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Toward a Diagnostic Score in Cushing's Syndrome

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Toward a Diagnostic Score in Cushing's Syndrome

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Cushing's syndrome (CS) is a classical rare disease: it is often suspected in patients who do not have the disease; at the same time, it takes a mean of 3 years to diagnose CS in affected individuals. The main reason is the extreme rarity (1–3/million/year) in combination with the lack of a single lead symptom. CS has to be suspected when a combination of signs and symptoms is present, which together make up the characteristic phenotype of cortisol excess. Unusual fat distribution affecting the face, neck, and trunk; skin changes including plethora, acne, hirsutism, livid striae, and easy bruising; and signs of protein catabolism such as thinned and vulnerable skin, osteoporotic fractures, and proximal myopathy indicate the need for biochemical screening for CS. In contrast, common symptoms like hypertension, weight gain, or diabetes also occur quite frequently in the general population and *per se* do not justify biochemical testing. First-line screening tests include urinary free cortisol excretion, dexamethasone suppression testing, and late-night salivary cortisol measurements. All three tests have overall reasonable sensitivity and specificity, and first-line testing should be selected on the basis of the physiologic conditions of the patient, drug intake, and available laboratory quality control measures. Two normal test results usually exclude the presence of CS. Other tests and laboratory parameters like the high-dose dexamethasone suppression test, plasma ACTH, the CRH test, and the bilateral inferior petrosal sinus sampling are not part of the initial biochemical screening. As a general rule, biochemical screening should only be performed if the pre-test probability for CS is reasonably high. This article provides an overview about the current standard in the diagnosis of CS starting with clinical scores and screenings, the clinical signs, relevant differential diagnoses, the first-line biochemical screening, and ending with a few exceptional cases.

Keywords: Cushing's syndrome, hypercortisolism, diagnostic score, Cushing's disease, low-dose-dexamethasone-suppression-test, urine cortisol

INCIDENCE, EPIDEMIOLOGY, AND TIME TO DIAGNOSE CS

Cushing's syndrome (CS) is a rare disease leading to atypical and rare symptoms for age, but it can be associated with disease features that are quite common in the general population. The incidence of endogenous CS has been estimated to be 0.7–2.4 to 0.2–5.0 per million people per year (1, 2). About 80% of cases are ACTH-dependent, whereas 20% of Cushing cases are of primary adrenal,

ACTH-independent origin. ACTH-dependent CS can be further divided into pituitary-dependent CS and ectopic, paraneoplastic CS. While the latter is quite seldom, causing not more than 10% of cases, CRH-producing tumors account for even fewer cases (3). Because of the universal expression of the glucocorticoid receptor, the list of symptoms and clinical signs is long and lacks a clear focus. This, and the overlap with symptoms of the metabolic syndrome, makes diagnostics challenging.

The rareness of the disease, the broad symptomatology without a lead symptom, and the clinical overlap with features of the metabolic syndrome often results in a delayed diagnosis. Generally, it can take up to 4 years and longer from the beginning of disease symptoms to the actual diagnosis (4). On average, 4.6 physicians will have to be consulted according to one study (4). This is hardly acceptable, considering that CS is an endocrinological “emergency” associated with relevant morbidity and mortality. Mortality is highest in patients with severe hypercortisolism and persisting and ectopic CS (5), and a relevant number of patients die before or within 90 days after initiation of treatment (6). The diagnostic delay might be one of the reasons for the long-term negative aspects and comorbidities (7–9) and high morbidity in these patients even after achieving remission of the actual disease. We have recently analyzed in a systematic meta-analysis of 45 studies on 5,560 patients with CS time to diagnosis and found that it has remained unchanged with a mean time of 34 months (10). This clearly argues for considerations beyond established awareness campaigns. New approaches including automated face recognition, easy-to-apply scoring systems, or focused rational diagnostics will be instrumental to overcome the current challenges. In the following, we will give a critical overview of the current standard in the diagnosis of CS, mainly focusing on the prevalence of different clinical symptoms in patients with CS.

CLINICAL REASONING PROBLEMS

Solving clinical cases correctly (11)—but also efficiently (12)—is one of the great challenges in daily clinical practice. Diagnostic error rate in internal medicine is generally high (at least 10%) (13) due to a lack of knowledge (14), premature closure, and forgetting to consider other diagnoses (15). As *Clinical Reasoning* is mainly based on pattern recognition and illness script formation (16, 17), it is quite clear that diagnosing CS correctly is even more challenging than many other diseases: statistically, a family practitioner diagnoses between 0 and 1 patient with CS in his work-life. Even endocrinologists seldom diagnose patients with CS, apart from endocrinologists in specialized outpatient clinics. Therefore, as physicians will only evaluate a small number of true Cushing patients, it is difficult for them to develop the corresponding illness scripts to diagnose the disease in time. To foster the clinical reasoning process, different attempts have been made.

ATTEMPTS TO FASTEN THE DIAGNOSTIC PROCESS

As the pretest probability is generally low because of the rareness of CS, it is mandatory to preselect patients with an appropriate likelihood of endogenous CS and use laboratory tests with high sensitivity and specificity. Different strategies starting from clinical scores, face recognition, and ending with extended screening have been used in the past decades to improve differential diagnostics:

Clinical Scores for CS

As early as 1964, Nugent et al. developed a clinical score to identify patients with CS (18) correctly. To simplify the diagnostic process, he used a combination of clinical signs and symptoms to discriminate patients with hypercortisolism from patients without it. The following 19 signs are part of his scoring: osteoporosis, central obesity, generalized obesity (muscle) weakness, plethora, WBC (white blood cells), acne, red and purple striae, diastolic blood pressure, edema, hirsutism, ecchymoses, serum potassium, oligomenorrhea, headaches, MCV, abnormal glucose tolerance, and age. However, only half of the patients with CS can be correctly identified using this scoring, and therefore, the score was not adapted into clinical routine. Importantly, “Cushing symptoms”—and even the combination of these symptoms—occur quite frequently in the general population (see **Table 1**).

More recently, Leon-Justel et al. developed a risk scoring system based on a prospective multicenter screening of 353 patients. Thirteen university hospitals in Spain took part in the study. All these patients consulted an endocrinologist due to different medical problems but not with the suspicion of CS. Patients were included in the screening when they had at least two of the following symptoms: obesity, a poorly controlled blood pressure, an uncontrolled diabetes, virilization syndrome, or osteoporosis. For screening purposes, the late-night salivary cortisol and the low-dose dexamethasone suppression test (LDDST) were used. Finally, 26 patients with CS were identified among this cohort (7.4%). Diagnosis was confirmed by a full biochemical screening and follow-up. The clinical features of patients with CS and patients without it were compared. In a univariate analysis, three clinical signs were significantly associated with CS: muscular atrophy, osteoporosis, and dorsocervical fat pad. Two clinical signs—obesity and type 2 diabetes—were more common in patients without a CS than in patients with CS. Different statistical models were studied, leading to a final score with just three clinical signs and one laboratory parameter: osteoporosis, dorsocervical fat pad, muscular atrophy, and late-night salivary cortisol levels (47). Muscular atrophy is higher scored than the other two clinical parameters, while salivary cortisol is divided into low, medium, and high with different scores. The maximum is a score of 12 points. The final form was as follows:

Risk for CS = 2 × presence of osteoporosis (yes/no) + 2 × dorsocervical fat pad (yes/no) + 3 × muscular atrophy (yes/no) + late-night salivary cortisol (low = 0, medium = 4, high = 5). Using

TABLE 1 | Clinical symptoms in Cushing's syndrome [*Prevalence in Cushing's syndrome adapted from Valassi et al. (19), Sharma et al. (20), Feelders et al. (21), and Newell-Price et al. (2)] and in the general population.

Symptom	Prevalence in Cushing's syndrome*	Prevalence in the general population
OBJECTIVELY ASSESSABLE		
Recent weight gain	70–95%	Up to 50% (22)
Overweight	21–48%	50%
Obesity	32–41%	20%
Hypertension (blood pressure > 140/90 mm mercury)	58–85%	Up to 44% (23)
Abnormal glucose tolerance	21–64%	7.4–16.4% (24)
Diabetes mellitus	20–47%	Age-dependent; 2–22% (25)
Osteopenia	60–80%	Dependent on age, sex; up to 30% in postmenopausal women (26), quite seldom in young men and women
Osteoporosis	31–50%	
Osteoporotic fractures	Asymptomatic fractures up to 80%	
Proximal muscle weakness, myopathy	60–82%	Unclear, dependent on medication, neurological diseases, and alcohol abuse
Dyslipidemia	38–71%	22% (27)
Purple striae	78% (children)	In healthy men: 11% (28)
Hypercoagulopathy (hemostatic abnormalities)	54%	Variable dependent on diseases
Atherosclerotic changes	27–31%	25% (29)
Round face	81–90%	Unclear
Plethora	70–90%	Unclear
Buffalo hump	50%	Unclear
Acne	59% (children)	12% in women, 3% in men (30)
Hirsutism	56–75%	Unclear, dependent on other diseases and ethnic, up to 83% in PCOS (31)
Stroke	6%	Age- and sex-dependent, 32–99/100.000/year (32)
Myocardial infarction	2%	About 5% lifetime-risk (33)
Major depression	50–81%	10–20% (34)
Lethargy, depression, labile mood	36%, maybe up to 80% (35)	Up to 50% in elderly people (36)
Cognitive changes	Prevalence unclear, but cognitive impairments are frequent even over the long-term (37)	Unclear
Hypocampal atrophy	27% (38)	High in patients with dementia or Alzheimer's disease, unclear in the younger population
Thrombosis	Incidence: 2.5–14.6/1,000 persons/year	Incidence: 1/1,000; higher in older people (39)
Lung embolism	4%	0,5/1.000 (40)

(Continued)

TABLE 1 | Continued

Symptom	Prevalence in Cushing's syndrome*	Prevalence in the general population
Nephrolithiasis	20–50%	1–20% (dependent on country) (41)
Thin skin	37%	Unclear
Lack of vitamin D	Unclear, but at least as frequent as in the general population	40–100% (42)
Edema	Unclear	Dependent on disease
Decreased growth in children	70–80%	3%
Asymptomatic urinary tract infections	Unclear	Very common (43)
Sleeping disorder, fatigue	60%	Up to 25% (44)
Easy bruising	35–65%	Unclear
Decreased libido	24–80%	29% (women) (45)
Poor wound healing	Unclear	Unclear
Menstrual changes (women)	70–80%	Dependent on illness
Hair loss	31%	Up to 65% (46)

4 points as a cutoff, sensitivity of this score is 96% and specificity is 83% (47).

This score is quite promising, as false-negative results are rare. However, specificity is not sufficiently high, so that further tests have to be conducted to confirm or exclude the diagnosis. Additionally, it is not described if muscular atrophy and osteoporosis were assessed objectively (for example, by a densitometry of a hand-grip test for muscle strength) or just by the medical history.

Automated Face Recognition

Face classification was primarily used to identify patients with acromegaly, for example, in a study by Learned-Miller et al. in 2006 (48). In a more recent study, accuracy rates up to 72% were achieved. Especially in patients with less-severe diseases, software classification outperformed medical experts significantly (49).

Face classification was also already used to distinguish between patients with CS and patients with metabolic syndrome, but only exploratively in a small cohort (50). Automatic face classification can detect CS in 85% of patients correctly and excludes the disease in 95% correctly. However, further studies with BMI-matched controls are needed to evaluate the use of automatic face recognition in CS (51). In a recent follow-up study, we enrolled 82 patients (22 male, 60 female) and 98 control subjects (32 male, 66 female) rigidly matched by age, gender, and body mass index. The control group consisted of patients with initially suspected, but biochemically excluded CS. The images were analyzed using specialized computer vision and classification software. The overall correct classification rates were 46 and 81% for male patients and controls, and 57 and 65% for female patients and controls, respectively. This moderate diagnostic accuracy is probably related to the clinical

characteristics of the control group (“rule-out CS patients”) who have shown some signs of CS, whereas discrimination from a normal control group would have been more efficient (52).

Extended Screening Approach for CS

Another screening approach to shorten time to diagnosis has been based on the assumption that the disease may be prevalent in an early form in certain at-risk population, such as the metabolic syndrome. It was based on the rationale that the prevalence of CS is expected to be high and screening those patient cohorts might detect cases in an oligosymptomatic state allowing before evolution of full-blown phenotype. Typical patient cohorts screened were patients with type 2 diabetes, uncontrolled hypertension, osteoporosis, or advanced metabolic syndrome. This concept, initially promoted by data from small studies with selection bias, has been called into question since the incidence of CS was quite low in extended studies.

In unselected patients with diabetes mellitus type 2, CS is very rare. In a study by Reimondo et al., a group of 100 patients with newly diagnosed diabetes mellitus was screened for CS. One patient had biochemical evidence of hypercortisolism but without any clinical features (53). In a study by Mullan et al., 79 controls and 201 patients with diabetes were screened for CS. None of these patients was diagnosed with CS (54). In a large multicenter study in Italy, 813 patients with diabetes were screened. 0.6% (6 persons) had a hypercortisolism but only four of them were successfully cured from CS by surgery (55). In altogether seven studies, a prevalence between 0 and 3% was reported (56).

In two studies, the prevalence of CS among patients with hypertension has been analyzed: a study from the US identified CS in 0.5% of 4,429 patients (57). In a study from Japan, over 1,000 patients were screened and about 1% were diagnosed with CS (58).

Surprisingly, 10% of patients with obesity were diagnosed with CS in one study (59), but these results are probably not reliable as standard screening tests can be falsely positive in very obese patients (60). The results of screening studies among obese patients are very controversial, most likely due to different methodological flaws in these studies according to a systematic literature review by Tabarin and Perez (56). All in all, screening for CS in this group does not seem to be efficient.

However, there are two groups, in which screening for CS is actually reasonable: young patients with osteoporosis and patients with adrenal incidentaloma.

CS is quite common in patients with osteoporosis and vertebral fractures: about 5% of these patients have CS, mostly caused by an adrenal adenoma (61). In another study with 219 patients, 11% of patients with vertebral fractures and a *T* score lower than 2.5 suffered from CS (62). Patients with an adrenal incidentaloma are another group, where standardized screening for CS is endorsed by current guidelines, as on average 5% of the patients have an autonomous cortisol secretion (63), in some studies even up to 10% (64). Screening studies for patients with depression are not available.

In conclusion, widespread screening appears to be neither efficient nor indicated. The prevalence of the metabolic

TABLE 2 | States of physiologic hypercortisolism (66, 67).

Causes	Prevalence of the condition in target populations
• Pregnancy	–
• Depression	8.6% (34) in general population
• Alcohol dependence	4.7% (68) in general population
• Glucocorticoid resistance	Unclear, but probably quite seldom
• Obesity	20% (US) in general population, dependent on country (22)
• Diabetes mellitus	6.4% (estimated) in general population (25)
• Physical stress	Unclear
• Malnutrition	Up to 40% in hospitalized patients (69)
• CBG excess	Unclear

syndrome among US-Americans is 22%. This is far more often than CS, even if the prevalence of the disease might be underestimated (65). Therefore, testing every patient with metabolic syndrome is uneconomical and inefficient and would be associated with an unreasonable number of false-positive results. Finally, physiological forms of hypercortisolism are quite common in a variety of conditions and diseases. Most of them are far more common than CS (see **Table 2**). However, biochemical testing may be indistinguishable from true CS. This reinforces the paradigm that the confirmed diagnosis of Cushing's syndromes requires both a clinical phenotype and biochemical confirmation.

GUIDELINES FOR SCREENING

There are only few guidelines regarding CS. The US Endocrine Society Practice guideline recommends a restricted screening approach for CS in the following instances (66):

- Patients with many clinical signs and symptoms that are typical for CS should be further tested.
- Patients with symptoms that are unusual for their age should be screened.
- Exceptional cases are children with diminished growth and increasing weight. CS should be considered early to avoid short stature in those children.
- Additionally, patients with an adrenal incidentaloma that could be an adenoma should be screened.
- In all other patient groups, regular screening is not recommended.

CLINICAL SIGNS AND SYMPTOMS

Many symptoms can go along with CS. Some of them are very common but not very specific, while others are quite specific but not common. Upfront, it is essential to realize that neither the appearance of a single symptom can by itself prove the disease nor the lack of a particular sign can exclude it. In the clinical reasoning process, the entire clinical appearance should be taken into account. The duration and extent of

the disease influence the clinical symptomatology (2). Also, sex and age affect clinical presentation (70). That explains the different appearance of patients with the same disease. A few symptoms can be helpful to discriminate patients with metabolic syndrome from CS. Most of them are related to increased protein breakdown due to the underlying catabolic state in hypercortisolism: wide purple striae, plethora, proximal muscle weakness, easy bruising (without a cause), and osteoporosis (without any other reason) (21, 67). In particular, patients with severe CS suffer from proximal muscle weakness and purple striae.

CLINICAL REASONING SHOULD BE SIMPLIFIED ACCORDING TO THE FOLLOWING PRINCIPLES

There are symptoms and signs that are quite typical for patients with CS as they seldom occur in healthy patients or patients with different diseases:

- Careful inspection of the skin: whereas no patient with CS has all symptoms, it is extremely likely that there will be at least some of the following skin changes (purple striae, ecchymosis, thin fragile skin, rubeosis, hirsutism, acne, impaired wound healing) (2, 21, 67, 71). Purple striae: The prevalence of striae among patients with CS is unclear, but lower than 50%. Its appearance, however, is quite specific for this disease. Cushing striae are livid and at least 1 cm wide. White, reddish, and smaller striae can occur in patients with CS but are far more often caused by obesity, pregnancy, or a fast growth in childhood (72).
- Careful watch-out for signs and symptoms of protein catabolism: Proximal muscle weakness (60–82%) affects usually the muscles of the thighs; patients have problems with stair climbing or squats. As patients often do not discriminate between problems climbing stairs caused by dyspnea or muscle weakness, physicians have to ask these aspects quite specifically when taking the history (73). Other catabolic signs include atrophic thin skin and osteoporosis (see below).
- Plethora (70–90%) (21, 74): Plethora can also be caused by mitral stenosis and different dermatological diseases (such as rosacea or lupus, which generally produce distinct erythema). Patients should be asked whether the color of their skin has changed. Photos of the patient taken a few years ago can help to determine the beginning of the hypercortisolism.
- Atypical fat distribution: in addition to intra-abdominal fat accumulation, the patients have increased fat pads in the buccal area (“moon face”), the neck, the fossa supraclavicularis, and the upper back (“buffalo hump”).
- Atypical symptoms for a specific age can be a hint for Cushing's disease: this includes osteoporotic fractures in premenopausal women and men under 50 years (prevalence up to 80% in fluoride CS) (61, 75, 76) and early onset arterial hypertension in patients below 40 years of age, after exclusion of other conditions like hyperaldosteronism or renal arterial stenosis (58).

Some symptoms can be caused by hypercortisolism, but patients might not be aware of this and do not mention them spontaneously:

- Change of taste perception and sense of smell.
- Cognitive changes, including forgetfulness (38).

DIFFERENTIAL DIAGNOSIS OF CUSHING PHENOTYPES

All the clinical signs and symptoms mentioned above allow a few differential diagnoses—most of them are much more common than CS and should be considered and possibly excluded (see **Table 3**). Common differential diagnoses are a PCO syndrome in young women, the metabolic syndrome, and depression. It can be difficult to distinguish between PCO syndrome and CS. We recommend that young women should undergo a gynecological examination. Also, androstenedione, testosterone, and insulin should be measured. In doubt or in biochemical unclear situations, a short-term follow-up (exemplarily after 3 months) can be helpful to differentiate the diagnoses. Depression and diabetes mellitus can also cause mild hypercortisolism. In these cases, a sufficient therapy (for example, with an anti-depressive medication) is helpful to distinguish between diagnoses. Naturally, a careful medication history should be taken to avoid overlooking an exogenous CS.

GENERAL LAB RESULTS

A broad laboratory investigation should be conducted as a few changes in laboratory parameters can occur in patients with CS—frequencies unfortunately unclear—and can be a first hint for a hypercortisolism (see **Table 4**). Hypokalemia occurs in severe hypercortisolism (up to 100% in patients with ectopic CS, 5–10% in Cushing's disease) (77).

BIOCHEMICAL SCREENING FOR CS

The US Endocrine Society Practice guideline (66) recommends three tests for biochemical screening: the 1-mg LDDST, the 24-h urine cortisol (UFC) determination, and the late-night salivary cortisol measurement. All other screening methods, such as the 2-mg dexamethasone suppression test, the midnight serum cortisol, or the desmopressin stimulation test, either have a lower sensitivity or are challenging to perform correctly. Therefore, these methods are not recommended for initial screening purposes. Also, the measurement of basal morning serum cortisol or basal ACTH is not diagnostically conclusive because of the diurnal rhythm with physiologically high morning levels. Only in severe cases (for example in ectopic CS) are basal cortisol levels elevated on a regular basis.

TABLE 3 | Differential diagnosis of Cushing's signs and symptoms.

Symptom/Sign	Possible differential diagnosis (selection)
Increased visceral fat tissue, weight gain	<ul style="list-style-type: none"> Eating disorder Pregnancy Alcohol abuse PCO syndrome Seldom: genetic disorders
Hypertension	<ul style="list-style-type: none"> Hypothyroidism Edema Medication Insulinoma Essential hypertension Primary aldosteronism Pheochromocytoma-Parangangioma syndrome Renal artery stenosis Sleep apnea Renal hypertension Hyperthyroidism Acromegaly Medication
Impaired glucose tolerance, diabetes	<ul style="list-style-type: none"> Diabetes mellitus type 1 and 2 Gestational diabetes Chronic pancreatitis Medication Other endocrinological disorders (hyperthyroidism, acromegaly) PCO syndrome
Proximal muscle weakness, myopathy	<ul style="list-style-type: none"> Age (sarcopenia) Neurological disorders Alcohol abuse
Osteoporosis, osteopenia, fractures	<ul style="list-style-type: none"> Postmenopausal osteoporosis Senile osteoporosis Medication Mastocytosis Primary hyperparathyroidism Idiopathic Hyperthyroidism Malabsorption Lifestyle Hypogonadism Diabetes mellitus Familial forms Liver and renal diseases Medication Alcohol abuse Obesity
Dyslipidemia	<ul style="list-style-type: none"> Obesity Medication Idiopathic
Livid striae	<ul style="list-style-type: none"> Pregnancy Medication Idiopathic
Round face	<ul style="list-style-type: none"> Obesity
Plethora	<ul style="list-style-type: none"> Rosacea Mitral stenosis Lupus
Buffalo neck	<ul style="list-style-type: none"> Madelung Syndrome Obesity
Acne	<ul style="list-style-type: none"> PCO syndrome Idiopathic Medication
Hirsutism	<ul style="list-style-type: none"> PCO syndrome Medication Ovarian tumors Menopause Acromegaly Idiopathic Congenital adrenal hyperplasia Obesity Diabetes
Lethargy, depression, labile mood	<ul style="list-style-type: none"> Depression Other psychiatric disorders
Cognitive changes	<ul style="list-style-type: none"> Depression Dementia Age
Thrombosis, lung embolism	<ul style="list-style-type: none"> Provoked (e.g., immobilization) Oral contraception Genetic disorders Nicotine abuse
Nephrolithiasis	<ul style="list-style-type: none"> Medication Nutrition
Thin skin	<ul style="list-style-type: none"> Age Medication Diabetes mellitus
Edema	<ul style="list-style-type: none"> Heart insufficiency Renal insufficiency, other renal disorders Lymphedema
Sleeping disorder	<ul style="list-style-type: none"> Depression Physical and psychological stress Sleep apnea Hyperthyroidism Alcohol abuse
Decreased libido	<ul style="list-style-type: none"> Depression Physiological stress Relationship problems Alcohol consume Medication Hypothyroidism Adrenal insufficiency

(Continued)

TABLE 3 | Continued

Symptom/Sign	Possible differential diagnosis (selection)
Recurrent infections	<ul style="list-style-type: none"> Other immune-deficiencies HIV infection
Poor wound healing	<ul style="list-style-type: none"> Malnutrition Age PAVK Diabetes mellitus Medication Nicotine abuse Cancer Anemia
Menstrual changes	<ul style="list-style-type: none"> PCO syndrome Pregnancy Hypo- and hyperthyroidism Menopause AGS Medication Adrenal insufficiency Weight loss Prolactinoma Anorexia
Hair loss	<ul style="list-style-type: none"> Androgenic alopecia Divers dermatologic disorders Adrenal insufficiency Hyperparathyroidism Lupus Medication Malnutrition

TABLE 4 | Laboratory changes in patients with Cushing's syndrome.

Lab parameter	Differential diagnoses (selection)
Hypokalemia (77)	Primary hyperaldosteronism, medication, renal diseases
Low testosterone (78)	Medication, hypogonadism
Eosinopenia (79)	Infections, stress, medication
Hypercalciuria (80)	Hyperparathyroidism, vitamin D intoxication, bone metastasis, sarcoidosis, idiopathic, renal resorption disorders
Leucocytosis, lymphopenia (81)	(Chronic) Infections, HIV, leukemia
Erythrocytosis (82)	Hypoxemia, renal, paraneoplastic

SENSITIVITY AND SPECIFICITY OF BIOCHEMICAL SCREENING TESTS

The abovementioned three screening tests all have a comparable accuracy (93). At least two of the screening tests should be performed. When two of them show an abnormal test result AND the patient has a high pre-test probability by clinical presentation, the diagnosis can be confirmed. The sensitivity and the specificity of individual tests is highly depending on factors such as the cutoffs and the assays used. Generally, higher sensitivity can be reached by lower cutoffs, but decreasing specificity—and the other way around. Sensitivity and specificity are almost equal in all three screening tests (shown in **Table 5**). However, not every test is as useful as the others in all patients (see **Tables 6, 7**).

FALSE-POSITIVE AND FALSE-NEGATIVE BIOCHEMICAL TEST RESULTS

Low-Dose Dexamethasone Suppression Test

The LDDST can be falsely positive in women who take oral contraception. Up to 50% of these women will have a positive test result (3). The oral contraception causes an increased CBG, which influences the test results (84). In these women, the cortisol measurement in the urine is more helpful as CBG

TABLE 5 | Sensitivity and specificity of first-line screening [adapted from Nieman et al. (66) and Reimondo et al. (83)].

Tests	Sensitivity (%)	Specificity (%)	Cutoffs
Low-dose dexamethasone suppression test	80–95	80–95	Depended on assay
Urine free cortisol	45–71	Up to 100	
Late-night salivary cortisol	92–100	85–100	

TABLE 6 | Biochemical tests: causes for false-positive and false-negative results (20, 84–92).

Screening test	False positive	False negative
Low-dose dexamethasone suppression test	Oral estrogens, increased CBG, medication, rapid metabolizer, lack of resorption of dexamethasone	Liver or renal failure
Urine free cortisol	Depended on the assay, volume over 5 L, medications	Dependent on the assay, GFR low, improper collection
Salivary cortisol	Age, hypertension, diabetes, dependent on the assay, depression, shift workers, smokers, stress, maybe blood	-

levels do not affect it. Otherwise, oral contraception should be paused at least 1 week but ideally for 3 months. Mitotane also increases CBG levels and may lead to incorrect test results. Other medications can influence the test results by induction or inhibition of CYP 3A4. Aprepitant, itraconazole, ritonavir, and diltiazem, for example, slow down dexamethasone metabolism (66). Phenobarbital, phenytoin, carbamazepine, rifampicin, and pioglitazone, however, will increase dexamethasone metabolism (66). Additionally, individual and variable absorption and metabolism of dexamethasone has become recently a focus and will have an impact on test results. A measurement of dexamethasone levels in spot urine or plasma can be helpful to evaluate the validity of adequate resorption and metabolism (94). Finally, liver or renal insufficiency may lead to reduced dexamethasone clearance, which could cause a false-negative test result.

Urine Free Cortisol (UFC)

The UFC has been used for over 50 years to diagnose CS (85). Unlike plasma cortisol, CBG levels do not affect the results as the free hormone is measured and not the CBG bound form. Therefore, UFC is the preferred test for women taking oral contraceptives. Nevertheless, different conditions can also lead to false-positive and -negative test results. A fluid intake of over 5 L per day increases cortisol levels (95), while an incomplete collection of the urine may cause false-negative test results. Also, the selection of urine is not useful in patients with chronic renal insufficiency, as UFC levels decrease with a decreasing creatinine clearance (86). Similar to the LDDST, medication can influence the test results. For example, carbamazepine, fenofibrate, and

TABLE 7 | Which screening method should be performed in which patient? [adapted from Nieman et al. (66)].

Patient group	Recommended methods	False-positive results	False-negative results
Patients with renal impairment	LDDST, salivary cortisol	-	UFC
Women under contraception	UFC	LDDST	-
Smokers	UFC, LDDST	Salivary cortisol	-
Mild or cyclic Cushing	Repeated measurements of UFC and/or salivary	-	LDDST
Adrenal Cushing	Salivary cortisol, LDDST	-	UFC
Pregnancy	UFC	-	LDDST
Patients with epilepsy	UFC, salivary cortisol	LDDST	-

synthetic glucocorticoids increase UFC results. Because of day-to-day fluctuations, current guidelines suggest collection of at least two 24-h urines.

Depending on calibration characteristics or cross-reactivity of first antibody with other steroids, commercial assays can both lead to false-positive and false-negative test results (83–85, 87).

Late-Night Salivary Cortisol

The sensitivity and specificity of salivary cortisol are described in the literature as quite high, but they are very much influenced by the methodology. Specificity varies between 85 and 100% depending on assay characteristics and technique. Hypertension and diabetes can cause elevated cortisol levels in up to 40% (88) as well as depression (89). Shift working may also be a reason for an impaired circadian rhythm. Smoking leads to elevated cortisol levels in saliva and should be avoided on the day of collection (90). Bleeding or oral infections can also influence the test results. Therefore, teeth brushing (which might lead to blood leaks) should be avoided before sampling the salivary. Because of a higher rate of test variability, collection of two late-night salivary cortisol samples is recommended.

Assay Problems

To make matters worse, the test results depend on the specific assays used to measure hormone concentrations. Basal cortisol (96), ACTH (97), and urine cortisol (98) levels diverge by up to 50% depending on the assays employed, heavily influencing classification of patients. Also, salivary cortisol levels are not comparable between assays from different manufacturers (99). Especially in women on oral contraception, different assays can result in highly discrepant results (100). Cortisol levels from different laboratories are only comparable if the same assay is used. Accordingly, recommended cutoffs and decision limits need to be defined in an assay-specific manner. Exemplarily, cortisol can be measured using immunoassays, gas chromatography mass spectrometry (GC-MS), or liquid chromatography mass spectrometry (LC-MS). Cortisol levels as measured by the latter two methods commonly are reported

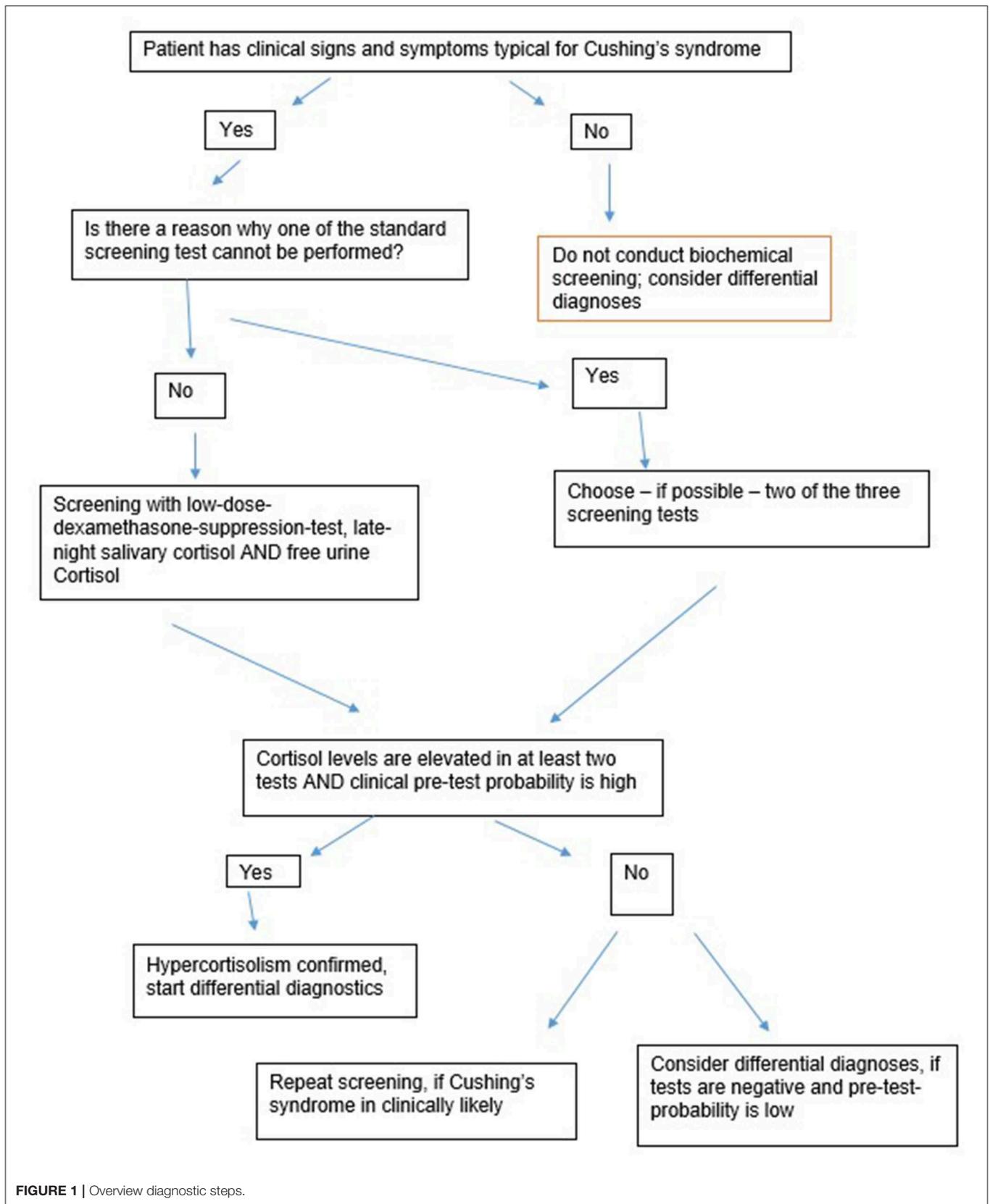


FIGURE 1 | Overview diagnostic steps.

to be lower (101) than those obtained by immunoassays. One reason is that immunoassays, particularly direct ones, can suffer from cross-reactions and thus have less specificity than the other two methods (101). The resulting over- or underestimation of cortisol concentrations can be a serious problem (96). Nevertheless, in clinical routine, immunoassays still are the most frequently used method (101). The same problems apply to the use of salivary cortisol, where concentrations measured by LC-MS and immunoassays exhibit poor agreement (92, 101).

SUMMARY OF THE FIRST-LINE SCREENING

Figure 1 gives an overview about the first-line-screening-steps. The following rules are essential regarding the first-line-screening:

1. Biochemical screening should only be conducted when the patient has clinical features suggesting CS.
2. At least two tests should be conducted.
3. False-negative and false-positive results are likely. A positive test result is therefore not a proof for a CS.
4. When in doubt, biochemical screening should be repeated.

EXCEPTIONAL CASES: DIAGNOSTICS IN MILD AND EPISODIC CS

Severe hypercortisolism can be relatively easily detected using the tests mentioned above. Mild or cyclic CS, however, requires a different procedure. Recent studies suggest that mild or episodic hypercortisolism is more frequent than assumed (102). In these cases, the clinical signs or symptoms of the patient are even more critical. If clinically a CS is likely, a single negative test should not lead to the exclusion of an endogenous hypercortisolism. Short-term follow-ups are essential to confirm or exclude the diagnosis. Diagnosing patients with just a few—or maybe even one—“Cushing symptoms” can be challenging as well. It is important to consider CS as a possible differential diagnosis in young patients with osteoporosis, hypertension, or even mental disorders that cannot be explained by anything else.

Just a few studies focused on cyclic CS so far. Generally, repeating the sampling of urine or salivary is recommended while the LDDST is less useful (102, 103). We recommend to carefully take a history in these patients to investigate whether they can identify a time pattern in their symptoms. Additionally, as it can be easily conducted at home, these patients should collect salivary samples or urine over a longer time period. Also, measuring cortisol in the hair has been advocated to detect cyclic CS (104). This assay is currently not commercially available.

NEW APPROACHES: PLASMA STEROID METABOLOME PROFILING

Using LC-MS, we recently explored the diagnostic accuracy of steroid profiling of basal plasma samples from patients

with CS. A panel of 15 plasma steroids were applied to 222 patient samples tested for CS. Disease was excluded in 138 and confirmed in 51 patients with pituitary CS, 12 with ectopic adrenocorticotropin secretion, and 21 with adrenal CS. We identified distinct steroid profiles among patients with CS that separated patients from healthy controls. Moreover, classification into the three Cushing subtypes was achieved with reasonable accuracy. With the use of 10 selected steroids, subjects with and without different CS subtypes could be discriminated nearly as good as with the use of a combination of the three conventional screening tests and plasma ACTH (misclassification of 9.5% of subjects with steroid profiling vs. 5.8% with conventional testing) (105). In another study, we demonstrated that the 15-plasma-steroid panel was able to discriminate 35 patients with subclinical CS from 21 with overt clinical CS and normal controls. Patients with subclinical CS had lower plasma concentrations of dehydroepiandrosterone and dehydroepiandrosterone-sulfate than normal subjects, but higher levels of 11-deoxycortisol and 11-deoxycorticosterone. The steroid combination provided superior diagnostic performance compared to each of the other routine biochemical tests (106). These data are in line with recent data obtained in patients with primary aldosteronism (107) and could pave the way to simplified biochemical confirmation of steroid excess syndromes if confirmed by other studies.

CONCLUSION AND OUTLOOK

Diagnosing CS is not straightforward: it remains challenging to preselect patients with an increased likelihood of CS and to select the best biochemical screening test for a given patient. Undoubtedly, current clinical and biochemical screening approaches are far from ideal, and simplified procedures are required from both a patient's and a physician's perspective. The clinical score by Leon-Justel could be a good starting point but it should be validated in other patient cohorts. Other laboratory tests like plasma steroid profiling are promising approaches to reduce the number of diagnostic tests (105).

To conclude, we suggest a rational clinical score for patients with CS: based on the scores by Leon-Justel and Nugent and the results from different screening studies, the following clinical signs should be included in a clinical score: osteoporosis, muscular atrophy [objectively assessed by a combination of the chair rising test, hand-grip measurement, and the bioelectrical impedance measurement (8)], livid striae, plethora, ecchymoses, and dorsocervical fat pat. Such a clinical score should be validated comparing a cohort of patients with CS and patients with an initial clinical suspicion for hypercortisolism in which CS was excluded.

AUTHOR CONTRIBUTIONS

LB and MR have written the first draft of the manuscript. AR, AO-K, JF, GR, MB, and FB substantially contributed to the revision of the manuscript. All authors read and approved the final version for submission.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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