-10/fiche_memo_hta_mel.pdf).

2.4. Osteoporosis

Hypercortisolism has direct and indirect impact on bone including hypogonadism and growth hormone deficit secondary to hypercortisolism. Demineralization is seen in 64–100% of patients with hypercortisolism, with osteopenia in 40–78% of cases and osteoporosis in 22–57%. Fractures are reported in 11–76% of patients [42]. Locations are usually the thoracic or lumbar vertebrae, hip, ribs or pelvis, and the fractures are often spontaneous or low-energy. Half of the vertebral fractures are asymptomatic [16]. In hypercortisolism, fracture risk not only correlates with demineralization on osteodensitometry but is also related to lesions of the bone microarchitecture [43].

Osteoporosis is more prevalent in case of adrenal Cushing's syndrome than in Cushing's disease, while osteoporosis is more severe, in terms of densitometry and/or fracture, in case of ectopic ACTH secretion than in Cushing's disease [12]. Low-level, infra-clinical or subclinical hypercortisolism is associated with increased risk of osteoporosis, with varying fracture risk [18].

Hypercortisolism is rare in case of evident idiopathic osteoporosis: in a series of 602 patients, infra-clinical hypercortisolism was diagnosed in 1.3% of cases [18,44]. Prevalence of infra- clinical hypercortisolism in patients referred for osteoporosis was 1.3–3.8%, but up to 11–18% in osteoporosis with fracture man- aged in tertiary centers [45]. The high rate of osteoporosis in patients with hypercortisolism makes it a specific sign in selected patients at-risk of hypercortisolism. Thus, a prospective multicen- ter study included 353 patients with at least 2 of the following 5 signs or symptoms: obesity, poorly controlled blood pressure, non-controlled diabetes, hirsutism, and menstrual disorder; prevalence of osteoporosis was 6.1% in patients without hypercortisolism and 23.1% in those with: odds ratio, 4.6 (1.66–12.75) [15]. Osteoporosis was predictive of hypercortisolism on uni- and multivariate analysis. Osteoporosis with fracture rate higher than expected from the level of demineralization on osteodensitometry also sug- gests hypercortisolism, especially in premenopausal women or under-50-year-old men [16,46]. Screening on interview and clinical examination at the time of diagnosis of osteoporosis is recommended, but hormonal screening depends on the severity of the osteoporosis, presence of vertebral fracture, gender, age and clinical examination findings [47].

2.5. Psychiatric disorder

Psychiatric disorder is frequent in case of hypercortisolism. It is seen in 50–80% of patients with Cushing's syndrome, intensity correlating with the severity of the Cushing's disease, both clinically and biologically, and with age and prior psychological status [12,32,48–50]. There are no prospective studies of systematic screening for Cushing's syndrome in psychiatric pathology. Studies are complicated by functional activation of the corticotropic axis (pseudo-Cushing's syndrome) on biologic tests. Even so, a

presentation of variable disorder without any particular rhythm, with predominantly depressive symptoms associated with irritability, sleep disorder (early waking) and cognitive disorder of declarative memory, is suggestive. These symptoms should be screened for in interview when Cushing's syndrome is suspected [51].

R1.1. We recommend biologic exploration in case of multiple progressive signs and symptoms entering in the spectrum of Cushing's syndrome, especially in case of faciotruncular fat distribution and signs of hypercatabolism (skin fragility, proximal amyotrophy, fractures/osteoporosis). ++

R1.2. We recommend biologic exploration in case of signs resembling corticosteroid side effects in a patient not taking corticosteroids. +

R1.3. We recommend screening for clinical signs of Cushing's syndrome in managing frequent pathologies such as obesity, type-2 diabetes, hypertension, postmenopausal osteoporosis, anxiety and depression disorder, etc., and in case of psychiatric symptomatology that is atypic and/or resistant to conventional psychiatric treatment. +

R1.4. In the latter context, we recommend:

- specialist consultation in case of the slightest doubt;
- biologic exploration:
  - in case of faciotruncular fat distribution or at least 1 sign of hypercatabolism,
  - if disease prevalence is low for age (hypertension in under-40 year-olds, osteoporosis in under-50 year-old males, etc.),
  - in case of unusual progression under conventional treatment. +

R1.5. We recommend screening for Cushing's syndrome in case of:

- any adrenal tumor;
- any pituitary adenoma;
- any tumor liable to induce Cushing's syndrome by ectopic ACTH secretion. ++

3. Biologic diagnosis of Cushing's syndrome

Recommendations for diagnosis of Cushing's syndrome by the American Association of Endocrinology and the French National Diagnosis and Care Protocol, published in 2008, advocate the following tests in first line: 24-hour urinary free cortisol (UFC) (taken at least twice), nocturnal salivary cortisol (taken at least twice), and 1 mg (or in some cases 2 mg) dexamethasone suppression test[14]. For the consensus, international publications since 2008 were reviewed, to draw up updated guidelines for positive diagnosis of Cushing's syndrome.

3.1. 24 h UFC

Urinary cortisol is the free glomerular-filtered plasma cortisol. It is essential to make sure no exogenous glucocorticoids havebeen taken that could interfere with immunoassay, and to avoid excessive hydration, as heavy diuresis (> 5 L) causes artifactual UFC elevation [52–54]. Intra-individual variation in UFC is about 50% in Cushing's disease [55], and several samples should therefore be taken. The diagnostic performance of UFC varies depending on the chosen threshold and assay method [56,57]. Mass spectrometry is the most specific and gives lower estimates than immunoassay, although the gain in diagnostic performance is slight when spe- cific immunoassays are used [58,59]. In a recent meta-analysis,mean sensitivity and specificity were respectively 94% and 93% [58]. Sensitivity is slightly lower than for midnight salivary corti- sol assay, and UFC may be normal in moderate Cushing syndrome. Specificity, on the other hand, is better than the overnight dexamethasone suppression test [58]. UFC values 4-fold higher than the upper limit of normal overwhelmingly confirm diagnosis of Cushing's syndrome [2,60,61]. The main pitfall with 24 h urinary cortisol assays is uncertainty about exhaustive sampling. Creatinuria is not systematically assayed in parallel to UFC, but is recommended [62]. UFC values may be falsely normal in case of kidney failure with clearance < 60 mL/min [63].

3.2. Midnight plasma cortisol measurement

Midnight plasma cortisol measurement shows 96–100% sensitivity in diagnosing Cushing's syndrome [58,64]. Sensitivityis still excellent in moderate Cushing's syndrome with normal 24 h UFC [64]. Specificity is around 93% [58]. A threshold at 7.5 μg/dL (205 nmoL/L) provides 87–96% specificity [57,65,66] and varies little whether the patient is awake or asleep [67]. Cortisolemia < 1.8 μg/dL (50 nmol/L) rules out Cushing's syndrome. The main limitation of midnight assay is the need for hospital admission. Also, synthetic estrogens increase cortisolemia by increasing CBG concentration [68,69].

3.3. Midnight salivary cortisol measurement

Salivary cortisol is an estimation of the free fraction of cortisol independently of variations in carrier proteins. It avoids the difficulties of 24 h urine collection and taking plasma samples at midnight in hospital. It is also independent of variations in dexamethasone metabolism. No differences were found between sampling at 10 pm or 11 pm [70,71]. As salivary cortisol shows intra-individual variations, it is recommended to take 2 midnight samples on non-consecutive days [72]. Midnight salivary cortisol measurement shows 90–100% sensitivity and 93–100% specificity [58,64,67,70,73–77]. It confirms diagnosis in patients with moderate Cushing's syndrome and only slightly elevated or normal UFC [64,78,79]. Specificity is not greatly impaired by combined oral contraception. The relevant threshold depends on the assay technique and should be validated by the biologists in each center. Ideally, normal values should be adapted to certain conditions as levels are higher in over-70 year-olds and patients with hypertension or diabetes [21,70,71,80,81]. When immunoassays are used, exogenous glucocorticoids are the main confounding factor in salivary cortisol assay. Plasma contamination can increase the concentration in case of bleeding gums. The assay can be associated to 1 mg dexamethasone suppression test. Sensitivity is 95–96% and specificity 96–98% [64,70]. This seems not to be better than for midnight salivary cortisol measurement in diagnosing Cushing's syndrome. The benefit in performance of simultaneously assaying salivary cortisone generated locally from salivary cortisol by 11beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) is only moderate [70,71,82]. Change in cortisol:cortisone ratio, becoming ≥ 1, indicates contamination by exogenous hydrocortisone [70,72].

3.4. Overnight dexamethasone suppression test (ONDST)

This test explores the sensitivity of the corticotrope axis to negative feedback by corticosteroids. Several cortisol thresholds after suppression have been advocated: 50 nmol/L (1.8 μg/dL) and 138 nmol/L (5 μg/dL). In a 2020 meta-analysis, the sensitivity of these thresholds was respectively 99.2% and 96.5% and speci- ficity 86.2% and 95.3% [58]. Whatever the threshold, the test provided better sensitivity but poorer specificity than the other tests assessed in the meta-analysis. As well as better sensitivity, required for screening, the 50 nmol/L threshold is supported by

studies of cardiovascular morbidity and mortality in adrenal incidentaloma [83–86]. The 1-mg ONDST showed the same diagnostic performance in obese subjects [87,88]. Combined oral contraception or pregnancy increase the concentration of cortisol binding globulin (CBG) and thus plasma cortisol levels [89], making the test difficult to interpret [64,90,91]. There is a dose-dependent effect on cortisol elevation according to the pill's estrogen content [90]. There is a sharp decrease in plasma cortisol down to near-normal values after 4 weeks, but some American authors advocate 2 months' estrogen withdrawal [89,92]. Variable absorption and liver metabolism of dexamethasone by cytochrome P145 isoform 3A4 (CYP3A4) is a frequent cause of false-positives; there are individual differences in CYP3A4 activity, which may also be due to use of CYP3A4-inducing medication, reducing plasma dexamethasone level and thus impairing test efficacy. Conversely, dexamethasone concentration can be increased by CYP3A4-inhibitors. To limit the rate of false-positives, plasma dexamethasone can be assayed to ensure sufficient suppression [93,94]. However, the optimal plasma dexamethasone threshold is uncertain [95,96]. Problems of feasibility in routine use and of cost make the test reserved to selected patients, notably to detect failure to take dexamethasone tablets [97,98].

3.5. Low-dose dexamethasone suppression test

The 2008 Endocrine Society guidelines see the low-dose dexamethasone suppression test (2 mg/day on 2 consecutive days) as an alternative to the 1 mg ONDST for positive diagnosis of Cushing's syndrome, with diagnostic performance comparable to other first-line tests, with a cortisolemia threshold of 50 nmol/L or 55 nmol/L [14]. Since these guidelines were published, 2 studies in particular confirmed that, for a 50 nmol/L threshold, the 2 mg × 2 dexamethasone suppression test showed overall performance equivalent to other tests [99,100]. Literature reviews [101,102] and a meta-analysis [58] confirmed these findings, although sensitivity (95.3%) was a little less than with the 1 mg test (98.6%). The factors affecting test performance were the same as for the 1 mg test. A study found that the specificity of the 2 mg × 2 dexamethasone suppression test was greater in patients taking oral contraceptives was greater when response was assessed on salivary than on plasma cortisol (90.5 versus 61.9%) [64]. Given the technical difficulties of the test, the above data point to the 2 mg × 2 dexamethasone suppression test as a second-line test when the first-line tests give results that are discordant or too close to the diagnostic thresholds.

3.6. Hair cortisol

Cortisol accumulates passively in hair; for a mean hair growth of about 1 cm per month, capillary cortisol can assess cortisol exposure over a period of months. It has been proposed as a diagnostic tool in Cushing's syndrome. It has the advantage of being simple to collect, in consultation, at any time, without need for repeat sampling; samples are kept dry, out of the light, and can be sent simply by mail. In some retrospective series, sensitivity and specificity with various assay techniques and thresholds were respectively 81–93% and 88–98% [103–106]. One study showed it to be contributive, although with poorer performance in mild Cushing's syndromewith normal or only slightly elevated UFC [105]. The diagnostic contribution of associating simultaneous hair cortisone assay has been discussed [105,106]. Hair cortisol concentration decreases under UV exposure [107] and with repeated washing, and can be affected by certain hair dyes [108,109]. The technique's great drawback lies in the technical requirements (notably, extraction), making it mainly interesting for diagnosing cyclic Cushing's syndrome [110]. The interest in moderate intensity Cushing's syndrome remains to be determined.

3.7. Situations impairing the performance of biological investigations

3.7.1. Kidney failure

Cushing's syndrome is very difficult to diagnose in a context of kidney failure, due to corticotroph axis activation, probably of hypothalamic origin related to stress and chronic inflammation caused by kidney failure and chronic dialysis [111,112]. Reports of Cushing's syndrome in kidney failure are few [113–116]. It is, however, an increasingly frequent issue, as kidney failure ratesare increasing in the developed world and survival is improvingfor long-course dialysis patients. Assaying 24 h UFC is not feasi-ble, even in patients with conserved diuresis, as values decrease when creatinine clearance reaches 60 mL/min [63]. Midnight cortisolemia is also elevated in case of kidney failure [117]. Some studies reported conserved plasma cortisol circadian rhythm [118], but with increased plasma and salivary cortisol concentrations [119]. Repeatedly normal nocturnal salivary cortisol assay makes Cushing's syndrome highly unlikely [111,112]. Likewise, while 1 mg dexamethasone suppression test can rule out Cushing's syndrome, kidney failure induces risk of false positive findings when creatinine clearance reaches < 60 mL/min/1.73 m^2 [120].

3.7.2. Shift-work

In most of shift-workers and night-workers, the circadian rhythm is usually not synchronized to night-work ("non-shifters") [121–123]. Cortisol secretion stays in daytime mode, like for day-workers [121]. Peak and trough may be shifted by 2 to 4 hours in night-workers [124–126]. In night-workers who are "shifters" (8 out of 30 in [121]), cortisolemia varies greatly according to circadian rhythm synchronization. Actimetry (analysis of the sleep/wake cycle) sheds useful light on circadian system status ahead of biologic exploration. After ceasing night shifts, the time to return to day-work rhythm varies greatly between individuals [127–131].

R2.1. We recommend checking that the patient is not taking exogenous glucocorticoids ahead of any diagnostic biologic testing.

R2.2. In first line, we recommend one of the following:

- 1 mg overnight dexamethasone suppression test;
- midnight salivary cortisol assay;
- or 24 h urinary free cortisol assay. ++

R2.3. We recommend choosing the first-line test according to the clinical context.

24 h UFC assay is preferable in clinically evident or severe Cushing's syndrome without even moderate kidney failure. UFC should be assayed on techniques with reference values less than 250 nmol/24 h. Other techniques, less specific to cortisol, should not be used.

Overnightsuppression tests are suited to less evident forms, having good sensitivity when the patient is not taking estrogens or other treatment interfering with dexamethasone metabolism.

Midnight salivary cortisol assay is especially recommended when the overnight suppression test or UFC assay are not possible. +

R2.4. Following the first-line test:

- if results are normal, we recommend an immediate or delayed repeat first-line test only in case of strong clinical suspicion;
- if results are pathological, we recommend an immediate repeat first-line test. +

R2.5. In case of 2 abnormal first-line tests, we do not recommend systematic second-line testing. +

R2.6. When first-line results are discordant or borderline, we suggest performing second-line tests in an expert center: midnight

cortisolemia or 24 h plasma cortisol assay, or 2 mg/day dexamethasone test for 48 h. +

R2.7. Particular situations. Kidney failure.

We recommend not relying on 24 h UFC in suspected Cushing's syndrome with kidney failure.

In first line, we advise:

- 1 mg dexamethasone test, which, if suppressive, rules out Cushing's syndrome;
- or overnight plasma cortisol or midnight salivary cortisol measurement, which, if normal, rule out Cushing's syndrome.

For all these investigations, threshold values in case of kidney failure are not precisely known. +

R2.8. Particular situations. Shift-work.

Most shift-workers, including night-workers, keep a day-work circadian rhythm, and biologic examination is as for non-shift-workers. For night-workers showing rhythm shift ("shifters"), we recommend biologic exploration after 7 days off night-work, when circadian rhythm has normalized. +.

4. Diagnosis of adrenal Cushing's syndrome (ACTH-independent)

4.1. Plasma ACTH threshold for diagnosing adrenal origin

Etiologic diagnosis of Cushing's syndrome is based firstly on plasma ACTH measurement. Plasma ACTH is suppressed in hypercortisolism of primary adrenal origin as autonomous adrenal secretion of cortisol inhibits pituitary production of ACTH. In contrast, it is above the lower limit of normal in case of inappropriate ACTH secretion, whether pituitary or ectopic [9].

ACTH is assessed on sandwich immunoassay. Blood sampling, between 8 and 9 am, uses an EDTA tube [132]. Recent studies reported relative stability of ACTH levels, and total blood samples can be kept for 4 h at room temperature or for 24 h at 4 °C [133–136]. The most sensitive techniques use a double antibody (sandwich method), either isotopic (immunoradiometric assay: IRMA) or non-isotopic (electrochemiluminescence). They have low detection thresholds, between 2 and 5 pg/mL, with lower limits of normal between 5 and 20 pg/mL, depending on the kit.

ACTH concentration < 10 pg/mL (2.2 pmol/L) on 2 tests confirms the primary adrenal origin of hypercortisolism [9]. Levels between 10 and 20 pg/mL are a gray zone where hypercortisolism of primary adrenal origin overlaps ACTH-dependent Cushing's syndrome; this is seen in moderate Cushing's syndrome, with fluctuating secretion, and in moderate adrenal hypercortisolism, with spontaneous fluctuation of cortisol secretion inducing transient loss of suppression of the hypothalamo-pituitary axis and incomplete ACTH suppression. In low ACTH ranges, assay quality is crucial to avoid misclassification [137]. If one or other ACTH value is in the gray zone, ACTH simulation by corticotropin-releasing hormone (CRH) can be used [138]. ACTH 50% above baseline or with a peak of > 30 pg/mL rules out hypercortisolism of primary adrenal origin. Finally, conventional adrenal CT is appropriate for ACTH 10–20 pg/mL [9].

4.2. Radiology

The aim of conventional imaging is to distinguish benign and malignant lesions [benign adrenal adenoma and adrenocortical carcinoma (ACC), respectively]. There are at present two conventional techniques that analyze the adrenal glands: CT and MRI. Most available studies are based on these for distinguishing malignant ACC from other benign lesions with adrenal incidentaloma. Thus, these studies were not devoted to study adrenal tumors responsible for Cushing's syndrome. On non-enhanced CT, the classic threshold of spontaneous density (SD) > 10 HU in the region of interest rules out many benign lesions, with > 95% specificity but low sensitivity, as about 30% of benign lesions are low-fat (SD > 10 HU) [139]. Moreover, up to 79% of benign secreting adenomas responsible for Cushing's syndrome may show SD > 10 HU [140]. It was therefore recently proposed to raise the threshold to 20 HU, increasing diagnostic specificity for ACC from 64% to 80%, while conserving excellent sensitivity (99%). In case of SD > 10 HU on acquisition without injection, the interest of absolute or relative wash-out with a late phase at 15 min is a matter of debate in adrenal incidentaloma, although absolute wash-out > 78% or relative wash-out > 63% has a positive predictive value of 100% for a benign lesion [140]. However, in secreting adrenal carcinoma, data are lacking to differentiate malignancy, using the relative wash-out [141]. Strategies associating criteria have been assessed. An association of size ≤ 30 mm and SD ≥ 20 HU or size ≥ 40 mm and SD ≤ 15 HU showed 100% positive predictive value for benign status [139,140,142,143]. Finally, there are other imaging criteria suggesting ACC: heterogeneous low-fat tumor with ≥ 20% growth on the long axis within 6 months [86,139,144]. MRI without injection with chemical displacement sequences can detect fat, the presence of which correlates strongly with benign status. A recent meta-analysis reported 94% sensitivity and 95% specificity for benign adenoma [145]. However, this technique has not been validated for secreting adrenal tumors.

4.3. Functional imaging

Functional imaging contributes to etiologic diagnosis and, for malign tumors, to staging assessment in ACC underlying Cushing's syndrome.

In case of typical adenoma on CT or MRI, FDG PET is generally negative or only slightly positive, although false-positives are possible. In multiple uni- or bilateral adenoma, FDG PET fails to distinguish between cortisolic adenoma and synchronous non-secreting adenoma. In case of a large and/or heterogeneous mass, malignant ACC may be suspected in presence of high uptake. Therefore, 18F-FDG PET contributes to etiologic diagnosis when conventional imaging is inconclusive [146,147]. A tumor/liver SUVmax ratio < 1 has strong negative predictive value for malignancy, while some benign tumors show higher ratios [148,149]. In a prospective study, tumor/liver SUVmax ratio > 1.5 was associated with high malignancy risk, with sensitivity, specificity, positive and negative predictive values and exactness of 86.7%, 86.1%, 56.5%, 96.9% and 86.2%, respectively [148]. In case of highly hypermetabolic mass (tumor/liver SUVmax ratio > 2), the main differential diagnosis in Cushing's syndrome is benign or malignant adrenal oncocytoma, with ratios often greater than 4.

The role of the adrenocortical tracer 131I-Norchol is more open to debate, but it can be useful for characterizing lesions inaccessible to biopsy. In case of uni- or bilateral micronodules or normal adrenal glands, diagnosis may be related to primary pigmented nodular adrenocortical disease (PPNAD). In this case, morphologic imaging may show an apparently normal adrenal gland, or uni- or bilateral abnormalities and possible coexistence of macronodules. Functional imaging of the adrenal cortex, with whatever tracer, generally shows bilateral adrenal tracer uptake, with possible asymmetry in case of macronodules [147]. In case of multiple macronodules suggesting primary bilateral macronodular adrenal hyperplasia (PBMAH), preoperative imaging of the adrenal cortex, with whatever tracer, may be considered ahead of unilateral adrenalectomy. Noriodocholesterol scintigraphy with visual or quantitative analysis may guide surgery, especially when the masses have similar volumes, although this remains to be assessed

[150,151]. PBMAH is often associated with a hypermetabolic phenotype on 18F-FDG PET [147].

4.4. Dynamic testing in micro- and macronodular adrenal hyperplasia

In some cases of ACTH-independent Cushing's syndrome and especially in case of PBMAH, cortisol excess is inducedby inappropriate expression of G protein-coupled receptors (GPCR), sometimes suggested by low fasting morning cortisolemia (< 280 nmol/L) followed by plasma cortisol increase after meals. Many dynamic tests have been studied to screen for abnor- mal cortisol secretion response, which indicates presence of one or more abnormal GPCRs in adrenal lesions but have been shown to be little if no influence on therapeutic management [152].

In Cushing's syndrome due to PPNAD, paradoxical > 50% increase in 24 h UFC over baseline was reported after 8 mg dexametha-sone suppression. This paradoxical response seems to be specific, but is seen in only 38% of patients undergoing surgery for PPNAD [153].

R3.1. For initial etiologic diagnosis of Cushing's syndrome, we recommend plasma ACTH measurement, performed between 8 and 9 am, and repeated at least twice, with concomitant plasma cortisol assay.

ACTH concentration < 10 pg/mL on 2 assays confirms the pri- mary adrenal origin of the hypercortisolism.

ACTH concentration > 20 pg/mL on 2 assays with concomitant hypercortisolism confirms ACTH-dependent Cushing's syndrome. ++

R3.2. In case of intermediate ACTH values (10–20 pg/mL), we recommend ACTH simulation by CRH. Absence of ACTH response (peak < 30 pg/mL) indicates ACTH-independent Cushing's syndrome. +

R3.3. We recommend multiphase adrenal CT except in case of homogeneous spontaneous lesion density < 10 HU throughout the non-injected phase; Adrenal imaging should be performed or analyzed in an expert center before considering any complementary diagnostic or therapeutic strategy. ++

R3.4. In case of unilateral adrenal tumor, CT can distinguish benign adenoma, with size < 4 cm + homogeneity + spontaneous density < 20 HU, or malignancy, with size > 4 cm + heterogeneity + density > 20 HU + locoregional extension or metastases. ++

R3.5. MRI shows no added benefit in lesions that are indeterminate on multiphase injected CT. It has a role to play when the CT contrast agent is contraindicated. Normal CT results do not justify complementary MRI. +

R3.6. In case of indeterminate unilateral adrenal mass on morphologic imaging, ^{18}F-FDG PET can determine its nature, strongly ruling out malignancy in case of tumor/liver SUVmax ratio $\leq$ 1.5. +

R3.7. In case of malignant ACC, ^{18}F-FDG contributes to extension assessment. +

R3.8. In case of bilateral macronodular adrenal hyperplasia, adrenocortical noriodocholesterol scintigraphy performed in an expert center may be considered, to define the extent of surgery. +

R3.9. In case of no detectable adrenal mass on morphologic imaging, adrenocortical noriodocholesterol scintigraphy can visualize the hypersecreting adrenal tissue (micronodular adrenal hyperplasia). +

R3.10. In case of bilateral macronodular adrenal hyperplasia, we do not recommend systematic diagnostic dynamic tests screening for inappropriate or abnormal GPCR expression. +

R3.11. In case of suspected PPNAD, a high dose (8 mg) dexamethasone suppression test screening for paradoxical UFC elevation should be considered in an expert center. +

5. Diagnosis of ACTH-dependent Cushing's syndrome

5.1. Clinical presentation and baseline biologic investigation

Although ACTH secretion is pituitary in more than two-thirds of Cushing's syndromes, endocrinologists sometimes encounter a clinical situation in which several features suggest ectopic secretion: advanced age, male gender, rapid symptom progression and intense Cushing's syndrome. Ectopic ACTH secretion is usually associated with clear catabolic symptoms (profound muscle weakness, osteoporotic fractures), little or no weight-gain, hypertension, and hypokalemia due to type-2 renal 11ßHSD saturation by excess cortisol. Strong elevation of UFC (> 5 times the upper limit of normal), serum cortisol (> 1000 nmol/L) and ACTH (> 150 pg/mL)is frequent [13,154]. It is important to take these parameters into account, as the probability of Cushing's disease exceeds 90% ahead of any complementary test in patients who are middle- aged women, with biologically moderate Cushing's syndrome and neither hypokalemia nor marked plasma ACTH elevation and progressive onset over more than 1 year [155]. This pre-test probability sometimes exceeds the sensitivity and specificity of certain dynamic tests [154].

5.2. Dynamic tests

Dynamic testing provides indirect in vivo assessment of molecular differences between corticotropic adenomas, typically witha corticotropic phenotype, and ectopic ACTH-secreting tumor cells. The corticotropic phenotype is characterized by: gluco- corticoid receptors (GRs) that conserve the capacity to inhibit ACTH secretion under high-dose dexamethasone; overexpression of the V3R receptor for vasopressin, and abnormal expression of the renal V2 receptor for vasopressin and its pharmacologic analog, desmopressin; and overexpression of the CRH receptor [156,157]. Suppression of cortisol production under high-dose dexamethasone and increased ACTH and cortisol secretion under CRH or desmopressin are expected in Cushing's disease. In general, ectopic ACTH-secreting tumors do not show these features and do not respond to dynamic tests. Well-differentiated neuroendocrine tumors, however, may express the GR, V2R/V3R and CRH receptors and respond in the same way as corticotropic adenomas [158–160]. Thus, no tests are free of false-positives and negatives. Moreover, thresholds reported in the literature are variable, depending among other factors on the type of ectopic tumor being studied (which is often not specified) and the number, which was very low in some studies, casting doubt on the reported specificity. It is therefore important to keep in mind that there are no universal thresholds for each test and that results are to be interpreted probabilistically. Cyclic Cushing's syndrome, which may be associated with both Cushing's disease and ectopic ACTH secretion, constitutes a pitfall, as test results during periods of inactivity may correspond to the activity of unsuppressed pituitary corticotropic cells. Finally, time-consuming dynamic tests should be renounced in urgent cases with intense hypercortisolism.

The CRH test is relatively noninvasive, well-tolerated, well-assessed and seems to be the most effective. With variable positivity criteria expressed as percentage increase in ACTH level (from 35% to 105%) and cortisol level (from 17% to 50%) from baseline, sensitivity and specificity are respectively reported to be around 80% and 92% [161–166]. Although performance is good, this is still, like other tests, a probabilistic approach for diagno-sis: the greater the elevation of ACTH and cortisol following CRH injection, the more probable the diagnosis of corticotropic ade-noma.

Ten studies of the desmopressin test have been published [160,163–165,167–171]. Most had methodological weaknesses,

mainly regarding predetermined interpretation criteria and small sample sizes. Sensitivity seems comparable to the CRH test, but some studies suggested lower specificity [160,163,171]. In the largest study, with a threshold of 18% for increase in cortisol and of 33% for ACTH, sensitivity, specificity and positive predictive value were roughly equivalent to the CRH test: respectively 83% and 83%, 81% and 85%, and 96% and 97%. The desmopressin test is relatively noninvasive and well-tolerated. Like the other tests, it is probabilistic: the greater the elevation of ACTH and cortisol, the more probable the diagnosis of corticotropic adenoma.

The high-dose dexamethasone suppression test (HDDS) was the earliest described, and is now implemented as a short "overnight" test, showing performance equivalent to long protocol involving UFC measurement [172]. Most HDDS studies are old, with few publications since the 2008 guidelines came out [162,170]. In older and more recent studies, 68–71% suppression of plasma cortisol was associated with 64–71% sensitivity and 99–100% specificity for diagnosis of Cushing's disease, but with great uncertainty regarding the ectopic tumors included [162,170,172–176]. Performance and contributiveness are debated. Association to CRH testing is still recommended by some teams [162], whereas it has been abandoned by others due to poor specificity and lack of value added to the pretest probability based on the above-mentioned simple criteria [155,176,177].

Given the limited performance of these tests in isolation, at least 2 should be combined, in a noninvasive strategy [9,154,178]. Concordant positive results reduce the risk of false-positives due to ectopic ACTH syndrome (EAS). Combining HDDS and CRH tests is the oldest such approach [9,154,178]. A large-scale study found identical diagnostic performance regarding 30% plasma cortisol suppression when the standard suppression test was associated to the CRH test [174]. More recently, other groups that had abandoned HDDS advocated combining CRH and desmopressin tests [164]. However, while concordant positive results on multiple tests increase the probability of diagnosis of Cushing's disease, ectopic ACTH secretion cannot be formally ruled out. Moreover, test results were reported to be discordant in at least a quarter of patients [13,154,164].

5.3. Pituitary imaging

Detection of corticotropic adenoma relies on pituitary MRI. Microadenomas present a challenge, with a median size of 5 mm [164]. The acquisition protocol should comprise coronal and sagittal spin-echo slices with gadolinium-enhanced T1 and T2 and millimetric 3D T1 slices [179,180]. Other MRI approaches have been proposed to improve microadenoma detection, but did not show systematic superiority, notably due to false- positives [179,181–184]. Sensitivity ranges between 42% and 85%[182,185–190], with performance improving in later studies thanks to technical progress, and especially the use of millimetric 3D sequences [187]. Correspondence to histology ranges from 76% to 93%, but is poorer for images smaller than 4 mm [184,186,189,191]. Several studies found that adenoma size was not the only relevant parameter and that, in an expert center, a trained neurosurgeon identified more microadenomas than found on preoperative MRI [187]. Millimetric pituitary lesions are encountered in case of ectopic ACTH secretion [189,192], reinforcing the need for expertise in interpreting MRI findings [193].

Molecular imaging has been studied for visualizing corticotropic pituitary adenoma. 18F-FDG PET showed poor performance, compared to MRI [194]. Some studies found 11C-methionine PET to be effective for visualizing corticotropic adenomas that were invisible on pre- and postoperative MRI [195–197], but this technique is still at the research stage.

5.4. Extra-pituitary conventional imaging

Conventional imaging seeks to detect ectopic tumors inducing ACTH secretion usually (49–79% of cases) at thoracic level or in the abdomen and especially the pancreas (see review in [13]). In case of suspected ectopic ACTH secretion, multiphase millimetric cervico-thoraco-abdomino-pelvic CT should be performed in first line, due to its excellent spatial resolution. Sequences are taken first without enhancement, followed by millimetric cervico-pelvic helicoid arterial-time acquisition with contrast enhancement and portal-time abdomino-pelvic acquisition. The sensitivity of first-line dedicated CT when there is no obvious neoplasia is around 67%, with specificity close to 100% when performed in an expert center [164]. Thoraco-abdomino-pelvic MRI may be performed in second line, notably in case of negative CT findings, although it is less effective for detecting bronchial tumors [198].

5.5. Petrosal sinus sampling

Bilateral inferior petrosal sinus sampling is an invasive procedure to distinguish between pituitary and ectopic ACTH secretion. Various technical points are fundamental. All situations other than Cushing's disease liable to induce pituitary ACTH secretion must first be ruled out: pseudo-Cushing's syndrome, cortisol hypersecretion via an adrenal tumor with incomplete ACTH suppression and resistance to glucocorticoids. Incomplete suppression of normal corticotropic cells in patients with ectopic ACTH secretion related to intermittent or cyclical cortisol secretion or to prior anticortisol treatment can thus lead to false-positives. It is therefore very important to perform inferior petrosal sinus sampling in a chronic hypercortisolism state confirmed by biological investigations. The catheters must be placed in the inferior petrosal sinuses, and this requires an expert team with angiography performed during and at end of procedure. Blood samples are taken simultaneously from both inferior petrosal sinuses before and after peripheral IV injection of 100 μg CRH [199–201] or desmopressin [202] or both [203].

In experienced expert hands, incidence of severe adverse events such as stroke or transient cranial nerve palsy is less than 1% [204]. The universally agreed diagnostic criterion for Cushing's disease is a central-to-peripheral plasma ACTH gradient $\geq$ 2.0 before or $\geq$ 3.0 after injection [199,200]. To date, inferior petrosal sinus sampling shows the best performance for diagnosis of Cushing's disease. In 11 single-center studies of $\geq$ 50 patients, overall sensitivity in 1309 Cushing's disease patients was 92% and specificity in 97 patients with ectopic ACTH secretion was 96% [200]. False negatives are the most frequent, generally resulting from inadequate placement of the catheter or variations in venous drainage diluting the pituitary blood [200,205,206]. Prolactin assay in blood samples has been advocated to determine whether pituitary blood had been sampled precisely by normalizing ACTH with respect to prolactin [207,208]. False-positives are rare in ectopic ACTH syndrome, and are not always fully accounted for.

Inferior petrosal sinus sampling with calculation of the intersinus ACTH ratio is not effective to locate corticotropic microadenomas [189,209,210] and is not improved by intracavernous catheter positioning.

Analysis of the literature discloses two main types of diagnostic algorithm: invasive or noninvasive, according to the role of petrosal sinus sampling. For some expert teams, it should be in first line in ACTH-dependent Cushing's syndrome if pituitary imagingis inconclusive [211,212]. However, progress in pituitary MRI and dynamic testing can avoid catheterization in many patients and, in this less invasive strategy, the most frequently recommended algorithm [9,154,178] places pituitary MRI in first line with at least 2 dynamic endocrine tests: CRH, high-dose dexamethasone suppression, and/or desmopressin test. Catheterization is resorted to

in case of negative or doubtful MRI or discordant tests. A large-scale retrospective study [164] advocated thin-slice cervico-thoraco-abdomino-pelvic CT before catheterization in case of diagnostic doubt: taken alongside biologic findings, it can reduce indications for catheterization by about half [193].

5.6. Functional imaging

Molecular imaging shows variable contribution to etiologic diagnosis. In case of positive morphologic imaging confirming ectopic Cushing's syndrome, it can:

- help determine tumor extension;
- confirm the endocrine nature of the suspected lesion, reducing the risk of false-positives [213];
- shed light on prognosis and treatment.

The type of molecular imaging depends on the type of tumor suggested on clinical and radiological examination, tumor or hormonal markers, and biopsy, including factors such as differentiation and grade for neuroendocrine tumors.

In 10–30% of studies, cross-sectional imaging fails to detect ectopic tumors [13,198]. In case of negative findings (occult ACTH secretion), PET scan targeting somatostatin receptors is the most effective technique and should be used in first line [13,198]. The global sensitivity of Octreoscan, taking all ectopic tumors together, is about 64%. PET with somatostatin analogs has been shown to be more effective than Octreoscan in neuroendocrinetumor outside Cushing's syndrome [214]. There are few reports of 68Ga-DOTATOC/DOTANOC PET, and performance varied accord- ing to context [215–218]. In one of the largest series, with 17 patients, sensitivity was around 65%, but still better than Octre- oscan [215]. 18F-DOPA PET is less sensitive [198,213,219], but may be useful in second line after negative 68Ga-DOTATOC PET results, to reveal bronchial carcinoids or very rare small-intestine neuroendocrine tumors. Uncertain findings suggestive of bronchialneuroendocrine tumor on 68Ga-DOTATOC or 18F-FDG PET can be supplemented by 18F-FDOPA PET to rule out inflammatory or infec- tious false-positives, notably in case of opportunistic infection. 18F-FDG PET is the examination of choice for rapidly progress-ing tumors (small-cell bronchial tumor, thymic carcinoma or some pancreatic neuroendocrine tumors) [198,213,220]. However, sen- sitivity as reported in a meta-analysis of neuroendocrine tumors implicated in ectopic ACTH syndrome was relatively good, rais-ing the question of whether the principle that FDG PET is positive only in case of aggressive neuroendocrine tumor also applies to ACTH-secreting tumor [13,198].

5.7. Tumor markers

Elevated plasma proopiomelanocortin (POMC) suggests aggressive ACTH-secreting tumor, but this assay is no more available and high levels in blood are generally associated with tumors easy to identify on imaging. Biological markers suggesting ectopic ACTH (notably, calcitonin and plasma or urinary metanephrin) can be included in the work-up as a complement to imaging in caseof strong suspicion of ectopic tumor [13]. Other markers can be assayed, depending on the context: e.g., urinary 5-HIAA in well-differentiated small-intestine neuroendocrine tumor [154]. Plasma chromogranin A and other non-specific markers have little diagnostic value.

5.8. First-line diagnostic algorithms

First-line diagnostic algorithms vary mainly in terms of the positioning of petrosal sinus sampling, more or less early in the process. It is important to bear in mind that knowledge is limited for establishing exploration algorithms for etiologic diagnosis of ACTH-dependent Cushing's syndrome:

- no prospective or retrospective studies compare global diagnostic strategies with relevant endpoints: histologic proof, postsurgical remission, etc. Thus, no particular algorithm can be recommended on evidence-base grounds;
- no study precisely assessed the benefit of various algorithms according to global context, notably including pre-test probability of Cushing's disease [155];
- literature reports often concern expert centers, which tend to optimize algorithm performance. Except in obvious cases, the differential diagnosis of Cushing's syndrome should therefore be made in expert centers;
- none of the reported algorithms show 100% sensitivity or specificity.

5.9. Follow-up in Cushing's syndrome with ectopic ACTH secretion by an occult tumor

Although reduced by progress in imaging, the prevalenceof occult ectopic ACTH secretion despite multiple investigation was 5–15% in recent series (see review in [13,154,198]). Reg-ular morphologic follow-up is therefore conducted alongsidemonitoring of hypercortisolism [13]. As most occult tumors are well-differentiated bronchial neuroendocrine tumors, priority isgiven to thin-slice cervico-thoraco-abdomino-pelvic CT analyzed by an expert radiologist, focusing mainly on the thorax, at 6 monthspost-diagnosis then annually. Nuclear imaging of somatostatin receptors by DOTATOC/DOTANOC PET, at longer intervals, is to bediscussed in the expert center [217,221]. Due to possible false nega-tives on petrosal sinus sampling [200,205], pituitary MRI screening for corticotropic microadenoma should also be considered if the tumor remains occult after several years' follow-up.

R4.1. We recommend taking account of parameters suggesting ectopic ACTH secretion, to guide complementary exploration:

- male gender;
- rapid onset of Cushing's syndrome (< 6 months);
- and intensity of biological parameters: UFC > 5 N, hypokalemia, ACTH > 100 pg/mL. +

R4.2. We recommend performing at least 2 of the follow- ing tests, associated to pituitary MRI, when etiologic diagnosis of ACTH-dependent Cushing's syndrome is uncertain: high-dose dexamethasone suppression, desmopressin test, and/or CRH test. ++

R4.3. We recommend caution in using literature thresholds, which are not universal. +

R4.4. We recommend giving priority to pituitary MRI, except in Cushing's syndrome with strong suspicion of ectopic ACTH secretion. ++

R4.5. Pituitary MRI should associate contrast-enhanced T1, T2 and millimetric 3D T1 sequences, and should be performed or analyzed in an expert center before determining any complementary diagnostic or therapeutic strategy. If interpretation is uncertain or acquisition conditions are unsatisfactory, the examination should be made again. +

R4.6. In case of strong suspicion of ectopic ACTH secretion,we recommend first-line multiphase cervico-thoraco-abdomino- pelvic CT. +

R4.7. We recommend performing inferior petrosal sinus sampling in an expert center, especially if diagnosis of Cushing's disease or ectopic ACTH secretion is not established after noninvasive exploration comprising 2 dynamic tests, pituitary MRI and cervico-thoraco-abdomino-pelvic CT. +

R4.8. In case of positive morphologic imaging with a presentation suggestive of Cushing's syndrome by ectopic ACTH secretion, we recommend functional molecular imaging to confirm the endocrine nature of the suspected lesion, determine locoregional or remote extension, and provide prognostic information. Choice of tracer depends on the clinical context and presumed tumor type. +

R4.9. In case of a presentation suggestive of Cushing's syndrome by ectopic ACTH secretion with negative morphologic imaging, we recommend first-line ^{68}Ga-DOTATOC PET. +

R4.10. In case of strong suspicion of ectopic ACTH secretion (notably after petrosal sinus sampling) with negative CT and ^{68}Ga-DOTATOC PET, we suggest ^{18}F-DOPA and/or ^{18}F-FDG PET. +

R4.11. POMC assay is not readily available and is not recommended. +

R4.12. In case of thyroid or adrenal tumor associated with ACTH-dependent Cushing's syndrome, tumor markers should be assayed: calcitonin or metanephrine to confirm respectively thyroid medullary carcinoma or ACTH-secreting pheochromocytoma. We do not recommend systematic assay of other tumor markers. +

R4.13. In the absence of any evidence-based algorithm, we recommend that etiologic diagnosis of ACTH-dependent Cushing's syndrome be performed in an expert center. +

R4.14. In case of suspected occult ectopic ACTH secretion, after inferior petrosal sinus sampling, we recommend:

- thin-slice cervico-thoraco-abdomino-pelvic CT in priority, by an expert radiologist, focusing mainly on the thorax, 6 months posttest then annually;
- discussing in an expert center the possibility of replacing or complementing CT by nuclear imaging of somatostatin receptors on DOTATOC/DOTANOC PET.

R4.15. If ACTH secretion remains occult for several years, we recommend associating pituitary MRI screening for corticotropic microadenoma.

6. Pseudo-Cushing's syndrome

Pseudo-Cushing's syndrome (PCS) is not clearly defined. The term is generally applied to patients with clinical signs compatible with Cushing's syndrome and biological signs of hypercortisolism but no tumoral pathology in the corticotrope axis. The prefix "pseudo" refers to this absence of tumoral pathology; PCS is a "functional" pathology, in which hypercortisolism is secondary to a non-tumoral pathology and disappears when this is resolved.In contrast, in pure "neoplastic" Cushing's syndrome, hypercortisolism depends on tumoral or hyperplastic tissue secreting ACTH or cortisol, and disappears only with resection of this tissue or control of the secretion.

The non-tumoral pathologies underlying functional hypercortisolism in PCS are mainly psychiatric (notably depression), chronic alcoholism, insulin-resistant obesity, and polycystic ovary syndrome [112]. Mechanisms suggested as underlying functional hypercortisolism comprise functional hypothalamic hypersecretion of CRH and/or increased cortisol production by inhibition or inactivation of metabolism, but without complete ACTH suppression. Thus, PCS resembles "ACTH-dependent" hypercortisolism, and an important point needs to be highlighted: in "pseudo- Cushing's syndrome", it is not hypercortisolism that is "pseudo"; while not tumoral in origin, it is perfectly real, although func- tional and generally moderate. Such functional hypercortisolism may be associated with some of the clinical signs seen in moder- ate Cushing's syndrome: generally, android obesity and elevated blood pressure; catabolic signs (vergetures, proximal amyotrophy) are rare, although no doubt prevalent in case of chronic alcoholism [100,112,222,223].

UFC is generally only moderately elevated in PCS; even in the most intense forms, often associated with neuropsychiatric contexts, UFC never exceeds 4 times normal values [119,224]. The use of midnight baseline cortisol measurement is based on the observation that, in functional Cushing's syndrome, the circadian rhythm of the corticotrope axis is abolished. Studies conducted before 2008 here reported > 95% sensitivity and specificity for diagnosing Cushing's disease versus functional Cushing's syndrome on midnight cortisolemia. The threshold, however, varies between studies, from 207 to 256 nmol/L [100,112,225]. It can be affected by differences in sampling and also by differences and progress in assay technique. Midnight cortisolemia shows about 98% sensitivity and 95% specificity [100]. Midnight salivary cortisol seems to show similar values but has been less thoroughly studied [100]. The midnight/morning cortisolemia ratio and analysis of the circadian cortisol cycle have also been proposed, but have been little studied.

Numerous tests have been proposed in case of persistent doubt between Cushing's disease and PSC, but none show absolute effectiveness. There is an important limitation in analyzing and comparing their performance. Sample sizes were small, criteriafor PCS and sometimes for "control" subjects varied, most stud-ies were retrospective, and assay techniques varied. The combined DEX-CRH test was introduced in 1993 [224] to enhance the sensitivity of the 48 h low-dose 2 mg/day dexamethasone suppression test (LDDST). The defining characteristic of Cushing's disease was stimulation of cortisolemia following CRH injection despite prior suppression, illustrating greater resistance to dexamethasone in Cushing's disease and increased corticotropic adenoma sensitivity to CRH. The test was credited with 100% sensitivity and specificity on this criterion. Subsequent reports, however, contested this performance [223,226,227]. Literature reviews [112,228] highlighted variable diagnostic performance but concluded that, although the combined test is relatively expensive and requires hospital admission, it is nevertheless useful when first-line results are equivocal or discordant, as it shows relatively good precision in excluding Cushing's syndrome. Thresholds, however, vary greatly between studies and the literature data do not demonstrate superiority over isolated 2 mg 2 dexamethasone suppression, CRH stimu- lation providing only slight diagnostic gain [223,226,229]. The desmopressin test contributes to differential diagnosis between Cushing's disease and PCS: ACTH stimulation by desmopressin is more intense in adenomatous than in normal corticotropic cells. This is related to 3 particularities of these adenomatous cells: strong V3 receptor expression, frequent ectopic expression of the V2 receptor, which shows the greatest affinity for desmopressin, and lower sensitivity to negative cortisol feedback. The desmo- pressin test is well-tolerated and can be implemented on an outpatient basis. Performance in differentiating between Cushing'ssyndrome and pseudo-Cushing's syndrome is at least as good or better than for first-line explorations (1 mg suppression, UFC, mid- night cortisol) and combined dexamethasone/CRH testing. Like for combined dexamethasone/CRH testing, analysis is uncertain due to the above-mentioned limitations, small sample sizes and definitions of "controls". The best results are obtained with absolute ACTH thresholds, according to measurement technique and defined a-posteriori in retrospective studies. In these series, analyzing variation in ACTH levels is certainly more effective than analyzing cortisol levels, as clearly demonstrated by Rollin [230]. An ACTH increment of 6 pmol/L has been advocated [231], with 87% sensitivity and 91% specificity, and this has been used in many studies [227]. On the other hand, a lower increment of 4 pmol/L associ- ated to a > 331 nmol/L cortisol concentration was also advocated [222]. The higher the peak stimulated ACTH value, the better the discriminative capacity [230].

Despite numerous investigations, differential diagnosis can be tricky, especially when pituitary MRI is normal. Inferior petrosal sinus sampling does not contribute to this differential diagnosis, and should not be performed. During follow-up, exploration should be repeated in an expert center to assess clinical and biological progression. Likewise, repeated exploration of the corticotropic axis is worth considering in cases in which it is possible to act upon the pathological situation underlying corticotropic axis activation: e.g., alcohol withdrawal, or treatment of depression [112].

R5.1. We recommend examining the differential diagnosis between PCS and moderate Cushing's disease in patients presenting moderate ACTH-dependent hypercortisolism with a pathology liable to induce non-controlled PCS, such as psychiatric disorder, and notably depression, alcoholism, diabetes and/or insulin-resistant obesity, or polycystic ovary syndrome. ++

R5.2. Differential diagnosis between PCS and tumoral Cushing's syndrome is based firstly on first-line tests: 24 h UFC, overnight dexamethasone suppression, and midnight salivary or plasma cortisol. ++

R5.3. In case of persistent suspicion of PCS, we advise one or more of the following tests in second line, although they cannot be ranked for performance:

- repeated midnight cortisolemia or salivary cortisol;
- desmopressin test;
- standard dexamethasone suppression (2 mg for 48 h);
- combined standard dexamethasone suppression + CRH stimulation. +

R5.4. In case of diagnostic difficulty, we recommend reassessment of the corticotropic axis function in a specialized center, at an interval or after treatment of the pathology liable to activate the corticotropic axis (alcoholism, depression, etc.). +

7. Diagnosis of severe Cushing's syndrome with intense hypercortisolism

Severe Cushing's syndrome shows high biological intensity with comorbidities and complications that may be life-threatening in the short- or mid-term [13,232,233]. It is thus an endocrine emergency requiring hospital admission and prompt treatment. In most cases, severe Cushing's syndrome is caused by ectopic ACTH secretion or, more rarely, by Cushing's disease or secreting adrenocortical carcinoma [13,234]. The diagnostic procedure aims simultaneously:

- to screen for, prevent and treat the comorbidities of the hypercortisolism [diabetes, hypokalemia, lung infection (especially opportunistic), phlebitis, pulmonary embolism, acute pulmonary edema due to cortisolic cardiomyopathy, diaphragmatic myopathy affecting breathing, agitation or steroid-induced psychosis];
- to achieve emergency etiologic diagnosis so as not to delay treatment of Cushing's syndrome.

The diagnostic procedure should be simplified, focusing only on essentials [13]. The local hormonology lab should be warned and send back serum and/or urinary cortisol and ACTH results rapidly. Just a few random serum cortisol assays should be performed, regardless of timing and all in the same day, generally revealing cortisolemia > 37 μg/dL (> 1000 nmol/L) [13,235]. 24 h urinary cortisol assay may be considered, depending on urgency, but will not be repeated. Serum ACTH assay in a few of the samplesin parallel to cortisolemia differentiates ACTH-dependent Cushing's syndrome from less frequent primary corticoadrenal causes. Very high ACTH levels indicate paraneoplastic Cushing's syndrome [13,175,192,236,237].

In emergency contexts, complex dynamic biological tests that delay control of hypercortisolism are to be avoided, and dexamethasone suppression tests are contraindicated. ACTH assay can guide imaging. In an emergency, CT may be performed before the assay results come back, given the high rate of ectopic ACTH-secreting and adrenal tumors, to detect threatening complications such as pulmonary embolism, lung infection, digestive perforation, etc. Petrosal sinus sampling for doubtful cases is often incompatible with the acute status of the hypercortisolism.

R6.1. Intense hypercortisolism (UFC > 5 times the upper limit of normal and serum cortisol > 1000 nmol/L) is an endocrine emergency that is life-threatening in the short term. Etiologic exploration should be conducted in an expert center, without delay, focusing only on essentials so that treatment of Cushing's syndrome can be initiated. The hormonology lab should be alerted. We recommend:

- limiting exploration to a few random blood samples for cortisol and ACTH assay within 24 h, possibly with UFC assay;
- abstaining from dexamethasone suppression;
- considering, on a case-by-case basis in an expert center, rapid dynamic testing (CRH, desmopressin), liable to delay treatment.

Expert opinion

R6.2. In case of intense hypercortisolism, we recommend emergency cervico-thoraco-abdomino-pelvic CT, independently of hormonal biology results:

- to guide etiologic diagnosis: ACTH-secreting extra-pituitary and adrenal tumor;
- to estimate oncologic urgency;
- to detect cortisol-related life-threatening complications: pulmonary embolism, lung infection, digestive perforation, etc. +

8. Diagnosis of Cushing's syndrome during pregnancy

8.1. Physiologic changes in HPA axis function during pregnancy

Cushing's syndrome is rare during pregnancy, with fewer than 300 cases reported between 1952 and 2020 [79]. Diagnosis is difficult to suspect and confirm in less severe forms. Exploration has to take account of physiological changes in hormone secretion, the lack of relevant studies, the limitations imposed by the pres- ence of the fetus, and the fact that most hypercortisolism is of adrenal origin. Changes in the HPA axis activity during pregnancy result from placenta production of CRH and ACTH, the plasma concentrations of which increase, but also from high concentrations of estrogens and progesterone. Hyperestrogenism increases liver production of transcortin (CBG) and thus raises cortisolemia. Progesterone induces cortisol resistance. Plasma free cortisol is slightly elevated. However, the circadian secretion rhythm is unchanged [238].

8.2. When should hypercortisolism be suspected during pregnancy?

Hypercortisolism is difficult to diagnose during pregnancy, especially when mild. Facial and trunk weight gain and vergetures are often attributed to the pregnancy. Diabetes and hypertension are also poorly specific. Signs of hypercatabolism (ecchymosis, amyotrophy) and of hyperandrogenism (acne, hirsutism) and hypokalemia are more specific, but rarer. Pre-eclampsia or delayed intrauterine growth may be revelatory. Fractures are reported [79,238,239].

8.3. How to make a positive diagnosis of hypercortisolism during pregnancy?

Serum cortisol rises as of the first trimester. Mean total cortisol levels on liquid chromatography with tandem mass spectrometry (LC-MS-MS) in the 1st, 2nd and 3rd trimesters were multiplied by respectively 1.6, 2.4 and 2.9 [240]. Thus, midnight plasma cortisol thresholds determined in control populations are inappropriate during pregnancy, although circadian rhythm is preserved. 24 h urinary cortisol increases progressively during pregnancy: levels on LC-MS-MS in the 1st, 2nd and 3rd trimesters were multipliedby respectively 1.7, 2.4 and 3.1 [240]; in this study, values were 30–35% higher when UFC was assayed on a commercial immunoas-say kit. UFC values 3 to 4 times the upper limit of normal aregenerally diagnostic of Cushing's syndrome at whatever stage of pregnancy. Midnight salivary cortisol, which reflects plasma free cortisol, increases slightly [69,240], mainly in the 3rd trimester. Midnight salivary cortisol is a good diagnostic assay to screen for Cushing's syndrome during pregnancy. The thresholds to be adopted during pregnancy are a matter of debate. Some authors advocate specific thresholds, others suggest that the normal val- ues for midnight salivary cortisol outside of pregnancy can be used for the first 2 trimesters [241], with an increase of about 20% for the 3rd trimester. It should be borne in mind that salivary cortisol assay is not presently covered by the French national health insur- ance system. Fewer than 40% of pregnant women show < 50 nmol/L plasma cortisol after 1 mg dexamethasone suppression [238]. This low suppression in pregnancy has not been sufficiently studied; suppression tests are thus unsuited to screening and diagnosis of hypercortisolism in pregnant women.

8.4. Establishing the cause of hypercortisolism during pregnancy

Unlike the situation outside pregnancy, during pregnancy etiology is most often adrenal: Caimiri reviewed publications from 1952 to 2015 and found a 60–75% rate of adrenal etiology [79]. Analysis of 44 case reports published between 2015 and 2020 confirmed this prevalence. Hypercortisolism may also be directly related to pregnancy, and adrenal LH/HCG receptor expression was demonstrated in some cases.

Plasma ACTH assay is the key to etiologic diagnosis, indicating adrenal (ACTH-independent) or ACTH-dependent hypercortisolism. However, several reports stressed that ACTH was not systematically suppressed in adrenal hypercortisolism discovered during pregnancy [238,242]. Dynamic testing has no role during pregnancy. Some studies of CRH or vasopressin tests in pregnant women without endocrine pathology reported variable ACTH and cortisol response [243]. The small number of cases and the heterogeneity of the results preclude determining normal values and sensitivity/specificity during pregnancy or even confirming the safety of the procedure. Safety and contribution likewise have not been established for strong suppression, with just a few published case reports. Inferior petrosal sinus sampling was reported in 1 pregnant woman; the risk of radiation to the fetus and the invasiveness of the procedure are obvious obstacles.

Imaging should firstly be non-irradiating. Adrenal ultrasound can be commonly used. Other imaging examinations should only be conducted if surgery is envisaged before delivery. If gadolinium injection is used, the patient needs to be informed of the risk/benefit ratio, although no maternal or fetal side effects have been reported (CRAT teratogen reference center). If injection is used, preference should be given to paramagnetic agents: MultiHance or ProHance [244]. If non-enhanced pituitary MRI is non-contributive, adrenal imaging may be used, given the difficulty of interpreting ACTHassay in pregnant women, before envisaging contrast enhance- ment.

R7.1. For etiologic diagnosis of Cushing's syndrome during pregnancy, we recommend:

- ACTH assay and adrenal ultrasound;
- non-enhanced adrenal MRI if plasma ACTH in undetectable or low-normal or in case of difficulty of interpretation;
- non-enhanced pituitary MRI if ACTH is normal or elevated;
- dynamic testing is not recommended.

Expert opinion

R7.2. We recommend suspecting hypercortisolism in pregnant women in case of poorly specific signs such as hypertension or diabetes, especially if associated with 1 or more of the follow-ing: faciotruncular fat distribution, severe vergetures, amyotrophy,acne, hirsutism, hypokalemia. ++

R7.3. For positive diagnosis of Cushing's syndrome during pregnancy, we recommend:

- salivary cortisol measurement, reflecting CBG-unbound cortisol, which is less altered especially in the 1st and 2nd trimesters;
- absence of circadian cortisol secretion rhythm is always pathological during pregnancy;
- urinary cortisol > 3–4 times the upper limit of normal is indicative of Cushing's syndrome during pregnancy, at whatever stage. +

R7.4. For positive diagnosis of Cushing's syndrome during pregnancy, we do not recommend:

- midnight plasma cortisol assay, as thresholds were determined in control populations and cannot be applied during pregnancy;
- overnight dexamethasone suppression test. +

9. Fluctuating Cushing's syndrome

Fluctuating (periodic, intermittent or cyclic) Cushing's syndrome (FCS) is a rare clinical form of endogenous Cushing's syndrome [56,245–247]. Progress in hormonal exploration and conventional imaging combined with better knowledge of this form by endocrinologists may explain why most cases were pub- lished from the 1990s onward. FCS involves repeated episodes of clinical signs of Cushing's syndrome associated with biologic hypercortisolism, separated by intervals of spontaneous remission of variable duration. Diagnosis should be suspected in case of discordance between clinical signs of Cushing's syndrome and hormonal results failing to confirm hypercortisolism. FCS is a pitfall, unawareness of which can lead to prolonged misdiagnosis. All etiologies of Cushing's syndrome may underlie FCS, although Cushing's disease is most frequently reported, followed by ectopic ACTH secretion and, less often, Cushing's syndrome secondary to primary adrenal diseases. Like Cushing's syndrome in general, FCS shows female predominance, with onset around 50 years of age [56,245–247]. Cases in children and elderly subjects have also been reported [248]. No single pathophysiological mechanism has been demonstrated, and mechanisms may vary from case to case and according to etiology.

The definition of FCS has changed over time; it features repeated episodes of hypercortisolism of varying duration and intensity, interspersed by periods in which cortisol secretion is not excessive even if not quite physiological. All authors concur in describ- ing episodic clinical symptoms suggestive of Cushing's syndrome, identical to those in other forms [247,249], and non-specific biological abnormalities (episodic hypokalemia or hyperglycemia with spontaneous regression) associated with episodes of hormonal hypercortisolism [245–247,249–251]. Episodes last between a few

hours and a few months, and spontaneous remission is highly variable and unpredictable, lasting a few days or a number of years.

When patients are assessed outside of the symptomatic episodes, diagnosis is based on more or less pronounced clinical signs contrasting with absence of hormonal hypercortisolism.

Two situations arise resembling FCS. Firstly, there is pseudo-Cushing's syndrome with (sometimes transiently) increased urinary or serum cortisol induced by functional hypercortisolism of various etiologies (see relevant section). Factitious hypercortisolism is another differential diagnosis in FCS, secondary to undeclared episodic consumption of glucocorticoids (hydrocortisone or prednisone) detected on cortisol immunoassay. These exogenous corticosteroids increase serum and urinary cortisol levels associated with low blood ACTH, suggesting an adrenal cause, whereas adrenal imaging shows no tumor or hyperplasia. In between episodes of taking corticosteroids, serum, urinary and salivary cortisol concentrations may collapse, as in true FCS. In exploration while the patient is under prednisone, diagnosis may be revealed by specific mass spectrometry showing a profile specific to prednisone. Undeclared consumption of hydrocortisone cannot be detected on usual mass spectrometry, and may be an insurmountable pitfall.

In the vast majority of cases, symptomatic episodes are accompanied by frank hypercortisolism that can quite easily be demonstrated on usual hormonal techniques. 24 h urinary cortisol and midnight serum or salivary cortisol are usually clearly elevated [252]. Likewise, in FCS secondary to Cushing's disease and ectopic ACTH syndrome, circulating ACTH concentration is increased [252]. In the rare reported cases of adrenal FCS, episodes are accompanied by increased urinary and serum (and/or salivary) cortisol, in contrast to suppressed circulating ACTH. In case of PPNAD, episodes may be triggered by administration of dexamethasone [253].

In exploration during an initial episode, diagnosis is suggested by spontaneous regression of clinical and biological signs with concomitant resolution of hypercortisolism ahead of any treatment. In these cases, authors who hypothesized FCS implemented long-term surveillance to detect any new episode. Detection methods for new episodes varied between cases: iterative 24 h urinary cortisol assay, concomitant urinary cortisol and creatinuria (simpler to perform on an outpatient basis), or salivary cortisol [254]. In some cases, the end of an episode may be followed by phases in which serum and/or urinary cortisol is very low, suggesting transient functional corticotropic deficit. Hormonal assessment outside episodes was highly variable in published reports of FCS and was often incomplete, precluding certainty as to recovery of physiological cortisol secretion or whether there are persistent abnormalities such as disrupted circadian rhythm and/or dynamic test disturbance. We should highlight cases of negative CRH test in FCS by Cushing's disease explored outside episodes and under corticotrope deficiency. Given these pitfalls [255] and uncertainties, we advise against dynamic exploration outside of clearly confirmed hypercortisolism episodes. This applies also to inferior petrosal sinus sampling, which can be misleading for etiologic diagnosis outside of episodes; in case of suspected FCS, we do not recommend this invasive examination outside of hypercortisolism episodes.

Analysis of reported cases shows that clinicians implement the usual conventional imaging in case of FCS. We believe this should be discussed on a case-by-case basis, preferably in an expert cen- ter, depending on clinical presentation and hormonal profile during episodes. When suspected FCS is explored during a spontaneous remission, it may be impossible to distinguish ACTH-dependent versus adrenal FCS; and the diagnosis may require complemen- tary pituitary imaging and thoraco-abdomino-pelvic CT, to detect any corticotropic adenoma, neuroendocrine tumor or adrenocor- tical tumor or hyperplasia. One especially difficult situation is FCS with low ACTH related to PPNAD, where adrenal imaging can be considered normal.

R8.1. Cyclic Cushing's syndrome may be suspected when hormonal exploration does not confirm hypercortisolism despite prior episodes that showed spontaneous regression:

- we advocate repeating hormonal exploration (UFC, midnight salivary cortisol, if available) during episodes;
- we recommend systematic follow-up in an expert center, so as to optimize long-term hormonal exploration. +

R8.2. We do not recommend dynamic testing (suppression and/or stimulation) or petrosal sinus sampling in suspected cyclic Cushing's syndrome, outside of demonstrated hypercortisolism episodes. ++

10. Cushing's syndrome in children

10.1. Etiologies of Cushing's syndrome in children

Cushing's syndrome in children shows particularities in clini- cal presentation, etiology and diagnostic procedure. As it is rare, with very few cases or studies of test sensitivity and specificity, the present recommendations are basically expert opinions.

In a Danish national cohort study between 1977 and 2012, 40 cases of Cushing's syndrome were found in pediatric patients (0–20 year-olds), estimating an annual incidence of 0.89/1 million children [256] and representing 10% of all cases of Cushing's syndrome [256]. Median age at diagnosis was 13.8 years, with 58% female predominance; corticotropic adenoma was implicated in 70% of cases.

In decreasing order of frequency, pediatric etiologies include: iatrogenic (main etiology in children of whatever age), pituitary (75–90% of endogenous Cushing's syndromes in children, with mainly peri-pubertal revelation) [256,257], adrenal (15%, adrenocortical tumors, with mainly neonatal revelation or at 2–5 years), and, very rarely, ectopic (< 1%).

Exogenous Cushing's syndrome may occur with all forms of administration, whether local or general. Corticosteroids may have been taken voluntarily, in which case they are more easily identifiable, or involuntarily. Thus, in suspected clinical hypercortisolism, screening should in priority focus on any source of exogenous glucocorticoids, especially if clinical presentation contrasts with very low plasma cortisol and ACTH levels.

Etiologic diagnosis should take account of age. Cushing's syndrome with peri-pubertal revelation is dominated by exogenous causes, followed by Cushing's disease, while adrenal (adrenocortical tumors) and ectopic causes are rare [256].

Endogenous Cushing's syndrome in under-8 year-olds is dominated by adrenal causes (adrenocortical tumors and PPNAD, whether isolated or part of a Carney complex); ectopic causes are exceptional. Adrenocortical tumors has a prevalence of 0.21/million children/year [258] and is the main cause of endogenous Cush- ing's syndrome at this age; about a third of adrenocortical tumors induce hypercortisolism [259]. Presence of Cushing's syndrome is of poor prognosis [260]. It may be associated with Li–Fraumeni syn-drome (p53 mutation) or Beckwith–Wiedemann syndrome [261], or more rarely with other genetic forms. Periodic or cyclic Cush- ing's syndrome is frequent in children and adolescents with PPNAD[257]. Ectopic Cushing's syndrome in children accounts for less than 1% of Cushing' syndromes, and is 80 times less frequent than Cushing's disease [262]. Onset can be at any age, but presen- tation is age-dependent. Generally, in young children (< 6 years) the causal tumor is revelatory and Cushing's syndrome is discov- ered secondarily or simultaneously. After 9 years of age, bronchial

or pancreatic neuroendocrine tumors are most often implicated, and the Cushing's syndrome is revelatory [263]. Cushing's syndrome with neonatal revelation is dominated by exogenous and adrenal causes; McCune–Albright syndrome is the main cause of endogenous Cushing's syndrome at this age, and can induce very early Cushing's syndrome, mainly in the first year of life [264]. Neonatal adrenocortical tumors are very rare [259]. Cushing's disease is extremely rare; in case of *DICER1* gene mutation, it is now reclassified as corticotropic blastoma or embryonic pituitary tumor.

10.2. Clinical presentation of Cushing's syndrome in children

The most frequent presentation of Cushing's syndrome in children is development of obesity associated with slowed growth velocity. Other signs include: faciotruncular fat distribution, purple vergetures, skin fragility, hypertension and hypertensive encephalopathy [265], skin infection (candidosis), recurrent infection [266], nephrocalcinosis and kidney stones [267], delayed bone mineralization [268], depression [269], fatigability, behavioral disorder [257], delayed puberty, glucose intolerance or diabetes. Clinical presentation also differs with age at diagnosis and etiology.

In Cushing's syndrome with neonatal onset in the context of McCune–Albright syndrome, clinical examination may, as of the first month of life, find characteristic skin marks and, in the following months, bone dysplasia and signs of early puberty in girls [270].

Given the possibility of malignant adrenocortical tumors (ACC) in children, there may be signs of hyperandrogenism: hirsutism, acne, genital virilization. Hyperandrogenism may abolish the classical slowing of growth [271]. PPNAD cyclic hypercortisolism is not systematically associated with delayed growth, especially before 7–8 years of age [272,273]. In case of associated Carney complex, presence of lentigines, myxomas and other endocrine abnormalities guide diagnosis [262]. In the peri-puberty period, Cushing's disease predominates. Slow growth associated with weight gain and increased BMI is classical. Slow growth may be aggravated by associated delayed puberty. Mood and behavioral disorder (aggressiveness, fatigability, poorer tolerance of physical exercise, increased appetite) is frequent [269,274]. Finally, the speed of the morphologic changes (ideally assessed using older photographs) can help clinical diagnosis.

10.3. Hormonal investigations for positive diagnosis of Cushing's syndrome in children

24 h UFC assay shows high diagnostic value, with 89% sensitivity and 100% specificity, in suspected Cushing's syndrome [275], and can be performed without hospital admission in older children. Sampling over 3 consecutive days improves performance [276]. Midnight cortisol shows very good sensitivity in children for diagnosing hypercortisolism at a threshold of > 4.4 µg/dL, but is difficult to implement in practice [277]. Blood or midnight saliva sampling can be used in children in whom 24 h urine sampling is not feasible.

In practice, UFC is sufficient for diagnosis. In case of doubt, dynamic testing may be used, with priority to overnight dexamethasone suppression test using a 15 µg/kg (max 1 mg) dose [278], by analogy with the procedure in adults, although there are no sensitivity or specificity values for the test in children.

More demanding dynamic tests are reported in the literature but little used in practice: the low-dose suppression test, with 8 doses of dexamethasone at 30 µg/kg/day (7.5 µg/kg per dose; maximum, 500 µg per dose), at 6-h intervals during 2 days. Serum cortisol 6 h after the last dexamethasone dose shows 94% sensitivity at 1.8 µg/dL (50 nmol/liter) [33,277].

10.4. Complementary exploration for etiologic diagnosis of Cushing's syndrome in children

ACTH < 5 pg/mL indicates adrenal Cushing's syndrome. If PPNAD is suspected, diagnosis is helped by findings of paradoxical cortisol/UFC elevation during the high dose dexamethasone suppression test (8 doses of dexamethasone 7.5 µg/kg/dose (maximum 0.5 mg/dose) administered every 6 hours followed by 8 at 30 µg/kg/dose (maximum 2 mg/dose) every 6 hours) [262].

To diagnose Cushing's disease, several tests can be used, none of which show perfect sensitivity or specificity. A morning ACTH threshold of > 29 pg/mL has 70% sensitivity, elevated midnight ACTH has 100% sensitivity [277] and a 20% drop in 8 o'clockcortisol from baseline after overnight high-dose dexamethasone (120 µg/kg at 11 pm, max. 8 mg) has 97.5% sensitivity [257].20% plasma cortisol elevation 30–45 minutes after CRH injection and 35% plasma ACTH elevation over baseline 15–30 minutes after CRH injection are diagnostic criteria for Cushing's disease[233]. Pituitary MRI should be performed if Cushing's disease is suspected, and shows 71% sensitivity with 43% NPV and 96% PPV [257,274]. Pediatric Cushing's disease is mainly associated with cor- ticotropic microadenoma, generally < 6 mm in diameter, generally hypointense on MRI and often non-enhanced by gadolinium. It is therefore necessary to perform thin-slice high-resolution MRI in a reference expert center for the diagnosis of Cushing's disease. Even so, depending on the report, MRI detects pituitary adenoma in only 16–71% of cases of pediatric Cushing's disease [274,279,280]. In case of intermediate ACTH concentration (5–29 pg/mL), adrenal CT and pituitary MRI should be associated [274].

In children, Cushing's syndrome may in extremely rare cases be of ectopic origin due to pulmonary, pancreatic or neuroendocrine CRH and/or ACTH hypersecretion [274,278,281]. If the causal tumor is unknown, findings of ACTH-dependent Cushing's syndrome, doubtful CRH test and normal pituitary MRI should be followed by extended imaging. In the peri-pubertal period, Cushing's syndrome by ectopic secretion remains rare and ACTH-dependent Cushing's syndrome, even with normal pituitary MRI, corresponds to Cushing's disease in the overwhelming majority of cases. This is why the diagnostic procedure may differ from that in adults. Thus, inferior petrosal sinus sampling, to differentiate Cushing's disease from ectopic forms, is rarely performed in pediatrics and its performance has been less well assessed. In adults, diagnostic failure of catheterization is partly due to technical issues, and the pediatricians involved in the present consensus statement considered this risk even greater in children. Catheterization is thus poorly contributive to etiologic diagnosis in pediatrics and should be used only exceptionally.

R9.1. Due to the rarity of Cushing's syndrome in children, we recommend that children with endogenous hypercortisolism suspected on biological exploration should be referred to an expert center for further management.

Expert opinion

R9.2. In case of suspected hypercortisolism, we recommended priority screening for any source of exogenous glucocorticoids. ++

R9.3. In case of suspected endogenous hypercortisolism, we recommended priority screening for:

- Cushing's disease in the peri-puberty period;
- adrenocortical adenoma in young children;
- McCune–Albright syndrome in the neonatal period. ++

R9.4. Cushing's syndrome should be screened in children and adolescents:

- at end of growth/puberty in case of classical clinical signs of hypercortisolism as in guidelines for adults;

- before end of growth/puberty in case of:
  - increased BMI associated with slowing growth,
  - signs of hyperandrogenism in under-8 year-olds or of severe hyperandrogenism at any age,
  - severe hypertension or iterative hyperglycemia without identifiable etiology in the neonatal period. ++

R9.5. We do not recommend screening for Cushing's syndrome in case of simple childhood obesity. ++

R9.6. Diagnosis of hypercortisolism in children with suggestive signs requires 24 h UFC assay when feasible and/or midnight cortisol (or cortisol cycle) assay in plasma (or saliva). ++

R9.7. In the peri-puberty period, in case of doubt, diagnosis should be confirmed by repeating UFC or midnight cortisol assay or overnight dexamethasone suppression test at 15 μg/kg (max 1 mg). ++

R9.8. In case of confirmed hypercortisolism, several 8 am ACTH assays should be performed.

Expert opinion

R9.9. ACTH < 5 pg/mL at 8 am confirms ACTH-independent Cushing's syndrome; adrenal CT should be performed. ++

R9.10. ACTH-dependent hypercortisolism (predominant in the peri-pubertal period) is confirmed by morning ACTH > 20 pg/mL, requiring pituitary MRI. +

R9.11. Ectopic ACTH-dependent Cushing's syndrome is exceptional in pediatrics, and we recommend considering ACTH-dependent Cushing's syndrome with normal pituitary MRI in the peri-pubertal period as Cushing's disease, unless proved otherwise. Exploration (CRH test, high dose dexamethasone suppression test and/or petrosal sinus sampling) are to be envisaged on a case-by-case basis. +

11. The genetics of Cushing's syndrome

11.1. Adrenal Cushing's syndrome with bilateral involvement

In Cushing's syndrome related to bilateral adrenal hyperplasia, the bilateral multifocal character suggests genetic predisposition. PPNAD is seen in 26–60% of cases of Carney complex and is isolated in about 12% of cases [282]. In about 70% of cases, Carney complex is related to mutations of the *PRKAR1A* gene coding for regulatory subunit R1α of protein kinase A. Primary bilateral macronodular adrenal hyperplasia (PBMAH) is related to a mutation of the ARMC5 gene in about 25% of sporadic cases and almost 80% of familial forms [283]. Index case patients with PBMAH due to *ARMC5* mutation showed more severe hypercortisolism and greater adrenal hyperplasia [284]. Association with meningioma was reported. Isolated PBMAH was reported in adults with amplification of *PRKACA* gene coding for protein kinase A catalytic subunit alpha [285]. Finally, PBMAH can be seen in syndromic diseases such as leiomyomatosis and hereditary renal cancer (*fumarate hydratase*), familial adenomatous polyposis coli (*APC*) or multiple endocrine neoplasia type 1 (*MEN1*) [286], but with low prevalence. Moreover, corti- solic adenoma or PBMAH is not a usual type of entry in thesediseases.

In very young children, the most frequent cause of adrenal Cushing's syndrome is McCune-Albright syndrome related to post-zygotic mutation of the *GNAS* gene coding for G protein subunit Gs [287]. Onset of neonatal Cushing's syndrome is associated with a particular form of nodular adrenal hyperplasia with zones of nodular hyperplasia and cortical atrophy and persistent fetal cells [288]. Finally, pediatric cases of Cushing's syndrome related to micro- or macronodular adrenal hyperplasia were reported in Beckman-Wiedemann syndrome with *PRKACA* amplification and *PDE8B* mutation.

Next-generation sequencing (NGS) is now preferred by most labs, identifying point mutations and larger rearrangements (described for *ARMC5*, *PRKACA*, *PRKAR1A*, *MEN1* and *APC*). If a candidate-gene approach fails to detect a genetic abnormality, a broader exome-type approach may be adopted, often after multidisciplinary team case discussion, to identify new causes of adrenal Cushing's syndrome.

11.2. Adrenal Cushing's syndrome by unilateral tumor

Unilateral cortisol-secreting adrenal tumor (adrenocortical adenoma) is mainly related to somatic changes. In 2020, genetic predisposition for cortisolic adenoma was reported exclusively in MEN type 1 and familial adenomatous polyposis. Cortisolic adenoma is rare in children, and may reveal MEN type 1. Early onset of adrenocortical tumors in children suggests Li–Fraumeni syndrome (mutations of *TP53*) [289].

11.3. Cushing's disease

Cushing's disease seems basically related to somatic abnormalities. It can, however, be found in certain syndromes such as MEN type 1 (1–2.5% of cases), or more rarely in MEN type 4 (*CDKN1B*) and yet more rarely in Carney complex [290–292]. In children, it is sometimes the first manifestation of the syndrome. It has also been associated with *AIP* gene mutation, in both adults and children, and is seen in 2.6–16% of cases of isolated familial pituitary adenoma [293,294]. *CABLES1* gene mutation was reported in 4 cases in the NIH series, but the prevalence of this mutation needs further confirmation.

The genetic investigation strategy is the same as for adrenal Cushing' syndrome, with initial NGS to explore the more frequent genetic causes, then discussion of a possible exome-type genome-wide approach.

R10.1. We recommend screening for a genetic cause of adrenal Cushing's syndrome in all children and adults presenting bilateral micro- or macronodular adrenal hyperplasia. ++

R10.2. We recommend a gene panel including the most frequently implicated gene:

- *ARMC5* in adults with macronodular adrenal hyperplasia;
- *GNAS* in children with neonatal Cushing's syndrome;
- *PRKAR1A* in children or adults with micronodular adrenal hyperplasia.

Choice of genes can also be guided by the syndromic context.

In second line, genome-wide study may be discussed (e.g., France Médecine Génomique 2025). +

R10.3. We recommend systematic screening for a genetic cause in case of unilateral adrenocortical tumor only in children. +

R10.4. We recommend screening for a genetic cause of Cushing's syndrome in case of:

- associated clinical manifestations suggesting a particular genetic syndrome;
- familial history of pituitary adenoma;
- corticotropic macroadenoma in patients < 30 years at diagnosis (TENGEN recommendation);
- corticotropic microadenoma in children (TENGEN recommendation). +

R10.5. We recommend a gene panel including at least the *MEN1* and *AIP* genes. Choice can also be guided by the syndromic context. +

R10.6. In second line, genome-wide study may be discussed (e.g., France Médecine Génomique 2025). +

12. Recurrence of Cushing's disease

12.1. Preoperative risk factors for recurrence, and follow-up of patients in remission after pituitary surgery

Transphenoidal surgery is the first-line treatment in Cush- ing's disease. Postoperative remission rates are globally 70–80%in the early postoperative period but longer-term remission is jeopardized by subsequent recurrence [295]. Long-term recurrence rates vary greatly between reports, due to patient heterogeneity, retrospective designs, variable follow-up and lack of a consen- sual definition of diagnostic criteria for recurrence. Two literature reviews in 2015 and 2020 reported the epidemiology of recurrence: Petersenn et al. collated 17 studies for 1376 patients in immediate remission following transsphenoid surgery [296]. The mean recurrence rate was 15% (n = 209), ranging between 11% and 47% on purely biochemical criteria and 5–21% on combined clinical and biochemical criteria. Mean time to recurrence was 51 months (range: 3–158 months). Ferriere et al. [297] collated25 studies reporting a mean rate of 18.5% at a mean 47 months (range: 3–345 months). In 2015, the Endocrine Society guidelines stressed the need to determine postoperative cortisolemia status, with prolonged follow-up [295], which was not put in ques- tion by recent studies. In case of corticotrope defiiciency, it was recommended to monitor 8 am cortisolemia (ahead of morning glucocorticoid replacement intake) and/or perform an ACTH analog stimulation test or insulin hypoglycemia to assess corticotropic axis recovery. Although studies indicated that some tests could predict recurrence risk, performances are insufficient to provide long-term certainty, thus requiring lifetime monitoring. Experts recommend screening for recurrence of Cushing's disease when the corticotropic axis recovers then annually or earlier in case of clinical manifestations, using Cushing's syndrome screening tests. Serum or nocturnal salivary cortisol provides one of the first indices of recurrence, ahead of UFC elevation [298,299]. Many patients progress toward significant hypercortisolism requiring complementary treatment, but it is not sure that there is any benefit in treating patients with mild early recurrence if they are asymptomatic.

The literature data are highly contradictory regarding preoperative recurrence risk factors; most are equally risk factors for immediate postoperative surgical failure. No studies assessed all parameters together on multivariate analysis. Younger age at diagnosis [300,301], tumor size [302,303], no visible MRI image of adenoma [303,304] and initial cortisolemia [305] were identified as recurrence risk factors in some studies, while others found no impact on final outcome. There seem to be no absolute preoperative risk factors for recurrence.

12.2. Postoperative risk factors for recurrence

Immediate postoperative corticotrope insufficiency not only indicated immediate remission status but was a protective factor against long-term recurrence in several studies [299,302,306–315], with 1.2–7-fold lower risk. However, postoperative eucortisolism is also, compatible with lasting remission [299,302,306,311,313]. Postoperative corticotrope insufficiency increases the chances of enduring remission, but there is no cortisolemia threshold below which recurrence can be ruled out in the long-term. The duration threshold for corticotrope deficiency ruling out long-term recurrence remains undetermined.

12.3. Postoperative dynamic desmopressin and CRH tests

Immediate postoperative (< 3–6 months) desmopressin response was a risk factor for long-term recurrence in sev- eral studies [312,315–322]. In a multicenter study of patients with positive preoperative desmopressin response and postoperative corticotrope insufficiency, abolition of the desmopressin response within 3 months postoperatively had a negative predictive value of nearly 94% (95% CI: 88–100) for recurrence over a mean 5-year follow-up [315]. Interpretation criteria, time to testing (immediate to 6 months) and test modalities (isolated or coupled to dexamethasone) varied between studies, precluding any consensual threshold. CRH-stimulated cortisol and ACTH levels in the first postoperative month were higher in patients who went on theshow long-term recurrence than in those with long-term remis- sion, but the overlap precluded determining a cut-off [321]. Other dynamic tests (CRH, dexamethasone-desmopressin) were also assessed during follow-up of patients successfully operated on for Cushing's disease. However, findings cannot easily be compared,as criteria differed (absolute values or percentages), definitions of recurrence varied (different biological markers, symptomatic or asymptomatic recurrence), comparison with a control group was not systematic, and follow-up was not long enough to exclude late recurrence.

Dynamic tests were also assessed for diagnosis of early recurrence during follow-up of patients successfully operated on for Cushing's disease. However, once again findings cannot easily be compared, as criteria differed (absolute values or percent- ages), definitions of recurrence varied (different biological markers, symptomatic or asymptomatic recurrence), comparison with a control group was not systematic, and follow-up was not long enough to exclude late recurrence; early positive response data included only patients showing recurrence, whereas tests may be positive without long-term recurrence; in several studies, positive desmopressin response preceded positive findings on the classical biomarkers of hypercortisolism by several months or sometimes years [299,312,322].

12.4. Diagnosis of recurrence

Diagnostic criteria for recurrence are not consensual. Clin- ical manifestations with reappearance of symptoms such as hypercatabolism, hypertension and diabetes or psychological manifestations occur too late to serve in isolation. Clinical assessment has to be combined with hormonal exploration, performed sequentially during follow-up, screening for incipient recurrence prior to onset of clinical signs. In case of postoperative corticotrope insufficiency, recovery of adrenal insufficiency raises the question of recovery of physiological corticotrope function or recurrence of Cushing's disease, requiring full hormonal work-up. Historically, the tests most often used were UFC and 1 mg suppression [296,323],but midnight salivary cortisol demonstrated good sensitivity and isnow widely used [324].

Studies showed UFC to be a marker that becomes positive late in case of recurrence, and is thus insufficiently sensitive for early diagnosis [325,326]; moreover, it shows inter-individual variations [327]. New assay techniques may be more sensitive, but remain to be assessed.

Late-night salivary cortisol was analyzed in 3 studies of recurrence of Cushing's disease [298,328,329]. The studies had limitations, but repeated measurement seemed to provide greater sensitivity than UFC.

Dexamethasone suppression, validated for diagnosis of Cushing's syndrome, has been little studied for specific diagnosis of

recurrence. The performance of the 1 mg test is considered satisfactory [325].

12.5. Procedure in case of early detection of recurrence

Treating biological recurrence when asymptomatic is controversial. It is well established that Cushing's syndrome should be actively treated once diagnosed and in case of failure of pituitary surgery [330], Extrapolation to asymptomatic biological recurrence is difficult. Diagnostic delay generally exceeds the interval between biological recurrence and clinical onset. There are only a few data on the impact of treatment for incipient recurrence. Carroll et al. reported clinical benefit in 7 out of 10 patients successfully treated for recurrence of Cushing's disease despite normal UFC [331].

R11.1. In the immediate postoperative phase, we recommend determining corticotrope insufficiency (low 8 am cortisolemia), eucortisolism (8 am cortisolemia and UFC within the normal range) or hypercortisolism. +

R11.2. After postsurgical remission of Cushing's disease, we recommend prolonged follow-up ($\geq$10 years) to screen for recurrence. ++

R11.3. There are no absolute preoperative criteria (age, adenoma size, image visible on MRI, hormone assays, etc.) defining recurrence risk profiles in corticotrope insufficiency or eucortisolism after pituitary surgery. +

R11.4. In the 3 postoperative months:

- corticotrope insufficiency is predictive of long-term remission, but does not rule out recurrence;
- eucortisolism is most often associated with risk of recurrence but is also compatible with lasting remission.

Postoperative cortisol level and duration of deficit influence the risk of recurrence, although no thresholds can be specified. ++

R11.5. In patients showing preoperative desmopressin response, we suggest repeating the test at 3–6 months postoperatively, to better estimate recurrence risk. ++

R11.6. In case of corticotrope insufficiency, we suggest performing just one 8 am cortisolemia assay (with or without Synacthene® test) to check corticotrope axis recovery. ++

R11.7. In case of eucortisolism and on recovery of the corticotrope axis, we suggest, as well as clinical assessment, 2 of the following 3 tests:

- $\geq$ 2 midnight salivary cortisol assays (although this may not be easily accessible);
- over-right dexamethasone suppression testing (although comparative data are lacking);
- 1 or 2 24 h UFC assays (despite late positivity), then, in the absence of clinical signs, repetition annually. ++

R11.8. Once biological recurrence has been diagnosed, we suggest that the risk/benefit ration of treatment be discussed in the multidisciplinary team meeting, especially if the patient shows no suggestive clinical symptomatology. ++

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