Subtyping of Cushing's Syndrome: A Step Ahead

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ABSTRACT

Cushing's Syndrome (CS) is a rare disease due to chronic endogenous cortisol secretion. In recent years, new developments have broadened the spectrum of differential diagnosis, traditionally categorized as adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent forms. Moreover, increased awareness of the detrimental effects of cortisol on cardiometabolic health and the risk of cardiovascular events lead to increased diagnosis of mild forms, especially in the context of adrenal incidentalomas.

This review provides an up-to-date narrative of the most recent literature regarding the challenges of CS diagnosis. After the description of the diagnostic tools available, the functional non-neoplastic hypercortisolism (formerly known as pseudo-Cushing state) is characterized, followed by the subtyping of the different conditions of hypercortisolism, including the differential diagnosis of ACTH-dependent forms and the management of adrenal hypercortisolism, with peculiar attention to the new genetic classification of adrenal CS, mild autonomous cortisol secretion, and bilateral adrenal adenomas.

Introduction

Cushing's syndrome (CS) is a rare disease caused by chronic exposure to inappropriately high levels of glucocorticoids. The clinical phenotype of CS is characterized by a wide spectrum of severity that varies from mild to severe presentation. If untreated, CS is potentially fatal, given the high incidence of cardiovascular events and opportunistic infections. One of the major goals of precision medicine is a correct and early diagnosis [1, 2]. According to the pathogenic mechanism, endogenous CS is traditionally classified into adrenocorticotropic hormone (ACTH)-dependent and ACTH-independent forms (respectively 70-80 % and 20-30 % of CS); in the latter ACTH is suppressed and cortisol is secreted directly from the adrenals [3, 4].

ACTH-dependent hypercortisolism is further classified into a pituitary source of ACTH secretion (from a corticotroph adenoma or hyperplasia), defined as Cushing's disease (CD), or ectopic ACTH secretion (EAS) characterized by the paraneoplastic corticotropin secretion in a neuroendocrine tumor (NET). ACTH-independent forms are primarily caused by unilateral adrenal cortisol hypersecretion and bilateral adrenal hypersecretion due to primary bilateral macronodular adrenal hypercortisolism (PBMAH) or primary pigmented nodular adrenocortical disease (PPNAD). The term PBMAH has been proposed because the dogma of "ACTH-independent" cortisol secretion has been questioned after the discovery of adrenal ACTH synthesis and paracrine ACTH-cortisol stimulation [5].

Furthermore, in some cases, non-neoplastic hypercortisolism (NNH, previously known as pseudo-Cushing) should be considered

in the differential diagnosis of hypercortisolism before the characterization of ACTH, especially in milder forms. In clinical practice, peculiar clinical features of hypercortisolism in some patients are commonly suspected to be secondary to a sustained or intermittent activation of the hypothalamic-pituitary-adrenal (HPA) axis caused by psychological diseases (major depression, eating disorders), metabolic conditions (obesity, polycystic ovary syndrome, poorly controlled diabetes mellitus), as well as physical (starvation/chronic intense exercise) or chemical (alcohol) stimuli [6, 7]. In these cases of NNH, the HPA activation is not secondary to neoplasia; nonetheless, their resulting biochemical features after first-line screening tests can be indistinguishable from neoplastic hypercortisolism.

After the exclusion of exogenous glucocorticoids for therapeutic purposes, the initial diagnosis of endogenous hypercortisolism is often challenging because many other common conditions (metabolic syndrome, osteoporosis, depression) overlap with CS in their clinical presentation. The clinical practice guidelines of the Endocrine Society and recent consensus of the Pituitary Society recommend CS screening with three first-line tests: 24-h urinary free cortisol (UFC), serum cortisol after 1-mg overnight dexamethasone suppression test (DST), and late-night salivary cortisol (LNSC) [1, 8]. All these tests present a high diagnostic accuracy to detect CS [8]; however, UFC is the less sensitive, especially in a mild form of hypercortisolism (as in the case of recurrent CD after surgery [9]). On the contrary, a false positive result of a screening test must be considered because it can capture the NNH form [10].

Therapy is based mostly on surgical options (in case of pituitary or adrenal adenoma, as well as in those localized NET with EAS) in addition to medical treatment (steroidogenesis inhibitors could be used in all forms of CS, pituitary-directed drugs only for CD) [11]. Therefore, when overt CS is established, the current differential diagnosis among subtypes of CS is of utmost importance. A correct diagnosis is the crucial crossroad in the pathway of CS and should never be delayed, except in those rare cases of extremely severe presentation of hypercortisolism (such as sepsis, fungal infection, pulmonary embolism, malignant hypertension, and acute psychosis) when the prompt reduction of cortisol excess with medical therapy and management of comorbidities is mandatory, but ideally should not delay the diagnostic process [12].

In our narrative review, we will discuss the most challenging situations in the diagnosis of hypercortisolism, from neoplastic CS (differential diagnosis of ACTH dependent and adrenal hypercortisolism) to non-neoplastic forms.

Tools available for the subtyping of different forms of Cushing's syndrome

The differential diagnosis of CS may be challenging. Many strategies have been proposed, but no single test reaches 100 % of diagnostic accuracy alone. Therefore, in clinical practice, the combination of at least two tools is used to define the subtyping of CS (as the combination of imaging and dynamic tests). The diagnostic tools available are mentioned in ▶ **Table 1**. After the exclusion of NNH (reassumed in a dedicated paragraph), the first step in patients with neoplastic hypercortisolism is to differentiate between ACTH-dependent and independent forms (▶ **Fig. 1**).

Morning unstimulated adrenocorticotropic hormone

Morning ACTH concentrations below detection levels or 10 pg/mL (2 pmol/L), combined with normal or elevated cortisol levels, suggest adrenal hypercortisolism [13]. Immediately after collection, blood should be stored in chilled tubes containing ethylenediaminetetraacetic acid, placed inside a container with ice, and quickly transported to the laboratory for ACTH measurement, as ACTH is rapidly degraded by plasma protease. However, ACTH may not be entirely suppressed in some patients with adrenal CS with mild or intermittent cortisol secretion; 11 % of cyclic forms are of adrenal origin [14]. ACTH levels > 20 pg/mL (4 pmol/L) indicate an ACTH-dependent CS. Commercially available ACTH immunoassays may be imprecise in patients with normal-low ACTH levels, leading to pre-analytical errors [15]; therefore, we suggest a corticotropin-releasing hormone (CRH) test to check neuroendocrine responsiveness and exclude ACTH-independent CS [16].

Dynamic tests

Blood samples for ACTH and cortisol levels are collected before (usually twice in 15 min) and 15, 30, 45, 60, 90, and 120 min after intravenous bolus injection of 100 µg ovine/synthetic human CRH. The diagnostic accuracy of ovine CRH seems superior to that of the human peptide [17]; nonetheless, the recent relative shortage of human CRH requires additional considerations [18]. The majority of corticotropinomas and a small number of NETs respond to synthetic CRH, with increased ACTH and cortisol concentrations. There is no consensus about the interpretation criteria, which vary according to the type of parameter considered (35-50 % increase in ACTH or 14-20 % increase in cortisol, compared to baseline). According to the literature, the sensitivity and specificity of ACTH peak are around 90 %. Ritzel et al. [19] reported that the duration of CRH test can be shortened: ACTH rise ≥ 43 % 15 min after CRH injection is the strongest predictor for CD, with a sensitivity of 83 % and a specificity of 94 %. A study in a larger series confirmed that a test duration longer than 1 h did not improve diagnostic performance [20]. We recently reported that an ACTH and cortisol increase of > 31 % or 20 % after human CRH, respectively, resulted in 91-86 % of sensitivity and 80 % of specificity [16].

Desmopressin is a long-acting arginine-vasopressin analog, which binds preferentially to V2 and V3 receptors, whose expression is increased in ACTH-secreting pituitary adenomas. The intravenous administration of 10 µg desmopressin stimulates an exaggerated ACTH or cortisol response in 80-90 % of patients with CD; nonetheless, those without ACTH-secreting tumors usually have a minimal (or absent in case of dexamethasone suppression) ACTH and cortisol response to desmopressin, suggesting NNH [6]. ACTH and cortisol are measured during the CRH test. The test is easily available, inexpensive, and can be useful also in the post-operative evaluation of corticotropinoma's recurrence [21]. Its application in the differential diagnosis of ACTH-dependent CS is, however, limited: 20-50 % of NETs express vasopressin receptors as well, and, therefore, can respond to desmopressin.

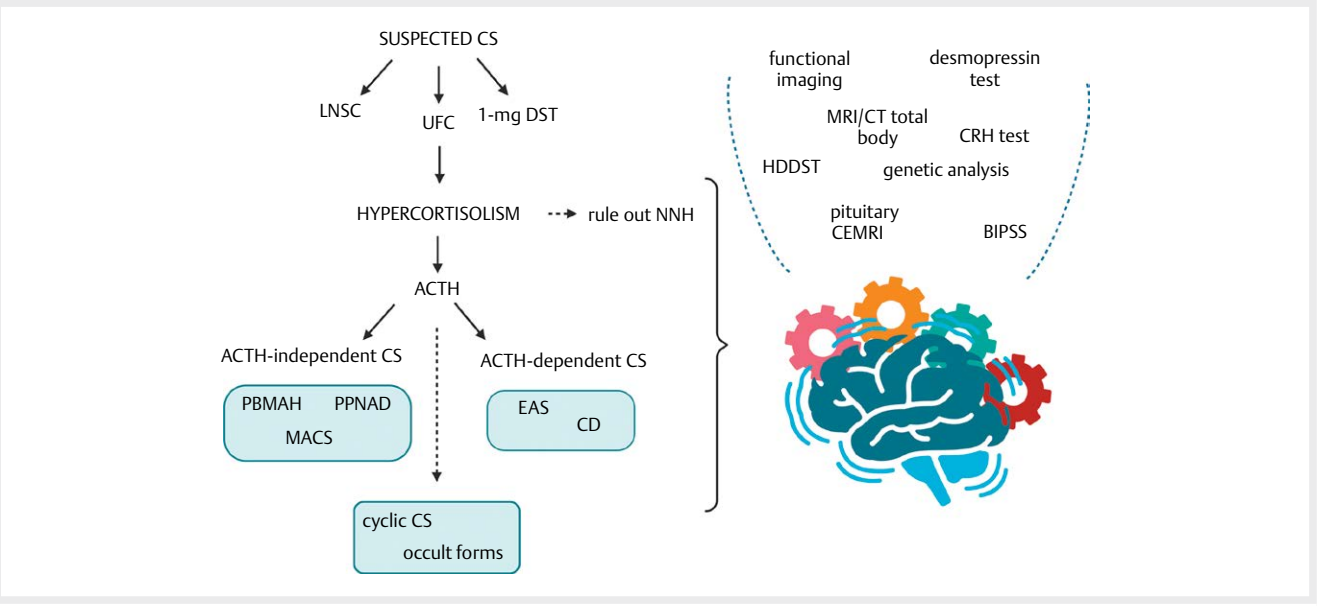
High dexamethasone (HD) concentrations can partially suppress ACTH secretion of most corticotroph adenomas with consequent reduction of cortisol levels. NET, on the contrary, are usually resistant to this negative feedback. It is the most reported test in literature (3057 tests in 43 studies, followed by the CRH test with 1715

▶ **Table 1** Diagnostic tools for the differential diagnosis of endogenous hypercortisolism (references in brackets).

Test	Suspected condition	Expected results for the suspected condition	Remarks (references in brackets)
CRH test	CD	Positive response: ACTH and/or cortisol response above baseline	▪ CRH shortage [18] ▪ presents the highest diagnostic accuracy to differentiate CD and EAS [22] ▪ Macro-corticotropinomas usually do not respond [52] ▪ No standardized cut-off
	EAS	Negative response	▪ Well-differentiated NET can respond [37–38]
	PBMAH	Negative response (ACTH always suppressed)	▪ Positive ACTH response predicts positive outcome of unilateral adrenalectomy [84]
	NNH	No ACTH or cortisol response (after dexamethasone suppression)	▪ Not standardized [51]
HDDST	CD	Positive response: cortisol suppression	▪ Overnight test with serum cortisol in Western countries [22] ▪ Dexamethasone levels ▪ Concerns: high dexamethasone levels can exacerbate hypertension, diabetes, psychosis ▪ No standardized cut-off
	EAS	Negative response	▪ Well-differentiated NET can suppress cortisol [37]
	PPNAD	Paradoxical urinary cortisol rise	
Desmopressin test	CD	Positive: ACTH and/or cortisol response above baseline	▪ Test with low diagnostic accuracy ▪ The position of the test should be evaluated: CRH shortage and HDDST concerns ▪ No standardized cut-off
	EAS	Negative	▪ Well-differentiated NET can respond
	NNH	No ACTH or cortisol response	▪ Easy and inexpensive
Pituitary CEMRI	CD	Adenoma detection	▪ 65 % CD with < 6 mm adenoma, 27 % negative ▪ High magnetic field ▪ Not standardized protocol of acquisition
	EAS	Negative	▪ Pituitary incidentaloma
	NNH	Negative	▪ Pituitary incidentaloma
BIPSS	CD	Central to peripheral gradient (at baseline and after stimulation)	▪ Invasive procedure ▪ Desmopressin stimulation if CRH shortage
	EAS	No gradient	▪ Consider rare cerebral NET that drain in the cavernous sinus
Conventional imaging	EAS		▪ Risk of occult EAS in 22 % after long-term follow-up [38]
	Adrenal CS	Single adenoma, bilateral macronodular or micronodular hyperplasia	▪ Consider ACTH-dependent bilateral enlargement ▪ Rule out those rare bilateral malignancies (adrenal cancer) ▪ Differential diagnosis between MACS and overt CS
Functional imaging	EAS	Positive 18-FDG or 68Ga-derivate uptakes	▪ High risk of false positives [43]
	PBMAH	Unilateral adrenalectomy in case of differential uptake	
Genetic evaluation	PBMAH	*ARMC5* or *KDM1A* mutation	▪ A large number of bilateral incidentalomas with MACS (especially considering ARMC5)
	PPNAD	PRKR1A	

ACTH, adrenocorticotropic hormone; CD, Cushing's disease; CS, Cushing's syndrome; EAS, ectopic ACTH secretion; NET, neuroendocrine tumor; MACS, mild autonomous cortisol secretion; PBMAH, primary bilateral macronodular adrenal hypercortisolism; NNH, non-neoplastic hypercortisolism; PPNAD, primary pigmented nodular adrenocortical disease.

patients and desmopressin with 1038 tests) [22]. There are different versions of the high-dexamethasone suppression test (HDDST): 8 mg of dexamethasone overnight and serum cortisol measurement is the most used in Western countries; on the contrary, 2 mg of dexamethasone every 6 h (for eight doses) with urinary cortisol is the most used in the Eastern world. Overnight HDDST comprises oral administration of 8 mg of dexamethasone between 23:00 and 24.00 and measurement of plasma cortisol at 8:00 the next morning. Using a cut-off for cortisol suppression > 80 % compared to the baseline levels, the sensitivity and specificity in differentiating pituitary disease from ectopic form varies between 60-90 %. The measurement of dexamethasone levels [23], available only in selected referral centers, may enhance the diagnostic accuracy of HDDST. The relative shortage of human CRH, combined with the



▶ **Fig. 1** Diagnostic flow chart of Cushing syndrome subtyping. After first-line screening tests, characterized by high evidence, none of the tools proposed on the right half of the panel is able to alone confirm the final diagnosis of hypercortisolism. Therefore, a skillful clinical interpretation is required to perform a correct diagnosis. Created with Biorender.com [rerif].

low specificity of the desmopressin test, can improve the role of HDDST in the differential diagnosis of ACTH-dependent hypercortisolism.

Bilateral Inferior Petrosal Sinus Sampling (BIPSS)

During BIPSS, the ACTH gradient between the right and left petrosal sinuses and a peripheral vein is assessed. Being an invasive procedure, we suggest that its use should be limited to patients with ACTH-dependent CS whose clinical, biochemical, and imaging data are discordant or doubtful [24], conversely to other authors that consider the sampling in all patients with ACTH-dependent form or in those with a pituitary lesion < 6 mm [25, 26]. An unstimulated gradient between the central and peripheral ACTH > 2, or > 3 after CRH or desmopressin, indicates CD. The reliability of BIPSS in localizing the left/right side of the adenoma within the pituitary gland is poor; a recent meta-analysis reported 69 % correct tumor lateralization with BIPSS, 53 % concordant with magnetic resonance, without association with postoperative remission [27].

The sensitivity of BIPSS for the diagnosis of CD is very high, either at baseline or after stimulation [28]. False-negative results include corticotroph adenomas with poor responsiveness to CRH, cyclic CS, or anomalous venous drainage. Occasional false positive results are reported in cerebral NET with venous drainage into the cavernous sinuses. Common minor complications after BIPSS are hematomas in the site of vascular access; significant adverse events are rare, including deep venous thrombosis, pulmonary embolism, and brain injury [29]. To avoid pitfalls in results interpretation, cortisol-lowering medications should be withdrawn at least 48-72 h before the procedure and overt hypercortisolism must be assured before BIPSS [30].

Desmopressin is an alternative to CRH during BIPSS [31], with the same procedure and diagnostic accuracy; however, it is less expensive [32]. Nonetheless, desmopressin is a pro-coagulant agent; its use during BIPSS needs extreme caution given the increased risk of thromboembolic events in CS [33]. Moreover, the number of patients with EAS evaluated after desmopressin-stimulated BIPSS is relatively small, making it difficult to calculate the effective specificity of the test.

Recently, studies have reported that the simultaneous measurement of prolactin might further improve the diagnostic performance of human CRH-stimulated BIPSS. The normalized ACTH to prolactin ratio was able to correctly detect ACTH source in 31 out of 32 confirmed ACTH-dependent CS [34].

Imaging

A contrast-enhanced magnetic resonance imaging (CEMRI) with gadolinium should be performed in all patients with ACTH-dependent CS [26]. CEMRI reveals a pituitary adenoma in up to 70 % of patients [35, 36]. In authors' opinion, the presence of a focal lesion (at least 2-3 mm) on CEMRI in a patient with a characteristic clinical presentation and concordant biochemical data in defining the ACTH-dependent form after dynamic tests suggests a definitive diagnosis and does not require any further investigation (as BIPSS) [35]. A microadenoma is hypointense and sometimes isointense compared to the surrounding normal tissue on a T1-weighted image without a contrast agent. After administration of gadolinium the lesion appears still hypointense because it is less vascularized compared to normal pituitary. Unilateral elevation of the sellar diaphragm or pituitary stalk deviation are indirect radiological signs that may indicate the presence of a microadenoma.

If the BIPSS does not show a significant ACTH gradient between the lower sinus and periphery, a total-body computed tomography (CT) is recommended in the suspicion of an EAS. Although the thorax is the most likely site of ACTH-secreting tumors [37–39], the

identification of these lesions is often difficult and they are detected only in 65 % of cases during the first assessment. Nuclear medicine improves the sensitivity of conventional radiology when the NET is not identified; however, its high diagnostic accuracy (75 % of patients with initial occult EAS with conventional imaging) is not confirmed in large series. A positive octreoscan was described in 67 % of CS (50 patients) and positive 18FDG-PET in 60 % (32 patients) [40]. There was no single diagnostic imaging technique with optimal accuracy [40]; in 23 % of EAS the NET can remain occult for years after the diagnosis of CS [38], and in 22 % of EAS, the detection of the primary tumor occurred in median 16 months after the diagnosis of hypercortisolism (defined as covert EAS) [37]. PET/CT using ^{68}Ga-conjugated somatostatin receptor targeting peptide (^{68}Ga-SSTR-PET/CT) reported by Goroshi et al. in 12 patients was useful in increasing the specificity of the suggestive CT-positive lesions [41] (in other words, to confirm the neuroendocrine origin and suggest ACTH secretion in a CT-detected node). In their series, the number of positive CT scans was higher than that of nuclear imaging. Similarly, Wannachalee et al. reported that 68Ga-SSTR-PET/CT is sensitive to detect primary and metastatic neoplasms in EAS, achieving a significant clinical impact in the diagnostic-therapeutic management of patients in 65 % of cases [42]. A careful interpretation of nuclear imaging with ^{68}Ga derivates is of utmost importance, because PET/CT presents a considerable number of indeterminate/false positive images [43]. In case of high cortisol levels, somatostatin receptors (especially type 2) are down-regulated [44], and a medical treatment with steroidogenesis inhibitors before nuclear imaging with somatostatin-based radioligand can enhance the identification of the NET [45].

Differential diagnosis of non-neoplastic hypercortisolism (formerly known as pseudo-Cushing's syndrome)

NNH is a functional and often mild form of hypercortisolism, characterized by a partial or complete recovery of the HPA axis after the treatment of the underlying condition. It is caused by chronic non-tumorous activation of the HPA axis by heterogenous conditions such as psychiatric disorders, uncontrolled diabetes mellitus, alcoholism, and severe obesity, with a consequent clinical presentation suggestive of CS [10]. The number of patients with obesity, cardiometabolic dysfunction, low bone density or fractures, adrenal nodules, and psychiatric disorders is increasing, therefore the diagnosis of NNH has emerged as an important topic [6]. The overlap with these disorders/conditions, that may also be associated with abnormalities in the HPA axis, increases the uncertainty in making the correct diagnosis. Imaging studies should not be used alone to confirm the presence or absence of neoplastic hypercortisolism [6] due to the large number of patients with pituitary or adrenal incidentaloma [46, 47].

Most CS patients present a criterion for major depressive disorder (MDD), while a small part can present other behavioral and cognitive disorders: MDD and hypercortisolism are mutually associated. In patients with MDD, some stress-related mediators over-activate the HPA axis, resulting in persistent hypercortisolism, which is non-suppressed in the dexamethasone suppression test (DST) and can be resolved by the administration of anti-depressive drugs. Several HPA axis alterations are reported in patients with eating disorders, such as anorexia or bulimia nervosa. The mechanisms involved in HPA deregulation and consequent hypercortisolism are increased CRH levels with normal ACTH, enhanced adrenal response (fasting/binge eating induced stress, but the regulation does not disappear after achieving a normal weight), reduced cortisol clearance, increased affinity versus corticosteroid-binding globulin (CBG), and resistance of glucocorticoid receptor (explaining the hypercortisolism without the increased lipogenesis necessary for the features suggestive of CS) [48, 49]. Likewise, alcohol abuse can present features suggestive of CS in patients with negative DST and circadian rhythm alterations due to chronic alcoholism or abstinence that lead to HPA axis activation (increase in CRH, ACTH, and cortisol), new-onset of alcohol-associated disorders in psychiatric (depression), and neurological (hippocampus neurotoxicity) spheres that can alter HPA axes regulation, as well as 11β-HSD1 induction secondary to alcoholic liver disease with increased conversion of cortisone to cortisol [6, 50]. Diabetes mellitus type 2 is associated with hypercortisolism due to central HPA axis activation, increased 11β-HSD1 expression in adipose tissue, and hippocampus damage due to glycemic decompensation. Obese patients present HPA axis activation due to increased responsiveness to neuropeptides and dietetic factors and increased 11β-HSD1 expression in adipose tissue. During pregnancy, physiological modifications in the HPA axis include estrogen excess that increase the levels of CBG (an increase of total cortisol with a constant free fraction), ACTH (explained by different CBG levels, placental synthesis, cortisol feedback desensitization, pituitary sensitization to CRH), and UFC [6].

In patients with NNH, the diagnostic accuracy of first-line screening tests for hypercortisolism is limited due to the mild activation of the HPA axis [10]. Therefore, several second-line tests have been proposed to distinguish NNH from neoplastic CS: although there is no agreement on the gold standard, CRH test after dexamethasone suppression and desmopressin test can be valid tools [51]. In authors' opinion, one of the critical factors in the differential diagnosis between CD and NNH is time. If we are approaching a patient with mild hypercorticism, in the suspicion of NNH form, a re-evaluation 3-6 months after the first diagnosis could be an optimal choice, especially if the underlying factor (e. g. alcohol abuse, depression, pregnancy) can be adequately managed.

Differential diagnosis of adrenocorticotropic hormone-dependent hypercortisolism (Cushing's disease vs. ectopic adrenocorticotropic hormone secretion)

CD is the most common cause (60-70 %) of CS and it is characterized by a pituitary ACTH hypersecretion. In most cases, it is due to a pituitary corticotroph basophilic adenoma and rarely due to diffuse corticotroph cell hyperplasia. Pituitary corticotroph adenoma is often < 10 mm in diameter (microadenoma), but in 10 % of the cases, it exceeds 10 mm (macroadenoma). Pituitary corticotroph adenomas present mild histological alterations compared to normal pituitary cells. The hallmark of pituitary corticotropinoma is partial resistance to the cortisol feedback, resulting in an ACTH hypersecretion and chronic hypercortisolism that is not suppressible after a low dose of dexamethasone (the screening test) but retains

the glucocorticoid feedback and is almost completely inhibited after a high dose of dexamethasone. While the presence of a 10 mm adenoma is considered sufficient to diagnose CD [26], endocrinologists should remember that attenuated response to dynamic tests is reported in macroadenoma [52].

EAS is diagnosed in 5-10 % of CS cases and derives mostly from NETs as bronchial or thymus or gastrointestinal carcinoids, small-cell pulmonary carcinoma, medullary thyroid carcinoma or pheochromocytoma [37]. EAS associated with malignant aggressive tumors is usually characterized by extremely high ACTH and cortisol circulating levels, producing a rapid and severe clinical presentation with hypokalemia, catabolic presentation, and opportunistic infections, rather than an insidious and mild outset CS that is more typical of small or occult tumors [53].

In some cases, the differentiating CD and EAS can be challenging. Aggressive pituitary adenomas with a large amount of cortisol secretion, rapid onset of catabolic signs of hypercortisolism, and acute severe comorbidities (such as hypertension with hypokalemia, mainly due to 11β-hydroxysteroid dehydrogenase type 2 saturation [54]) mimic a paraneoplastic cortisol excess. On the contrary, some patients with indolent and small, well-differentiated (mainly bronchial) EAS present only with the common signs of cortisol excess [39]. In the context of ACTH-dependent forms, ACTH levels tend to be higher in the case of ectopic secretion compared to those from a pituitary origin; however, there is considerable overlap, so the ACTH measurement alone is not sufficient to distinguish the two conditions [37, 54].

Although the evidence of a > 6 mm pituitary adenoma in the diagnostic work-up for ACTH-dependent hypercortisolism is highly suggestive of the pituitary source of ACTH secretion [26], pituitary incidentalomas are not uncommon in the context of EAS (reported in 5 out of 26 patients with EAS [55]) and can further complicate the diagnosis in case of discordant dynamic tests. Moreover, in clinical practice, 65.5 % of patients with CD displayed a < 6 mm pituitary adenoma at CEMRI [36]. CRH test has emerged as the most reliable non-invasive test for the differential diagnosis of ACTH-dependent forms in terms of diagnostic odds ratio [22]. However, it cannot guarantee an absolute differentiation between the pituitary and ectopic origin, since some ectopic ACTH-secreting tumors may respond to CRH. Similarly, some well-differentiated NETs (especially bronchial carcinoids) may express glucocorticoid receptors and, therefore, be sensitive to the suppression by HDDST. High-resolution conventional imaging is now the technique of choice in patients with EAS, because it is the most used approach to localize the source of ectopic ACTH secretion (sensitivity is 98 % for CT and 93 % for MRI) [40]. By definition, an occult EAS is not detected during the initial management of hypercortisolism: in more than 30 % of EAS, the ACTH source was detected during follow-up [40], with 22 % occult tumors after at least two years of follow-up [38].

A skillful combination of dynamic tests, BIPSS, and imaging is required to differentiate ACTH-dependent CS. The diagnostic accuracy of BIPSS is high and has long been the gold standard to reliably exclude EAS [26]. Nonetheless, several authors proposed diagnostic strategies to reduce the number of invasive procedures. Vilar et al. documented an increased diagnostic accuracy by combining the results of different dynamic tests; ACTH response to CRH or desmopressin (≥ 35 % above basal) and cortisol suppression > 50 % after HDDST was found only in patients with CD, with a sensitivity of 63.3 % and a specificity of 100 % [56]. Similar results were described in a large Italian series, where none of the patients with EAS had positive responses in HDDST and CRH tests [57]. A recent large European study in 205 patients (197 CD and 8 EAS) reported that different combinations (ACTH and/or cortisol) of the human CRH test and the overnight HDDST revealed similar diagnostic accuracy to the single tests [20], even if the reduced cohort of patients with EAS could partially limit the impact of the results. During the evaluation of combined tests, overall performance depends on whether discordant results can be interpreted as positive or negative; in the case of the discordant test, the CRH test was more likely to be positive in the CD group compared with EAS, while the HDDST was more often incorrect in both ACTH-dependent CS [58], suggesting that CRH performs better than the other tests [22]. The innovative combination strategy proposed by Frete et al. [35], also reported in the consensus of the Pituitary Society [26], suggested that a noninvasive diagnostic strategy that combined dynamic tests and imaging enabled to avoid the BIPSS in half the number of patients. They excluded evident cases of cancer and paraneoplastic ACTH secretion to reflect an "endocrinological" setting recruitment and considered positive a 2 mm adenoma detected after CEMRI thanks to the high expertise of pituitary-dedicated neuro-radiologist. Their strategy detected all cases of CD with positive response of ACTH and cortisol to dynamic tests (CRH and desmopressin) and positive CEMRI, as well as in those cases of CD with negative CEMRI, or detected all EAS with negative test response and positive CT (and negative CEMRI) [35].

From a surgical perspective, the identification of a pituitary adenoma at CEMRI is of utmost importance, even if in referral centers, the outcome of surgery is not affected by adenoma identification [36]. In clinical practice, whichever sequences are considered, the most important "soft skill" is the expertise of the neuroradiologist supported by a pituitary multidisciplinary team. Most corticotroph tumors are microadenomas (defined also as "picoadenomas"), and up to 50 % of them are not readily visualized using lower field strength (up to 1.5 Tesla), especially if image acquisition is performed using 2–3 mm slice thickness with gaps between consecutive slices [59]. The core protocol of a pituitary CEMRI for the detection of corticotropinoma should consist of coronal and sagittal T1-weighted spin-echo pre- and post-gadolinium and coronal T2w fast (turbo) spin echo [59], acquired with 1-2 mm slice thickness and minimal spacing, using a 3 Tesla field. Suggested additional supplementary sequences (ideally immediately in the same session) are volumetric 1 mm T1w gadolinium-enhanced 3D-spoiled gradient echo (useful to provide better soft tissue contrast and improve detection of smaller adenomas [60]) and dynamic T1w gadolinium-enhanced acquisition every 10–20 seconds over 1–2 min starting with contrast injection (some authors reported that the high number of false positive images after dynamic CEMRI results in reduced final diagnostic accuracy [61]).

Recently, several radioligands have been developed. Functional pituitary imaging can confirm a suspected microadenoma or reveal a previously unsuspected one [62]. Modern functional pituitary imaging is predominantly performed using PET due to the limited spatial resolution of gamma cameras, reflecting its superior

spatial resolution compared to SPECT. Hybrid imaging techniques co-registered with volumetric magnetic resonance have been successfully used with ^{11}C-methionine in patients with newly diagnosed CD [63]. The same cellular pathway (peptide synthesis via the l-type amino acid transporter) has been further studied in 9 patients with ^{18}F-fluoroethyltyrosine [64], characterized by a longer half-life (~110 min vs. 20 min) and therefore the possibility to transport the radioligand out of the on-site cyclotron. The detection of the CRH receptor on pituitary adenoma with a ^{68}Ga-DOTA was studied in 24 patients with CD (17 microadenoma, 10 with < 6 mm adenoma) [65], and should be further investigated in patients with negative CEMRI.

Differential diagnosis of adrenal hypercortisolism

The mild autonomous cortisol secretion (MACS) that characterizes 20-40 % of incidentally discovered adrenocortical adenomas, defined as abnormal cortisol suppression (> 50 nmol/L) after 1mg-DST [66], is probably the most frequent form of hypercortisolism. The prevalence of cortisol-related comorbidities is increased in patients with MACS [67], leading to increased cardiovascular mortality, especially in women younger than 65 years [46]. Only the signs and symptoms of overt CS are able to differentiate adrenal hypercortisolism in MACS because 1-mg DST is positive (unsuppressed) by definition; therefore, clinical expertise and additional biochemical tests to assess the degree of cortisol secretion should be used in clinical practice. In this scenario, we reported that UFC achieved the highest diagnostic accuracy in detecting CS in a patient with adrenal incidentaloma [68].

A Unilateral adrenal adenoma is the main cause of ACTH-independent CS; it can be associated with alterations of cAMP-dependent or β-catenin pathways [69]. PBMAH is rare and characterized by multiple bilateral adrenal nodules > 10 mm in diameter, usually sporadic or sometimes familial [70, 71]. Steroidogenesis is dysregulated in PBMAH: the aberrant expression of ectopic and/or eutopic G-protein coupled receptors combined with autocrine non-CRH-dependent ACTH secretion (gastric inhibitory polypeptide, β-adrenergic ligands, 5-hydroxytryptamine, luteinizing hormone , and antidiuretic hormone) [72] enables cortisol secretion. Recently, a genetic landscape of PBMAH has been reported. Inactivating bi-allelic mutations (first a germline and then somatic) of the ARMC5 gene (armadillo repeat containing 5) have been identified either in familial or sporadic PBMAH cases [73]. Patients with ARMC5 mutations are characterized by increased cortisol-related comorbidities (especially arterial hypertension and diabetes mellitus) and meningiomas [74, 75]. Another recent acquisition is the discovery that a two-hit inactivation of KDM1A (a tumor suppressor gene member of the lysine demethylase family involved in human tumorigenesis) explains the GIP receptor upregulation in food-dependent PBMAH with CS [76, 77].

Bilateral micronodular adrenal hypercortisolism is characterized by a nodule diameter < 10 mm. Familial cases are reported as part of a Carney complex (CNC), a genetic syndrome characterized by endocrine tumors, atrial myxomas, skin pigmentation anomalies, and peripheral nerve tumors. Isolated PPNAD and Carney complex usually affect children and young adults and are characterized by normal dimensioned adrenal glands with multiple nodular lesions. Germline PRKAR1A gene mutations are detected in more than 80 % of patients with CNC and are transmitted with an autosomal dominant trait. Inactivating mutations cause the haploinsufficiency of PRKAR1A, which encodes for the type 1α regulatory subunit of PKA, leading to constitutive activation in cAMP/PKA signaling in the affected tissues. Somatic mutations and loss of PRKAR1A locus have also been identified in sporadic adrenal masses supporting a tumor suppressor role of PRKAR1A in the adrenal cortex [78–80]. The HDDST leads to a paradoxical increase in UFC in patients with PPNAD, with a high sensitivity [81].

The differential diagnosis of bilateral adrenal lesions is not always immediate. First, endocrinologists must remember that ACTH-induced bilateral adrenal nodes are detected in 37 % of patients with ACTH-dependent CS, especially in older patients with CD or a longer disease duration [82]. Furthermore, PRKACA somatic mutations can be found in adrenal nodules of patients with CD [83]. Therefore, in the case of low ACTH levels and bilateral adrenal nodes, a dynamic test (such as the CRH test) is useful to assess adrenal autonomous CS [16]. CRH test has a predictive role in the management of patients with PBMAH; an increase in ACTH > 50 % above baseline value was associated with higher remission rates after unilateral adrenalectomy [84]. Specific dynamic tests can be used to study the behavior of adrenal nodes further. In PBMAH, selective provocative tests (with GnRH, TRH, desmopressin, metoclopramide, upright posture test, oral glucose tolerance test or mixed meal) can suggest specific treatments, such as octreotide LAR or propranolol in case of food-dependent CS or positive postural test, respectively [85].

In subtyping bilateral adrenal lesions with imaging, each adrenal mass should be assessed individually, considering that two different types of adrenal lesions could be detected concomitantly [86]. Normal adrenal glands show an attenuation close to the liver on CT and have homogeneous enhancement after contrast injection [87]. On unenhanced CT scans, adrenal adenomas are characterized by attenuation values lower than 10 Hounsfield Units (HU), secondary to a high lipid content [88]. However, almost 30 % of adenomas are lipid-poor, thus presenting HU > 10, especially in the case of MACS [89]. MRI is a second-line imaging modality in the assessment of adrenal lesions [87], and it is fundamental when CT is contraindicated (pregnancy or allergy to iodine contrast reagent) or in young people. In addition, MRI evaluates the fat content of adrenal masses (called chemical shift), defined by a drop of signal on the T1-weighted out-of-phase images compared with the T1-weighted in-phase images (signal drop > 16.5 %) [90]. The major role of metabolic imaging with fluorine-18 deoxyglucose positron emission tomography/computed tomography (18-FDG PET/CT) regards the discrimination of benign from malignant adrenal masses [91, 92]. Regarding PBMAH, 18-FDG PET/CT value has not been assessed yet, but high standardized uptake values have been reported, suggesting that cortisol-secreting masses have higher FDG uptake than non-secreting lesions [93].

In opposition to its important role in primary aldosteronism, the use of adrenal vein sampling in subtyping bilateral adrenal hypercortisolism has not proven to be more accurate than conventional imaging. Given the need to use plasma metanephrine as a marker for lateralization, it is associated with a consistent risk of inadequate bilateral selectivity [94, 95].

Conclusions

The combination of a skilled multidisciplinary team (not a single endocrinologist or neurosurgeon, although expert) is necessary for a correct diagnosis of CS, as summarized in ▶ **Fig. 1**. Usually, referral or academic medical centers include all facilities, combined with a dedicated laboratory, interventional radiology, and conventional and nuclear medicine.

After the suspect of activation of the HPA axis, we suggest a step-by-step approach:

1. Rule out NNH, especially in case of mild cortisol excess (UFC below 2-4 times the upper limit of normality). If safe for the patient, a wait-and-see approach with retesting after 3-6 months is a good choice. In case of positive imaging and suspected NNH, an incidentaloma should be considered.
2. Define CS as ACTH-dependent or ACTH-independent form. In case of bilateral adrenal disease, consider a CRH test in case of normal-low ACTH levels or genetic testing in case of food-dependent hypercortisolism.
3. Consider BIPSS as the gold standard to differentiate pituitary from ectopic CS; however, it can be avoided if dynamic tests are concordant with imaging.
4. Carefully revise imaging (either pituitary, adrenal or every suspected lesion) before surgery.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Nieman LK, Biller BMK, Findling JW et al. The diagnosis of Cushing's syndrome: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2008; 93: 1526–1540. DOI: 10.1210/jc.2008-0125

[2] Gadelha M, Gatto F, Wildemberg LE et al. Cushing's syndrome. Lancet 2023; 402: 2237–2252. DOI: 10.1016/S0140-6736(23)01961-X

[3] Reincke M, Fleseriu M. Cushing syndrome. JAMA 2023; 330: 170. DOI: 10.1001/jama.2023.11305

[4] Ceccato F, Boscaro M. Cushing's syndrome: Screening and diagnosis. High Blood Press Cardiovasc Prev 2016; 23: 209–215. DOI: 10.1007/s40292-016-0153-4

[5] Louiset E, Duparc C, Young J et al. Intraadrenal corticotropin in bilateral macronodular adrenal hyperplasia. N Engl J Med 2013; 369: 2115–2125. DOI: 10.1056/NEJMoa1215245

[6] Findling JW, Raff H. Recognition of nonneoplastic hypercortisolism in the evaluation of patients with Cushing syndrome. J Endocr Soc 2023; 7. DOI: 10.1210/jendso/bvad087

[7] Scaroni C, Albiger NM, Palmieri S et al. Approach to patients with pseudo-Cushing's states. Endocr Connect 2020; 9: R1–R13. DOI: 10.1530/EC-19-0435

[8] Galm BP, Qiao N, Klibanski A et al. Accuracy of laboratory tests for the diagnosis of Cushing syndrome. J Clin Endocrinol Metab 2020; 105: 1–14. DOI: 10.1210/clinem/dgaa105

[9] Khalil RB, Baudry C, Guignat L et al. Sequential hormonal changes in 21 patients with recurrent Cushing's disease after successful pituitary surgery. Eur J Endocrinol 2011; 165: 729–737. DOI: 10.1530/EJE-11-0424

[10] Scaroni C, Mondin A, Ceccato F. How to rule out non-neoplastic hypercortisolemia (previously known as pseudo-Cushing. Pituitary 2022; 25: 701–704. DOI: 10.1007/s11102-022-01222-2

[11] Fleseriu M, Varlamov EV, Hinojosa-Amaya JM et al. An individualized approach to the management of Cushing disease. Nat Rev Endocrinol 2023; 19: 581–599. DOI: 10.1038/s41574-023-00868-7

[12] Young J, Haissaguerre M, Viera-Pinto O et al. Cushing's syndrome due to ectopic ACTH secretion: An expert operational opinion. Eur J Endocrinol 2020; 182: R29–R58. DOI: 10.1530/EJE-19-0877

[13] Boscaro M, Arnaldi G. Approach to the patient with possible Cushing's syndrome. J Clin Endocrinol Metab 2009; 94: 3121–3131. DOI: 10.1210/jc.2009-0612

[14] Nowak E, Vogel F, Albani A et al. Diagnostic challenges in cyclic Cushing's syndrome: A systematic review. Lancet Diabetes Endocrinol 2023; 11: 593–606. DOI: 10.1016/S2213-8587(23)00150-X

[15] Pecori Giraldi F, Saccani A, Cavagnini F et al. Assessment of ACTH assay variability: A multicenter study. Eur J Endocrinol 2011; 164: 505–512. DOI: 10.1530/EJE-10-0962

[16] Ceccato F, Tizianel I, Vedolin CK. Human corticotropin-releasing hormone tests: 10 years of real-life experience in pituitary and adrenal disease. J Clin Endocrinol Metab 2020; 105: e3938–e3949. DOI: 10.1210/clinem/dgaa564

[17] Pecori Giraldi F, Invitti C, Cavagnini F. Study group of the Italian Society of Endocrinology on the pathophysiology of the hypothalamic-pituitary-adrenal axis. The corticotropin-releasing hormone test in the diagnosis of ACTH-dependent Cushing's syndrome: A reappraisal. Clin Endocrinol (Oxf) 2001; 54: 601–607. DOI: 10.1046/j.1365-2265.2001.01258.x

[18] Ceccato F, Di Dalmazi G. Shortage of hCRH for the diagnosis of endogenous CS: The end of an era or the beginning of a new journey? J Endocrinol Invest 2023; 46: 2189–2191. DOI: 10.1007/s40618-023-02113-4

[19] Ritzel K, Beuschlein F, Berr C et al. ACTH after 15 min distinguishes between Cushing's disease and ectopic Cushing's syndrome: A proposal for a short and simple CRH test. Eur J Endocrinol 2015; 173: 197–204. DOI: 10.1530/EJE-14-0912

[20] Detomas M, Ritzel K, Nasi-Kordhishti I et al. Outcome of CRH stimulation test and overnight 8 mg dexamethasone suppression test in 469 patients with ACTH-dependent Cushing's syndrome. Front Endocrinol (Lausanne) 2022; 13. DOI: 10.3389/fendo.2022.955945

[21] Barbot M, Albiger N, Koutroumpi S et al. Predicting late recurrence in surgically treated patients with Cushing's disease. Clin Endocrinol (Oxf) 2013; 79: 394–401. DOI: 10.1111/cen.12133

[22] Ceccato F, Barbot M, Mondin A et al. Dynamic testing for differential diagnosis of ACTH-dependent Cushing syndrome: A systematic review and meta-analysis. J Clin Endocrinol Metab 2023; 108: e178–e188. DOI: 10.1210/clinem/dgac686

[23] Ceccato F, Artusi C, Barbot M et al. Dexamethasone measurement during low-dose suppression test for suspected hypercortisolism: Threshold development with and validation. J Endocrinol Invest 2020; 43: 1105–1113. DOI: 10.1007/s40618-020-01197-6

[24] Nieman LK. Is it time for a new approach to the differential diagnosis of ACTH-dependent Cushing syndrome. J Clin Endocrinol Metab 2020; 105: e4964–e4966. DOI: 10.1210/clinem/dgaa493

[25] Loriaux DL. Diagnosis and differential diagnosis of Cushing's syndrome. N Engl J Med 2017; 376: 1451–1459. DOI: 10.1056/NEJMra1505550

[26] Fleseriu M, Auchus R, Bancos I et al. Consensus on diagnosis and management of Cushing's disease: A guideline update. Lancet Diabetes Endocrinol 2021; 9: 847–875. DOI: 10.1016/S2213-8587(21)00235-7

[27] Webb KL, Hinkle ML, Walsh MT et al. Inferior petrosal sinus sampling tumor lateralization and the surgical treatment of Cushing disease: A meta-analysis and systematic review. World Neurosurg 2024; 182: e712–e720. DOI: 10.1016/j.wneu.2023.12.024

[28] Wang H, Ba Y, Xing Q et al. Differential diagnostic value of bilateral inferior petrosal sinus sampling (BIPSS) in ACTH-dependent Cushing syndrome: A systematic review and meta-analysis. BMC Endocr Disord 2020; 20: 143. DOI: 10.1186/s12902-020-00623-3

[29] Pecori Giraldi F, Cavallo LM, Tortora F et al. The role of inferior petrosal sinus sampling in ACTH-dependent Cushing's syndrome: Review and joint opinion statement by members of the Italian Society for Endocrinology, Italian Society for Neurosurgery, and Italian Society for Neuroradiology. Neurosurg Focus 2015; 38: E5. DOI: 10.3171/2014.11.FOCUS14766

[30] Perlman JE, Johnston PC, Hui F et al. Pitfalls in Performing and interpreting inferior petrosal sinus sampling: Personal experience and literature review. J Clin Endocrinol Metab 2021; 106: e1953–e1967. DOI: 10.1210/clinem/dgab012

[31] Almeida de TS, Rodrigues T, da CCostenaro F et al. Enhancing Cushing's disease diagnosis: Exploring the impact of desmopressin on ACTH gradient during BIPSS. Front Endocrinol (Lausanne) 2023; 14. DOI: 10.3389/fendo.2023.1224001

[32] Chen S, Chen K, Wang S et al. The optimal cut-off of BIPSS in differential diagnosis of ACTH-dependent Cushing's syndrome: Is stimulation necessary? J Clin Endocrinol Metab 2020; 105. DOI: 10.1210/clinem/dgz194

[33] Pecori Giraldi F, Federici AB, Cavagnini F. Caution during use of desmopressin in IPSS. J Neurointerv Surg 2013; 5: e27. DOI: 10.1136/neurintsurg-2012-010580

[34] Detomas M, Ritzel K, Nasi-Kordhishti I et al. Bilateral inferior petrosal sinus sampling with human CRH stimulation in ACTH-dependent Cushing's syndrome: Results from a retrospective multicenter study. Eur J Endocrinol 2023; 188: 448–456. DOI: 10.1093/ejendo/lvad050

[35] Frete C, Corcuff J-B, Kuhn E et al. Non-invasive diagnostic strategy in ACTH-dependent Cushing's syndrome. J Clin Endocrinol Metab 2020; 105: 3273–3284. DOI: 10.1210/clinem/dgaa409

[36] Ferrante E, Barbot M, Serban AL et al. Indication to dynamic and invasive testing in Cushing's disease according to different neuroradiological findings. J Endocrinol Invest 2022; 45: 629–637. DOI: 10.1007/s40618-021-01695-1

[37] Isidori AM, Kaltsas GA, Pozza C et al. The ectopic adrenocorticotropin syndrome: Clinical features, diagnosis, management, and long-term follow-up. J Clin Endocrinol Metab 2006; 91: 371–377. DOI: 10.1210/jc.2005-1542

[38] Davi' MV, Cosaro E, Piacentini S et al. Prognostic factors in ectopic Cushing's syndrome due to neuroendocrine tumors: A multicenter study. Eur J Endocrinol 2017; 176: 453–461. DOI: 10.1530/EJE-16-0809

[39] Ragnarsson O, Juhlin CC, Torpy DJ et al. A clinical perspective on ectopic Cushing's syndrome. Trends Endocrinol Metab 2024; 35: 347–360. DOI: 10.1016/j.tem.2023.12.003

[40] Isidori AM, Sbardella E, Zatelli MC et al. Conventional and nuclear medicine imaging in ectopic Cushing's syndrome: A systematic review. J Clin Endocrinol Metab 2015; 100: 3231–3244. DOI: 10.1210/JC.2015-1589

[41] Goroshi MR, Jadhav SS, Lila AR et al. Comparison of 68Ga-DOTANOC PET/CT and contrast-enhanced CT in localisation of tumours in ectopic ACTH syndrome. Endocr Connect 2016; 5: 83–91. DOI: 10.1530/EC-16-0010

[42] Wannachalee T, Turcu AF, Bancos I et al. The clinical impact of [68Ga]-DOTATATE PET/CT for the diagnosis and management of ectopic adrenocorticotropic hormone – secreting tumours. Clin Endocrinol (Oxf) 2019; 91: 288–294. DOI: 10.1111/cen.14008

[43] Ceccato F, Cecchin D, Gregianin M et al. The role of68ga-dota derivatives PET-CT in patients with ectopic ACTH syndrome. Endocr Connect 2020; 9: 337–345. DOI: 10.1530/EC-20-0089

[44] de Bruin C, Feelders RA, Waaijers AM et al. Differential regulation of human dopamine D2 and somatostatin receptor subtype expression by glucocorticoids in vitro. J Mol Endocrinol 2009; 42: 47–56. DOI: 10.1677/JME-08-0110

[45] Davi' MV, Salgarello M, Francia G. Positive 68Ga-DOTATOC-PET/CT after cortisol level control during ketoconazole treatment in a patient with liver metastases from a pancreatic neuroendocrine tumor and ectopic Cushing syndrome. Endocrine 2015; 49: 566–567. DOI: 10.1007/s12020-014-0391-y

[46] Deutschbein T, Reimondo G, Di Dalmazi G et al. Age-dependent and sex-dependent disparity in mortality in patients with adrenal incidentalomas and autonomous cortisol secretion: An international, retrospective, cohort study. Lancet Diabetes Endocrinol 2022; 10: 499–508. DOI: 10.1016/S2213-8587(22)00100-0

[47] Toini A, Dolci A, Ferrante E et al. Screening for ACTH-dependent hypercortisolism in patients affected with pituitary incidentaloma. Eur J Endocrinol 2015; 172: 363–369. DOI: 10.1530/EJE-14-0599

[48] Misra M, Klibanski A. Endocrine consequences of anorexia nervosa. Lancet Diabetes Endocrinol 2014; 2: 581–592. DOI: 10.1016/S2213-8587(13)70180-3

[49] Schorr M, Miller KK. The endocrine manifestations of anorexia nervosa: Mechanisms and management. Nat Rev Endocrinol 2017; 13: 174–186. DOI: 10.1038/nrendo.2016.175

[50] Badrick E, Bobak M, Britton A et al. The relationship between alcohol consumption and cortisol secretion in an aging cohort. J Clin Endocrinol Metab 2008; 93: 750–757. DOI: 10.1210/jc.2007-0737

[51] Mondin A, Barbot M, Voltan G et al. Second-line tests in the differential diagnosis of neoplastic and non-neoplastic hypercortisolism: A systematic review and meta-analysis. J Endocrinol Invest 2023; 46: 1947–1959. DOI: 10.1007/s40618-023-02099-z

[52] Woo YS, Isidori AM, Wat WZ et al. Clinical and biochemical characteristics of adrenocorticotropin-secreting macroadenomas. J Clin Endocrinol Metab 2005; 90: 4963–4969. DOI: 10.1210/jc.2005-0070

[53] Toivanen S, Leijon H, Arola A et al. Characteristics and outcomes of the Finnish ectopic ACTH syndrome cohort. Endocrine 2021; 74: 387–395. DOI: 10.1007/s12020-021-02768-0

[54] Ceccato F, Trementino L, Barbot M et al. Diagnostic accuracy of increased urinary cortisol/cortisone ratio to differentiate ACTH-dependent Cushing's syndrome. Clin Endocrinol (Oxf) 2017; 87: 500–507. DOI: 10.1111/cen.13391

[55] Yogi-Morren D, Habra MA, Faiman C et al. Pituitary MRI findings in patients with pituitary and ectopic ACTH-dependent Cushing syndrome: Does a 6-mm pituitary tumor size cut-off value exclude ectopic ACTH syndrome. Endocr Pract 2015; 21: 1098–1103. DOI: 10.4158/EP15662.OR

[56] Vilar L, Freitas MC, Naves LA et al. The role of non-invasive dynamic tests in the diagnosis of Cushing's syndrome. J Endocrinol Invest 2008; 31: 1008–1013. DOI: 10.1007/BF03345640

[57] Barbot M, Trementino L, Zilio M et al. Second-line tests in the differential diagnosis of ACTH-dependent Cushing's syndrome. Pituitary 2016; 19: 488–495. DOI: 10.1007/s11102-016-0729-y

[58] Elenius H, McGlotten R, Nieman LK. Ovine CRH stimulation and 8 mg dexamethasone suppression tests in 323 patients with ACTH-dependent Cushing's syndrome. J Clin Endocrinol Metab 2023; 109: e182–e189. DOI: 10.1210/clinem/dgad454

[59] Bashari WA, Gillett D, MacFarlane J et al. Modern imaging in Cushing's disease. Pituitary 2022; 25: 709–712. DOI: 10.1007/s11102-022-01236-w

[60] Kasaliwal R, Sankhe SS, Lila AR et al. Volume interpolated 3D-spoiled gradient echo sequence is better than dynamic contrast spin echo sequence for MRI detection of corticotropin secreting pituitary microadenomas. Clin Endocrinol (Oxf) 2013; 78: 825–830. DOI: 10.1111/cen.12069

[61] Grober Y, Grober H, Wintermark M et al. Comparison of MRI techniques for detecting microadenomas in Cushing's disease. J Neurosurg 2018; 128: 1051–1057. DOI: 10.3171/2017.3.JNS163122

[62] Gillett D, MacFarlane J, Bashari W et al. Molecular imaging of pituitary tumors. Semin Nucl Med 2023; 53: 530–538. DOI: 10.1053/j.semnuclmed.2023.02.005

[63] Koulouri O, Steuwe A, Gillett D et al. A role for 11C-methionine PET imaging in ACTH-dependent Cushing's syndrome. Eur J Endocrinol 2015; 173: M107–M120. DOI: 10.1530/EJE-15-0616

[64] Berkmann S, Roethlisberger M, Mueller B et al. Selective resection of Cushing microadenoma guided by preoperative hybrid 18-fluoroethyl-L-tyrosine and 11-C-methionine PET/MRI. Pituitary 2021; 24: 878–886. DOI: 10.1007/s11102-021-01160-5

[65] Walia R, Gupta R, Bhansali A et al. Molecular imaging targeting corticotropin-releasing hormone receptor for corticotropinoma: A changing paradigm. J Clin Endocrinol Metab 2021; 106: 1816–1826. DOI: 10.1210/clinem/dgaa755

[66] Fassnacht M, Tsagarakis S, Terzolo M et al. European Society of Endocrinology clinical practice guidelines on the management of adrenal incidentalomas, in collaboration with the European Network for the Study of Adrenal Tumors. Eur J Endocrinol 2023; 189: G1–G42. DOI: 10.1093/ejendo/lvad066

[67] Pelsma ICM, Fassnacht M, Tsagarakis S et al. Comorbidities in mild autonomous cortisol secretion and the effect of treatment: Systematic review and meta-analysis. Eur J Endocrinol 2023; 189: S88–S101. DOI: 10.1093/ejendo/lvad134

[68] Ceccato F, Antonelli G, Frigo AC et al. First-line screening tests for Cushing's syndrome in patients with adrenal incidentaloma: The role of urinary free cortisol measured by LC-MS/MS. J Endocrinol Invest 2017; 40: 753–760. DOI: 10.1007/s40618-017-0644-8

[69] Di Dalmazi G, Altieri B, Scholz C et al. RNA sequencing and somatic mutation status of adrenocortical tumors: Novel pathogenetic insights. J Clin Endocrinol Metab 2020; 105: dgaa616. DOI: 10.1210/clinem/dgaa616

[70] Araujo-Castro M, Marazuela M. Cushing´s syndrome due to bilateral adrenal cortical disease: Bilateral macronodular adrenal cortical disease and bilateral micronodular adrenal cortical disease. Front Endocrinol (Lausanne) 2022; 13: 913253. DOI: 10.3389/fendo.2022.913253

[71] Ceccato F, Barbot M, Scaroni C et al. Frequently asked questions and answers (if any) in patients with adrenal incidentaloma. J Endocrinol Invest 2021; 44: 2749–2763. DOI: 10.1007/s40618-021-01615-3

[72] Cavalcante IP, Berthon A, Fragoso MC et al. Primary bilateral macronodular adrenal hyperplasia: Definitely a genetic disease. Nat Rev Endocrinol 2022; 18: 699–711. DOI: 10.1038/s41574-022-00718-y

[73] Espiard S, Drougat L, Libé R et al. ARMC5 mutations in a large cohort of primary macronodular adrenal hyperplasia: Clinical and functional consequences. J Clin Endocrinol Metab 2015; 100: E926–E935. DOI: 10.1210/jc.2014-4204

[74] Albiger NM, Regazzo D, Rubin B et al. A multicenter experience on the prevalence of ARMC5 mutations in patients with primary bilateral macronodular adrenal hyperplasia: From genetic characterization to clinical phenotype. Endocrine 2017; 55: 959–968. DOI: 10.1007/s12020-016-0956-z

[75] Jojima T, Kogai T, Iijima T et al. Genetic alteration of ARMC5 in a patient diagnosed with meningioma and primary macronodular adrenal hyperplasia: A case report. Eur J Endocrinol 2020; 183: K7–K12. DOI: 10.1530/EJE-20-0014

[76] Chasseloup F, Bourdeau I, Tabarin A et al. Loss of KDM1A in GIP-dependent primary bilateral macronodular adrenal hyperplasia with Cushing's syndrome: A multicentre, retrospective, cohort study. Lancet Diabetes Endocrinol 2021; 9: 813–824. DOI: 10.1016/S2213-8587(21)00236-9

[77] Vaczlavik A, Bouys L, Violon F et al. KDM1A inactivation causes hereditary food-dependent Cushing syndrome. Genet Med 2022; 24: 374–383. DOI: 10.1016/j.gim.2021.09.018

[78] Kamilaris C, Faucz F, Voutetakis A et al. Carney complex. Exp Clin Endocrinol Diabetes 2019; 127: 156–164. DOI: 10.1055/a-0753-4943

[79] Kirschner LS, Carney JA, Pack SD et al. Mutations of the gene encoding the protein kinase A type I-α regulatory subunit in patients with the Carney complex. Nat Genet 2000; 26: 89–92. DOI: 10.1038/79238

[80] Bertherat J, Groussin L, Sandrini F et al. Molecular and functional analysis of PRKAR1A and its locus (17q22-24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase A expression and activity. Cancer Res 2003; 63: 5308–5319. http://www.ncbi.nlm.nih.gov/pubmed/14500362

[81] Stratakis CA, Sarlis N, Kirschner LS et al. Paradoxical response to dexamethasone in the diagnosis of primary pigmented nodular adrenocortical disease. Ann Intern Med 1999; 131: 585. DOI: 10.7326/0003-4819-131-8-199910190-00006

[82] Albiger NM, Occhi G, Sanguin F et al. Adrenal nodules in patients with Cushing's disease: Prevalence, clinical significance and follow-up. J Endocrinol Invest 2011; 34: 1–20. DOI: 10.3275/7349

[83] Di Dalmazi G, Timmers HJLM, Arnaldi G et al. Somatic PRKACA mutations: Association with transition from pituitary-dependent to adrenal-dependent Cushing syndrome. J Clin Endocrinol Metab 2019; 104: 5651–5657. DOI: 10.1210/jc.2018-02209

[84] Tizianel I, Detomas M, Deutschbein T et al. Corticotropin-releasing hormone test predicts the outcome of unilateral adrenalectomy in primary bilateral macronodular adrenal hyperplasia. J Endocrinol Invest 2024; 47: 749–756. DOI: 10.1007/s40618-023-02204-2

[85] Albiger NM, Ceccato F, Zilio M et al. An analysis of different therapeutic options in patients with Cushing's syndrome due to bilateral macronodular adrenal hyperplasia: A single-centre experience. Clin Endocrinol (Oxf) 2015; 82. DOI: 10.1111/cen.12763

[86] Katabathina VS, Flaherty E, Kaza R et al. Adrenal collision tumors and their mimics: Multimodality imaging findings. Cancer Imaging 2013; 13: 602–610. DOI: 10.1102/1470-7330.2013.0053

[87] Barat M, Cottereau A-S, Gaujoux S et al. Adrenal mass characterization in the era of quantitative imaging: State of the art. Cancers (Basel) 2022; 14: 569. DOI: 10.3390/cancers14030569

[88] Boland GW, Lee MJ, Gazelle GS et al. Characterization of adrenal masses using unenhanced CT: An analysis of the CT literature. Am J Roentgenol 1998; 171: 201–204. DOI: 10.2214/ajr.171.1.9648789

[89] Ceccato F, Tizianel I, Voltan G et al. Attenuation value in adrenal incidentalomas: A longitudinal study. Front Endocrinol (Lausanne) 2021; 12: 1–9. DOI: 10.3389/fendo.2021.794197

[90] Nandra G, Duxbury O, Patel P et al. Technical and interpretive pitfalls in adrenal imaging. RadioGraphics 2020; 40: 1041–1060. DOI: 10.1148/rg.2020190080

[91] Boland GWL, Dwamena BA, Jagtiani Sangwaiya M et al. Characterization of adrenal masses by using FDG PET: A systematic review and meta-analysis of diagnostic test performance. Radiology 2011; 259: 117–126. DOI: 10.1148/radiol.11100569

[92] Kim SJ, Lee SW, Pak K et al. Diagnostic accuracy of 18F-FDG PET or PET/CT for the characterization of adrenal masses: A systematic review and meta-analysis. Br J Radiol 2018; 91: 20170520. DOI: 10.1259/bjr.20170520

[93] Patel D, Gara SK, Ellis RJ et al. FDG PET/CT scan and functional adrenal tumors: A pilot study for lateralization. World J Surg 2016; 40: 683–689. DOI: 10.1007/s00268-015-3242-y

[94] Acharya R, Dhir M, Bandi R et al. Outcomes of adrenal venous sampling in patients with bilateral adrenal masses and ACTH-independent Cushing's syndrome. World J Surg 2019; 43: 527–533. DOI: 10.1007/s00268-018-4788-2

[95] Rubinstein G, Osswald A, Braun LT et al. The role of adrenal venous sampling (AVS) in primary bilateral macronodular adrenocortical hyperplasia (PBMAH): A study of 16 patients. Endocrine 2022; 76: 434–445. DOI: 10.1007/s12020-022-03020-z