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STATE-OF-THE-ART REVIEW IN ENDOCRINOLOGY

Management of Cushing's disease in the initial phase ~From detection to surgery~

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Abstract. Cushing's disease is a rare endocrine disorder that presents many systemic complications, and its initial phase management can be difficult in atypical and severe cases or at institutes with limited experience. It is a disease in which several complications may have already progressed at the time of diagnosis, and complications may become more severe during the initial management phase, potentially becoming life-threatening. In addition, many patients are young, and depending on this phase management, their quality of life will significantly decline later on. Therefore, this review summarizes the evidence accumulated to date and outlines strategies for disease management, focusing on the initial stages from detection, diagnosis, and referral of patients to surgery.

Key words: Cushing's disease, Screening, Diagnosis

Introduction

A century has passed since Harvey Cushing proposed the concept that a disease presenting with systemic symptoms, including central obesity, hirsutism, and amenorrhea caused by basophil adenoma developed in the pituitary grand [1]. With the subsequent discovery of cortisone [2] and ACTH [3], the development of hormone measurement assay, the elucidation of the steroid synthesis pathway in the adrenal cortex, and the clarification of the effects of each hormone, the full picture of this disease is gradually becoming clearer [4]. The elucidations of this pathology have led to the development of accurate disease diagnosis, better surgical therapy, radiotherapy, and drug therapy for this challenging pituitary tumor; and, the prognosis of this disease, which had been extremely poor, has improved considerably [5, 6]. However, many challenges remain, including early detection, diagnostic strategy in atypical cases, and treatment not only in the initial phase but also in the chronic phase. Despite the considerable improvement in mortality, the overall SMR is still 2.5 (95% CI 2.1–2.9) in recent reports [7], and there is no doubt that it has a still poor prognosis among pituitary diseases [8]. Furthermore, a major problem remains that many patients suffer from decreased QoL even if they achieve remission after successful treatment [9]. This review focuses on the clinical process from detection, to diagnosis, to requesting a pituitary surgeon for operation, and provides an overview from the perspective of an endocrinologist.

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Pathophysiology of Corticotroph Tumors

Before beginning the clinical review, it is necessary to summarize the pathology of pituitary tumors: the essence of this disease. Despite much insight into the pathogenesis of ACTH-secreting pituitary tumors, the primary causal genetic abnormality of the tumors had not been identified until recently. Since retinoblastoma (*Rb*) heterozygous knockout mice [10] and *p27* homozygous knockout mice [11] frequently exhibit pituitary tumors expressing ACTH, abnormalities in the cell cycle—particularly in the G1 to S phase entry—have been the initial focus in the pathogenesis of Cushing's disease. In the meantime, a germline *CDKN1B* variant, which encodes p27 (Kip1), has been identified in patients with multiple endocrine neoplasm (MEN) syndrome, including Cushing's disease, and has been named MEN4 [12]. However, germline genetic abnormalities in Cushing's disease are quite rare, representing approximately less than 3%, even if including MEN1, Carney Complex, and familial isolated pituitary adenoma syndrome (FIPA). Further investigation in genetically modified model mice, such as cyclin E transgenic mice, showed that they exhibited ACTH-producing

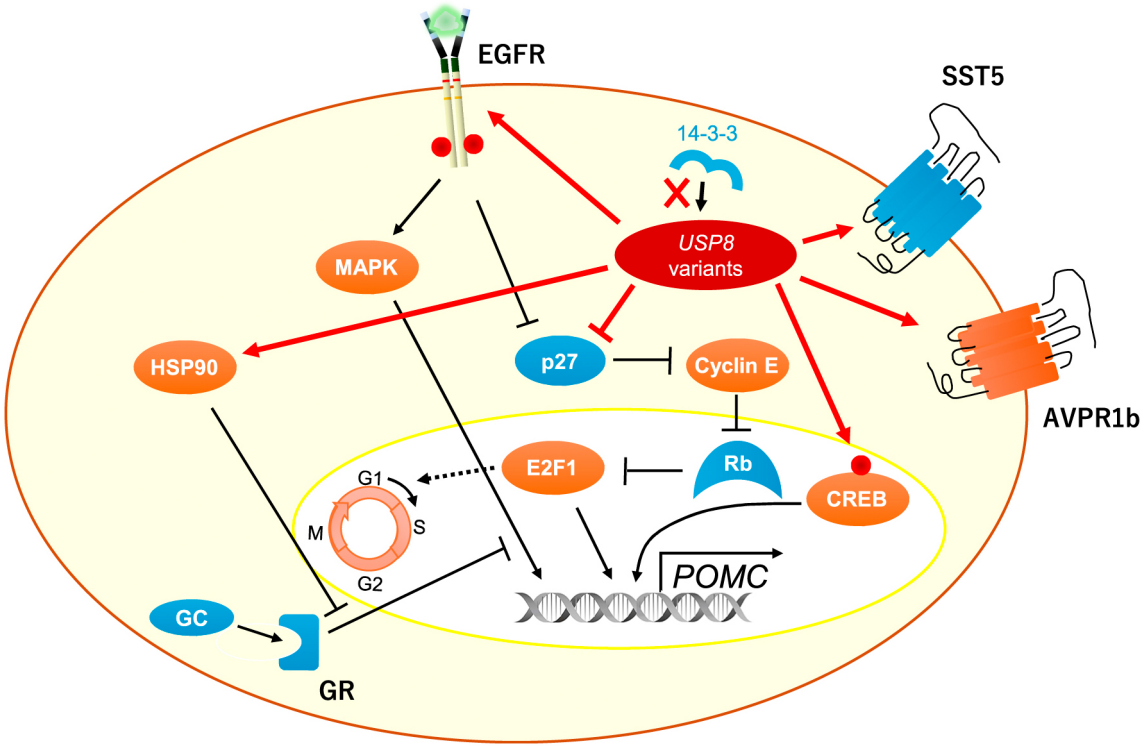


Fig. 1 Overview of change in ACTH-producing pituitary tumors with *USP8* pathogenic variants
Impaired binding of 14-3-3 protein to USP8 due to its variants induces enhanced expression of several receptors, including EGFR, SST5, and AVPR1b. These variants are also related to high expression of HSP90, low expression of P27, and enhanced phosphorylation of CREB. The EGFR-P27-E2F1 pathway can be associated with induction of POMC expression resulting in hyper ACTH secretion. HSP90 has a role in inhibiting the nuclear translocation of GR as a chaperone.
USP8: ubiquitin-specific protease 8; EGFR: epithelial growth factor receptor; SST5: somatostatin receptor type 5; AVPR1b: arginine vasopressin receptor type 1b; HSP90: heat shock protein 90; CREB: cAMP response element binding protein; GC: glucocorticoid; GR: glucocorticoid receptor; POMC: pro-opiomelanocortin.

pituitary tumors [13], supporting the importance of the CDK-Rb pathway in this condition. Due to the negative association between EGF receptor (EGFR) and P27 expression from immunohistochemical analysis [14], we have identified EGFR tyrosine kinase activity as a positive regulator of ACTH synthesis *via* cell cycle dysregulation [15]. Moreover, we have generated corticotroph-specific EGFR overexpressing mice, which resulted in corticotroph tumor or hyperplasia showing clinical features of Cushing's syndrome *via* an E2F1-dependent manner [16]. In the meantime, next-generation sequence analysis of human ACTH-producing pituitary tumor specimens identified novel somatic variants in a deubiquitinase *USP8* gene, which is found in 30–60% of these tumors. These variants are located within an extremely limited region around the 14-3-3 binding motif, which leads to proteolytic cleavage resulting in a gain-of-function of its deubiquitinase activity [17, 18]. This enhanced deubiquitinase activity is thought to induce ACTH hypersecretion and tumorigenesis through increased expression of EGFR, HSP90, and AVPR1b, downregulation of P27, and enhanced phosphorylation of CREB (Fig. 1) [17-20]. The clinical features of patients with these variants were female dominant, younger at diagnosis, more responsive to high dose dexamethasone (Dex) suppression test or desmopressin, small size, and more commonly experienced curative remission [20-24]. Among the same USP family, the *USP48* gene abnormality has also been identified at a single hotspot variant in the catalytic domain (p.Met415lle, p.Met415Val) and further described in the splice site (p.Pro433IVS + 2T>A) in 8-23% of Cushing's disease [25-28]. This is also thought to be gain-of-function, and the underlying mechanisms to induce ACTH synthesis are thought to be mediated by enhanced NFκ-B signaling rather than MAPK [25], and/or by increased Gli1 and H2A levels *via* enhanced CRH activity [26, 27]. Other somatic gene variants identified as rare abnormalities include *BRAF* V600E, *NR3C1*, *ASCL1*, *TP53* and

ATRX. Among them, *TP53* or *ATRX* variants have been confirmed to be associated with a highly proliferative phenotype [25, 29-33]. Recently, a single-cell RNA sequencing analysis of ACTH-secreting pituitary tumors has reported that *PMAIP1*, a BH3-only Bcl2 family that encodes NOXA, has highly expressed compared with other pituitary tumors and normal corticotroph, and was enhanced by EGFR overexpression. However, NOXA expression in ACTH-secreting pituitary tumors was paradoxically lower than in normal corticotroph, which was shown to be caused by proteasomal degradation. Although they showed that a selective proteasomal inhibitor can induce NOXA protein, leading to apoptosis, which can be a potential drug for these tumors, the reason for this enhanced proteasomal degradation remains unclear [34].

Detection

Epidemiology

The incidence of Cushing's disease is quite rare, with approximately 1.2–2.6 cases per million per person-years. If endogenous Cushing's syndrome, such as cortisol secreting adrenocortical tumors and hyperplasia and ectopic ACTH syndrome are included, annual incidence rate increases to 2.3–3.2 per million person-years [35-37], and its prevalence had been reported as 6.21/100,000 in a nationwide study from Iceland [38]. Data from Europe and New Zealand indicate that Cushing's disease accounts for 60 to 74% of endogenous Cushing's syndrome [37]. In contrast, the reported figure in Japan is 47%, with adrenocortical adenoma-derived Cushing's syndrome being more frequently observed than those caused by pituitary tumors [39]. However, the frequency of the disease may be underestimated due to diagnostic difficulties, especially in cases with mild clinical features or cyclic Cushing's syndrome [40, 41]. In fact, the prevalence of hypercortisolism was shown as 1.4% (95% CI: 0.4–2.9) among cases of type 2 diabetes mellitus [42] and 0.5–1.0% among hypertension [43, 44]. Based on these results, the estimated prevalence of Cushing's syndrome must be 20–35/100,000. Furthermore, according to the screening of hypercortisolemia among patients with obesity, up to 9.4% were diagnosed with Cushing's syndrome, while false positive patients were detected at 2.3% [45]. Notably, our previous case series of multiple-institute studies in Japan showed that the mean body mass index (BMI) of 92 patients with Cushing's disease was 23.1 kg/m^2 [46], indicating that more than half of these patients do not exhibit obesity based on BMI. Nevertheless, weight gain remains the most common symptom, and this change in body composition should be noted regardless non-obese BMI score.

Who needs to be screened?

Meta-analysis revealed that it took 38 months (95% CI: 33–43) for patients to be diagnosed after they noticed the first symptoms of this disease [47]. By then, they had visited 4.6 ± 3.8 clinics [48], suggesting that challenges remain in early diagnosis. According to the Endocrine Society Clinical Practice Guideline, the following patients are recommended for a screening test: patients with unusual early onset metabolic abnormalities; those with progressive symptoms, especially when more predictive of Cushing's syndrome; those with adrenal incidentaloma; and, children with decreasing height with increasing weight [49]. However, the guidelines also emphasize the need to exclude the use of exogenous glucocorticoids before screening for Cushing's syndrome. This criterion was validated and confirmed as effective in detecting patients who need to be screened [50]. In this validation study, patients with osteoporosis, adrenal incidentaloma, metabolic syndrome, and the presence of multiple symptoms were more common in patients with Cushing's syndrome than in those who had been suspected of having Cushing's but were ruled out. Particular attention should be paid to patients who have recently gained weight rather than obese patients. If there is any doubt, specific symptoms should be confirmed through a detailed interview and examination to further confirm suspicions (Fig. 2) [51]. Moon face can sometimes be difficult to diagnose, but if symmetrical swelling or severe sagging of the cheeks is observed with facial flushing (Fig. 3a) [52], it is useful to ask the patient if she or he has noticed any recent changes in cheek appearance. Comparing previous photos, if possible, is also helpful for the diagnosis. Furthermore, central obesity is difficult to determine when examined from the front but is easier to detect when observed from the side and judged with the contrast between abdominal distension and brachial atrophy. Gowers' sign helps diagnose muscular atrophy. Additionally, when asked certain questions, patients often complain of progressive difficulty climbing stairs (Fig. 2). Further, thin skin at the extremities (Fig. 3b) and a buffalo hump can be detectable. From physical examination, pigmentation is a useful sign to differentiate between ACTH-dependent and ACTH-independent Cushing's syndrome, which is frequently observed at mucosal membranes, hand joints, and nail beds (Fig. 3c). Striae are a distinctive physical finding in this disease, characterized by their wide, purple appearance, which differentiates them from the thin, pink striae associated with rapid weight gain (Fig. 3d). Based on the skin findings, fungal infection has been shown to be more common with exogenous glucocorticoid excess, and hirsutism is more likely with endogenous excess (Fig. 3a), which may be helpful for differential diagnosis [53].

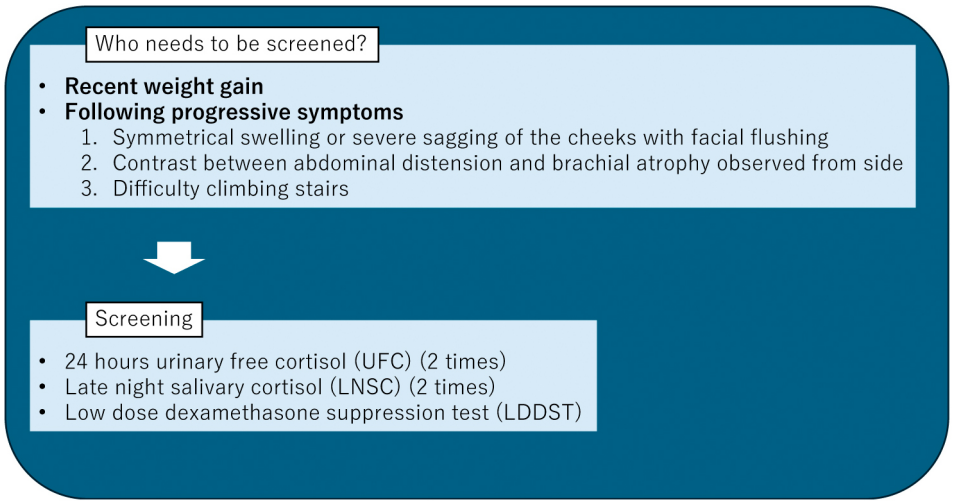


Fig. 2 Summary of detection to screening

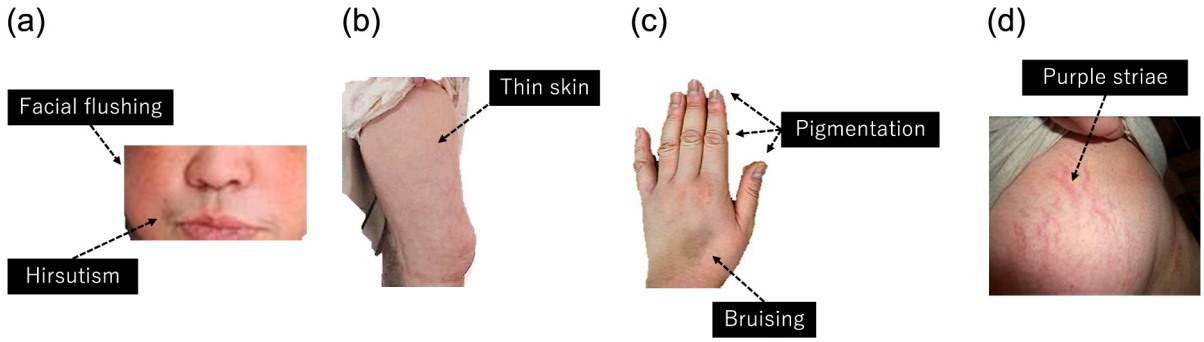


Fig. 3 Typical clinical signs in patients with Cushing's disease

(a) Facial features in a female patient showing flushing of the cheek, and beard as hirsutism. (b) Skin thinning observed on the thighs. The fragile-looking veins stand out, and the muscles show signs of atrophy. (c) Subcutaneous hemorrhage on the back of the hand, and pigmentation of the finger joints and nail beds. (d) Purple striae on the abdomen, characterized by thickness and color.

Diagnosis

Screening

Once Cushing's syndrome is suspected, the best screening test is multi-measuring of either urinary free cortisol (UFC) or late-night salivary cortisol (which is not covered by insurance in Japan), or a single overnight low dose Dex suppression test (LDDST) (Fig. 2) [54, 55]. In these tests, attention needs to be paid to false-positive or false-negative results. False-positive results can be caused by non-neoplastic hypercortisolism (NNH), also known as pseudo-Cushing's syndrome due to depression, obesity, polycystic ovary syndrome (PCOS), poorly controlled diabetes mellitus, chronic alcoholism, chronic kidney disease, and glucocorticoid resistance [56, 57]. Furthermore, elevated serum cortisol levels can occur due to increased corticosteroid-binding globulin (CBG), which is induced by estrogen or chronic active hepatitis [58]. We encountered a man who had elevated serum cortisol levels with positive results for LDDST without any Cushingoid appearance. A careful interview revealed that he suffered from a gender identity disorder and was taking conjugated estrogen pills, resulting in NNH [59]. Another pitfall of LDDST is that metabolism of this drug is mainly mediated by CYP3A4. If patients are taking drugs that induce or inhibit CYP3A4, these need to be discontinued in consultation with the prescribing physician. Otherwise, this test cannot accurately assess a diagnosis of Cushing's syndrome [49, 54]. Therefore, it is recommended that these tests should be used in combination rather than single. It is also necessary to confirm the existence of effects associated with hypercortisolemia. This requires at least the presence of Cushingoid appearance, as well as laboratory findings including elevated white blood cell counts with high neutrophil despite suppressed eosinophil and lymphoid cells [59, 60], shortened

Table 1 Sensitivity and specificity of dynamic test for differentiating from NNH or EAS

		Sensitivity (%)	95% CI	Specificity (%)	95% CI	OR (%)	95% CI
CD *vs.* NNH	Dex-CRH test	97	88–99	92	8–96	138.83	49.38–390.32
	Desmopressin test (10 mg)	88	77–95	94	83–98	110.44	32.13–379.63
CD *vs.* EAS	CRH test	86.9	82.1–90.6	93.9	87.0–98.3	57.88	43.25–77.47
	HDDST	80.8	75.1–85.5	84.5	79.6–88.4	15.82	11.1–22.55
	Desmopressin test (10 mg)	84.8	81.4–87.8	69.9	61.4–77.3	11.4	5.86–22.16
	Desmopressin test (4 mg)	86		55.6			

CD: Cushing's disease, NNH: non-neoplastic hypercortisolism, EAS, ectopic ACTH syndrome, Dex-CRH: corticotropin releasing hormone after dexamethasone suppression test, HDDST: high dose dexamethasone suppression test.

activated partial thromboplastin time (aPTT) [61, 62], and low albumin levels [63]. These can help determine the presence or absence of Cushing's syndrome.

Differentiating Cushing's syndrome from NNH is sometimes difficult and requires further dynamic tests, including CRH after Dex suppression test (Dex-CRH), and desmopressin test. Sensitivity and specificity of these dynamic tests are shown in Table 1. These tests aim to determine whether the source of ACTH is a pituitary tumor or a normal pituitary gland, and their principle is to utilize glucocorticoid negative feedback resistance and high expression of vasopressin receptors type 1b (AVPR1b) in tumors. From this perspective, we investigated the characteristics of Cushing's disease patients who showed responsiveness to the desmopressin test (4 μg) (which is not covered by insurance in Japan) and those who showed negative results. When an increase in ACTH of 1.5 times or more was considered positive [55], 15 out of 47 cases showed a negative result in the desmopressin test, which was more frequent in men. In reactive patients, *USP8* pathogenic variants are more common in tumors, which may increase *AVPR1b* expression, and desmopressin test results should be interpreted with this in mind [20]. If these tests are negative but there is a clear Cushingoid appearance or a history of these findings, careful follow-up is required in consideration of cyclic Cushing's syndrome [40, 41].

Next step after diagnosis of Cushing's syndrome

Once the presence of Cushing's syndrome is confirmed, physicians need to determine whether hypercortisolemia is severe enough to be life-threatening, presenting infectious, psychiatric, metabolic, cardiovascular, and thromboembolic complications. If it seems severe, or a certain amount of time is needed before finalizing further evaluation or performing surgical treatment, physicians should consider prioritizing a correction of hypercortisolemia (see "Management in the initial phase" below).

Further evaluation aims to determine the localization of the tumor responsible for Cushing's syndrome. ACTH-independent Cushing's syndrome is relatively easy to localize with both hormonal evaluation and imaging findings. In contrast, differentiating between Cushing's disease and ectopic ACTH syndrome remains clinically challenging. It is also important to note that the tests available and recommended vary depending on the country and guidelines. There are several recommended confirmatory tests, including CRH test, desmopressin test, MRI, and intra petrosal sinus sampling (IPSS). However, CRH testing is now unavailable in Western countries due to breakdowns in key manufacturing facilities, while insurance does not cover the desmopressin test in Japan. High-dose (8 mg) Dex suppression test (HDDST) is also an important confirmatory test that has long been used. However, it is no longer included in the algorithm in the Pituitary Society's Guideline due to its low accuracy and the additional hormonal burden placed on patients with glucocorticoid overload [54]. However, according to the systemic review and meta-analysis of these dynamic tests, HDDST has the second highest sensitivity and specificity (80.8 [75.1–85.5], 84.5 [79.6–88.4]) following the CRH test (86.9 [82.1–90.6], 93.9 [87.0–98.3]), while the results of the desmopressin test are inferior (84.8 [81.4–87.8], 69.9 [61.4–77.3]). This also applies to diagnostic odds ratios (Table 1) [64]. It must be noted that these results are influenced by various factors, such as differences in the criteria used in each study and the number of studies. In Japan, Dex 0.5 mg is used for LDDST as a test showing higher specificity than Dex 1 mg (100 *vs.* 96), and the desmopressin test is also proposed as a test with a smaller amount of 4 μg, which is comparable [55, 65]. Further verification of these tests is necessary in order to identify tests that can provide sufficient results with a smaller amount of test reagents.

Management in the Initial Phase

Pre-operative management for complications

Some of the most severe complications of active Cushing's syndrome include infection, acute cardiovascular

events, pulmonary embolism, and suicide [66]. Therefore, in severe hypercortisolemia, or when suspecting a life-threating state, immediate initiation of treatment is required under a strict management system with an experienced endocrinologist (Fig. 4) [54, 67].

Since pneumocystis pneumonia (PcP) has a high mortality rate (20–60%), initiation of trimethoprim-sulfamethoxazole (TMP-SMX) for prophylaxis of *pneumocystis jirovecii* is recommended in such conditions, especially in patients who are seriously ill and/or have UFC levels more than 5 to 10-fold the upper-normal limit, and those with lymphopenia, especially when the number of $CD4^+$ T cells is less than 200 /mm^3 based on data for HIV patients. Caution is required as a sudden drop in serum cortisol concentration may cause immune reconstitution inflammatory syndrome (IRIS), leading to PcP (Fig. 5). According to the previous case series, the risk of PcP increases just after initiating cortisol inhibitors, with PcP onset after an average of 5.5 days [68], making it essential to start prophylaxis medications in advance in high-risk patients. However, PcP development is also reported without cortisol suppression, suggesting hypercortisolemia itself could be a risk factor for PcP. Although there is little evidence regarding the timing of discontinuation of this prophylactic drug, it is considered desirable to continue it after curative surgery or normalization of hypercortisolemia with medication for at least 2–6 weeks [68, 69].

The prevalence of venous thromboembolism (VTE) events in patients with Cushing's disease has been shown as 14.6 per 1,000 person-years, which is a more than 10 times higher risk than in normal subjects [70]. In the currently largest Cushing's syndrome patient database, the European Registry on Cushing's Syndrome (ERCUSYN), VTE risk in Cushing's syndrome was analyzed, showing that predictors include: male sex, high UFC levels, and

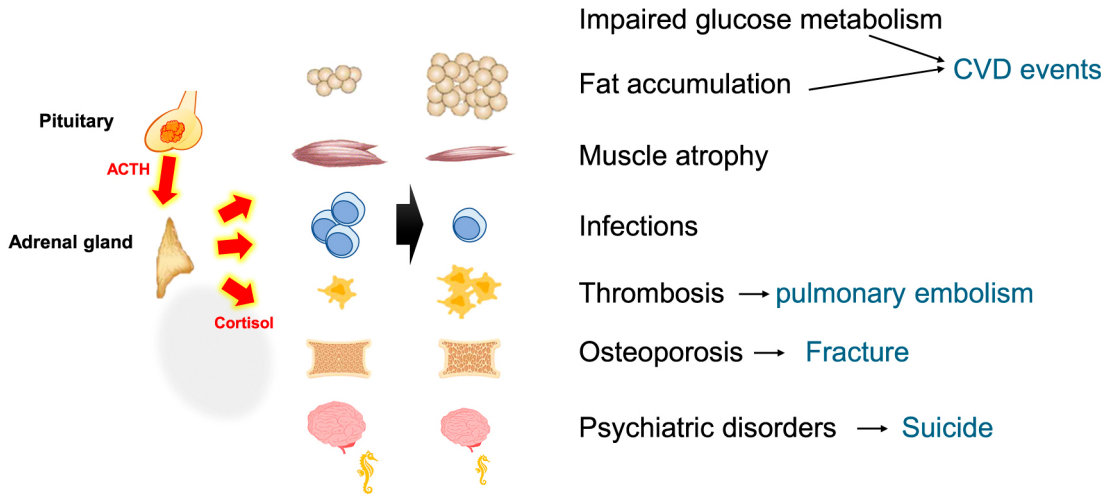


Fig. 4 Complications induced by ACTH hypersecretion mediated *via* cortisol excess
ACTH hypersecretion from pituitary tumors induces hyperplasia of the adrenal cortex and causes excessive secretion of cortisol. These affect several tissues such as fat, muscle, lymphocytes, coagulation system, bones, and brain. These result in impaired glucose metabolism, fat accumulation, muscle atrophy, infections, thrombosis, osteoporosis, and psychiatric disorders.

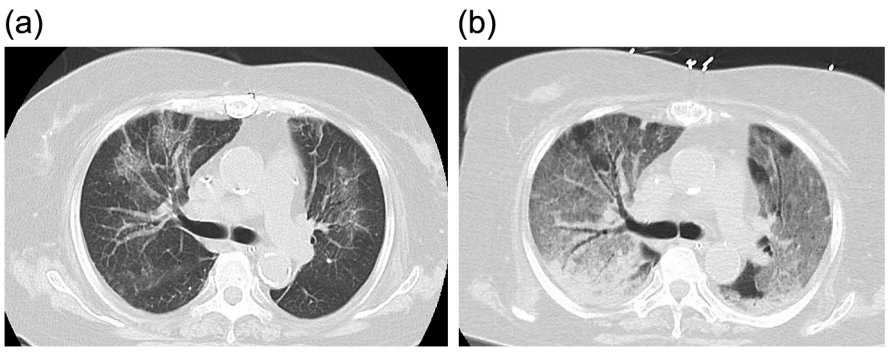


Fig. 5 CT scan imaging of pneumocystis pneumonia development in patient with Cushing's disease after metyrapone initiation
(a) Day 6 after initiation of metyrapone, (b) Day 11 after initiation of metyrapone.

repeat operation [71]. In both reports, the VTE is more common in Cushing's disease than adrenal Cushing's syndrome, especially in the perioperative period. Regarding prophylactic anticoagulants, fewer than half of the facilities participating in the ERCUSYN registry routinely prescribed them. Currently, the indication criteria for initiating anticoagulant treatment in this condition are not shown in any treatment guidelines about Cushing's syndrome and are still in debate. Also, no standardized method exists for the routine preoperative detection of VTE in patients with Cushing's disease. However, plasma D-dimer measurement is widely used as a screening tool. In cases of elevated D-dimer levels, lower extremity venous ultrasound serves as the next step for confirmatory diagnosis. It is important to acknowledge that plasma D-dimer levels may increase due to hospital stay alone. It has been shown that the mechanism of hypercoagulation caused by excess glucocorticoids is an increase in von Willebrand Factor (vWF) in the coagulation system and also plasminogen activator inhibitor type 1 (PAI-1), which suppresses the fibrinolytic system. From this perspective, D-dimer, commonly used as a biomarker for deep vein thrombosis, is undervalued in this pathology, leading to a difficulty in detecting early development [61].

Pre-operative management for hypercortisolemia

Correcting hypercortisolemia before surgery was performed only in 20% of cases in the ERCUSYN registry, and was more in Cushing's disease (23%) than in adrenal Cushing's syndrome (7%) [72]. Characteristics of these patients include a high prevalence of signs related to hypercortisolemia, hypertension, impaired QoL score, and tumors not visible on MRI. There are concerns that it has not led to improved post-operative remission rate as a surgical outcome and that pre-operative treatment restores ACTH secretion from normal corticotroph, which may complicate the determination of surgical cure. Therefore, pre-operative treatment is not routinely recommended by most treatment guidelines. However, this may benefit patients with potentially life-threatening complications with severe hypercortisolemia; those with difficult-to-control complications, including psychiatric disorders, thrombosis, and infections; patients with significantly decreased QoL; and patients who may take time until surgery [54, 66].

It is noteworthy that high cortisol is known to cause irreversible atrophy in the hippocampus, amygdala, and prefrontal cortex, in addition to enlarged ventricles [73, 74]. After surgical remission for Cushing's syndrome, previous reports indicate that some patients' cognitive ability remains decreased while others show improvement, and this point requires further investigation. However, the decline in QoL continues over a long period of time in post-operative patients with this disease, and the period of exposure to hypercortisolemia has been shown to be a risk factor [75]. Considering the time required for tumor localization, I believe there may be an advantage in initiating pre-operative medical treatment immediately after diagnosis. Furthermore, very few studies have evaluated outcomes other than postoperative remission for preoperative medical treatment, and this point should be considered in the future.

Treatment for Cushing's disease is mainly divided into three targets: pituitary tumor, adrenal gland, and peripheral glucocorticoid target organs. As pre-operative treatment drugs, steroidogenesis inhibitors including ketoconazole and metyrapone, dopamine receptor agonist cabergoline, glucocorticoid receptor antagonist mifepristone, and their combinations have been previously reported and now levoketoconazole, rather than ketoconazole, and osilodrostat have also been added as candidate drugs, while cabergoline, ketoconazole, levoketoconazole, and mifepristone are not covered by insurance in Japan at this moment. The most commonly used drugs reported so far are ketoconazole and metyrapone, an inhibitor of CYP11B2 and CYP11B1 [72]. There are two treatment regimens for steroidogenesis inhibitors, namely "Titration to normalization" and "Block-and-replace." Generally, block-and-replace regimens, which include a combination of steroidogenesis inhibitor and glucocorticoid replacement, are used for severe hypercortisolism or cyclic Cushing's syndrome [76]. At the initiation of steroidogenesis inhibitors, the risk of PcP induced by a sudden drop in hypercortisolemia needs to be noted. It is difficult to monitor the effect of these steroidogenesis inhibitors due to the lack of a gold standard biomarker. Early morning serum cortisol levels, 24-hour UFC levels, late night salivary cortisol levels, and multiple daily serum cortisol levels are frequently used for the assessment of drug efficacy in many studies, especially during titration to normalization regimens [76, 77]. We have reported multiple measurements of salivary cortisol levels during titration to normalization regimens as a useful biomarker. In this report, the dosage and timing of the drug were determined based on the suppression rate of salivary cortisol levels and the time to reach the nadir, which was proposed as a new method to normalize UFC levels [78]. In the block-and-replacement regimen, monitoring cortisol levels is quite difficult except when using dexamethasone as a replacement therapy. Also, determining the adequate dose for replacement is challenging, especially in this initial management phase. To date, there are limited reports describing the use of osilodrostat, which is a recently approved steroidogenic inhibitor thought to suppress not only

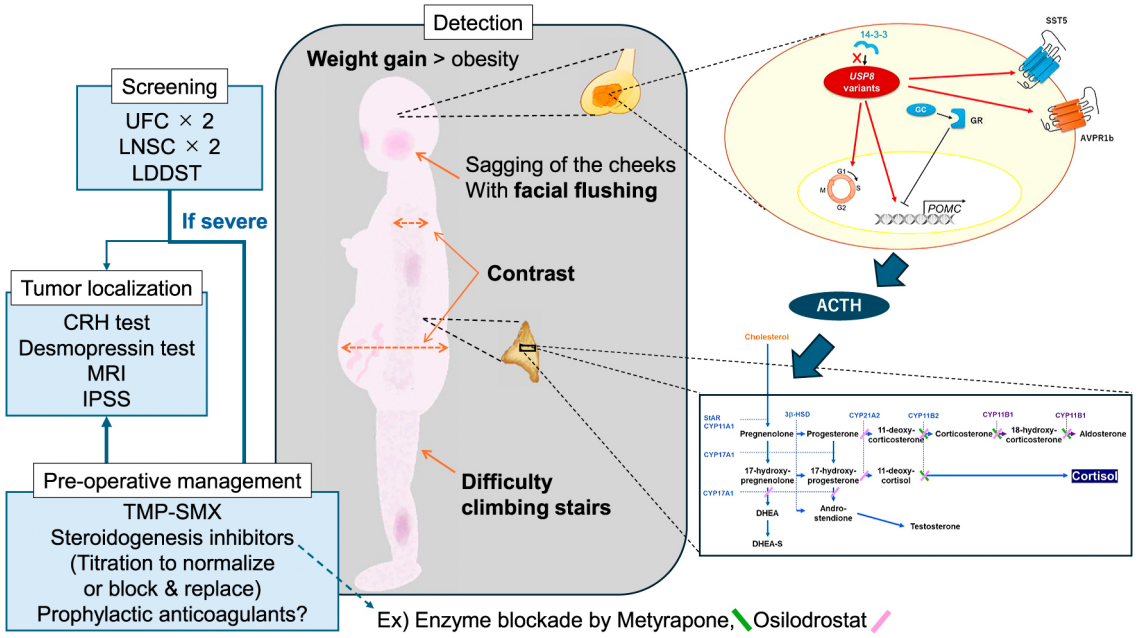
CYP11B2 and CYP11B1 but also CYP21A2 and CYP17A1 as preoperative treatment. However, this drug is believed to offer more reliable and specific cortisol suppression, and an increase in real-world data is anticipated [72, 79]. Again, optimal pre-operative medical treatment is still in debate. Undoubtedly, the endocrinologist's mission is to transport patients to the best expert pituitary surgeon in the safest and best way possible. For the treatment of inoperable Cushing's disease for some reason, drug therapy targeting ACTH-producing tumors, such as pasireotide and cabergoline, may be used first or in combination with steroid synthesis inhibitors. If these drugs could suppress tumor-specific ACTH secretion, they would be a more essential treatment and would likely shrink the tumor simultaneously. Therefore, their use should be considered, especially in cases where long-term management is required.

Future Perspective

Despite the recent development of diagnostic and treatment guidelines and their updates, many concerns remain regarding initial phase management of patients with Cushing's disease. First, to shorten the current 38 months required for diagnosis, efforts should be made to make the public and non-specialists, especially the departments that serve as the points of contact for this rare disease, aware of it, and to increase the number of endocrinologists. In addition, it is considered necessary to promote the development of a digital diagnosis system as a breakthrough in the future for the initial detection of this disease. Hormone evaluation after detection can be relatively difficult, even for endocrinologists, especially in atypical cases. Therefore, new diagnostic biomarkers, such as those reflected by hypercortisolemia, and tumor-specific markers, will be needed in the future. Markers that reflect such cortisol effects can also be used as monitoring markers during treatment. To continue the molecular, technological, and engineering developments that have been made to date, further breakthroughs must be opened up by fully using the remarkable developments made possible in information science, artificial intelligence, and not only in genetics but also in epigenomes. This is expected to improve the prognosis of this rare disease and lead to applications in various fields related to the HPA axis.

Conclusions

Cushing's disease is a rare disease that has seen remarkable progress in diagnosis and treatment over the hundred years since its concept was first proposed, leading to significant improvements in its prognosis today. However, among pituitary diseases, it is a disease that still has many clinically challenging issues. It takes time to detect, many hormone assessments and specialized tests are required to achieve a diagnosis, and complex judgments are required in these tests. Complications due to hypercortisolemia are frequently significant and sometimes life-threatening, requiring prompt and comprehensive management. In this review, I have presented the management of this initial phase as sequentially as in the real approach (Graphical Abstract). I hope that this



Graphical Abstract

review will assist in the treatment of this disease and raise issues for future research.

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References

1. Cushing H (1932) The basophil adenomas of the pituitary body and their clinical manifestations (pituitary basophilism). *Bull Johns Hopkins Hosp* 50: 137–195.
2. Mason HL, Meyers CS, Kendall EC (1936) Chemical studies of the suprarenal cortex: II. The identification of a substance which possesses the qualitative action of cortin; its conversion into a diketone closely related to androstenedione. *J Biol Chem* 116: 267–276.
3. Li CH, Geschwind II, Levy AL, Harris JI, Dixon JS, *et al.* (1954) Isolation and properties of alpha-corticotrophin from sheep pituitary glands. *Nature* 173: 251–253.
4. Melmed S, Kaiser UB, Lopes MB, Bertherat J, Syro LV, *et al.* (2022) Clinical biology of the pituitary adenoma. *Endocr Rev* 43: 1003–1037.
5. Lacroix A, Feelders RA, Stratakis CA, Nieman LK (2015) Cushing's syndrome. *Lancet* 386: 913–927.
6. Fleseriu M, Varlamov EV, Hinojosa-Amaya JM, Langlois F, Melmed S (2023) An individualized approach to the management of Cushing disease. *Nat Rev Endocrinol* 19: 581–599.
7. Ragnarsson O, Olsson DS, Papakokkinou E, Chantzichristos D, Dahlqvist P, *et al.* (2019) Overall and disease-specific mortality in patients with cushing disease: a swedish nationwide study. *J Clin Endocrinol Metab* 104: 2375–2384.
8. Melmed S (2020) Pituitary-tumor endocrinopathies. *N Engl J Med* 382: 937–950.
9. Webb SM, Santos A, Resmini E, Martínez-Momblán MA, Martel L, *et al.* (2018) Quality of life in Cushing's disease: a long term issue? *Ann Endocrinol (Paris)* 79: 132–137.
10. Jacks T, Fazeli A, Schmitt EM, Bronson RT, Goodell MA, *et al.* (1992) Effects of an Rb mutation in the mouse. *Nature* 359: 295–300.
11. Kiyokawa H, Kineman RD, Manova-Todorova KO, Soares VC, Hoffman ES, *et al.* (1996) Enhanced growth of mice lacking the cyclin-dependent kinase inhibitor function of p27(Kip1). *Cell* 85: 721–732.
12. Pellegata NS, Quintanilla-Martinez L, Siggelkow H, Samson E, Bink K, *et al.* (2006) Germ-line mutations in p27Kip1 cause a multiple endocrine neoplasia syndrome in rats and humans. *Proc Natl Acad Sci U S A* 103: 15558–15563.
13. Roussel-Gervais A, Bilodeau S, Vallette S, Berthelet F, Lacroix A, *et al.* (2010) Cooperation between cyclin E and p27(Kip1) in pituitary tumorigenesis. *Mol Endocrinol* 24: 1835–1845.
14. Theodoropoulou M, Arzberger T, Gruebler Y, Jaffrain-Rea ML, Schlegel J, *et al.* (2004) Expression of epidermal growth factor receptor in neoplastic pituitary cells: evidence for a role in corticotropinoma cells. *J Endocrinol* 183: 385–394.
15. Fukuoka H, Cooper O, Ben-Shlomo A, Mamelak A, Ren SG, *et al.* (2011) EGFR as a therapeutic target for human, canine, and mouse ACTH-secreting pituitary adenomas. *J Clin Invest* 121: 4712–4721.
16. Araki T, Liu X, Kameda H, Tone Y, Fukuoka H, *et al.* (2017) EGFR induces E2F1-mediated corticotroph tumorigenesis. *J Endocr Soc* 1: 127–143.
17. Reincke M, Sbiera S, Hayakawa A, Theodoropoulou M, Osswald A, *et al.* (2015) Mutations in the deubiquitinase gene USP8 cause Cushing's disease. *Nat Genet* 47: 31–38.
18. Ma ZY, Song ZJ, Chen JH, Wang YF, Li SQ, *et al.* (2015) Recurrent gain-of-function USP8 mutations in Cushing's disease. *Cell Res* 25: 306–317.
19. Weigand I, Knobloch L, Flitsch J, Saeger W, Monoranu CM, *et al.* (2019) Impact of USP8 gene mutations on protein deregulation in Cushing disease. *J Clin Endocrinol Metab* 104: 2535–2546.
20. Shichi H, Fukuoka H, Kanzawa M, Yamamoto M, Yamamoto N, *et al.* (2022) Responsiveness to DDAVP in Cushing's disease is associated with USP8 mutations through enhancing AVPR1B promoter activity. *Pituitary* 25: 496–507.
21. Perez-Rivas LG, Theodoropoulou M, Ferraù F, Nusser C, Kawaguchi K, *et al.* (2015) The gene of the ubiquitin-specific protease 8 is frequently mutated in adenomas causing Cushing's disease. *J Clin Endocrinol Metab* 100: E997–E1004.
22. Hayashi K, Inoshita N, Kawaguchi K, Ibrahim Ardisasmita A, Suzuki H, *et al.* (2016) The USP8 mutational status may predict drug susceptibility in corticotroph adenomas of Cushing's disease. *Eur J Endocrinol* 174: 213–226.
23. Losa M, Mortini P, Pagnano A, Detomas M, Cassarino MF, *et al.* (2019) Clinical characteristics and surgical outcome in USP8-mutated human adrenocorticotropic hormone-secreting pituitary adenomas. *Endocrine* 63: 240–246.

24. Sesta A, Cassarino MF, Terreni M, Ambrogio AG, Libera L, *et al.* (2020) Ubiquitin-specific protease 8 mutant corticotrope adenomas present unique secretory and molecular features and shed light on the role of ubiquitylation on ACTH processing. *Neuroendocrinology* 110: 119–129.
25. Chen J, Jian X, Deng S, Ma Z, Shou X, *et al.* (2018) Identification of recurrent USP48 and BRAF mutations in Cushing's disease. *Nat Commun* 9: 3171.
26. Sbiera S, Perez-Rivas LG, Taranets L, Weigand I, Flitsch J, *et al.* (2019) Driver mutations in USP8 wild-type Cushing's disease. *Neuro Oncol* 21: 1273–1283.
27. Nadhamuni VS, Korbonits M (2020) Novel insights into pituitary tumorigenesis: genetic and epigenetic mechanisms. *Endocr Rev* 41: 821–846.
28. Abraham AP, Pai R, Beno DL, Chacko G, Asha HS, *et al.* (2022) USP8, USP48, and BRAF mutations differ in their genotype-phenotype correlation in Asian Indian patients with Cushing's disease. *Endocrine* 75: 549–559.
29. Miao H, Liu Y, Lu L, Gong F, Wang L, *et al.* (2021) Effect of 3 NR3C1 mutations in the pathogenesis of pituitary ACTH adenoma. *Endocrinology* 162: bqab167.
30. Chen Z, Jia Q, Zhao Z, Zhang Q, Chen Y, *et al.* (2022) Transcription factor ASCL1 acts as a novel potential therapeutic target for the treatment of the Cushing's disease. *J Clin Endocrinol Metab* 107: 2296–2306.
31. Uzilov AV, Taik P, Cheesman KC, Javanmard P, Ying K, *et al.* (2021) USP8 and TP53 drivers are associated with CNV in a corticotroph adenoma cohort enriched for aggressive tumors. *J Clin Endocrinol Metab* 106: 826–842.
32. Perez-Rivas LG, Simon J, Albani A, Tang S, Roeber S, *et al.* (2022) TP53 mutations in functional corticotroph tumors are linked to invasion and worse clinical outcome. *Acta Neuropathol Commun* 10: 139.
33. Casar-Borota O, Boldt HB, Engström BE, Andersen MS, Baussart B, *et al.* (2021) Corticotroph aggressive pituitary tumors and carcinomas frequently harbor ATRX mutations. *J Clin Endocrinol Metab* 106: 1183–1194.
34. Asuzu DT, Alvarez R, Fletcher PA, Mandal D, Johnson K, *et al.* (2022) Pituitary adenomas evade apoptosis *via* noxa deregulation in Cushing's disease. *Cell Rep* 40: 111223.
35. Lindholm J, Juul S, Jørgensen JO, Astrup J, Bjerre P, *et al.* (2001) Incidence and late prognosis of cushing's syndrome: a population-based study. *J Clin Endocrinol Metab* 86: 117–123.
36. Arnardóttir S, Sigurjonsdóttir HA (2011) The incidence and prevalence of Cushing's disease may be higher than previously thought: results from a retrospective study in Iceland 1955 through 2009. *Clin Endocrinol (Oxf)* 74: 792–793.
37. Wengander S, Trimpou P, Papakokkinou E, Ragnarsson O (2019) The incidence of endogenous Cushing's syndrome in the modern era. *Clin Endocrinol (Oxf)* 91: 263–270.
38. Agustsson TT, Baldvinsdottir T, Jonasson JG, Olafsdottir E, Steinthorsdottir V, *et al.* (2015) The epidemiology of pituitary adenomas in Iceland, 1955–2012: a nationwide population-based study. *Eur J Endocrinol* 173: 655–664.
39. Takayanagi R, Miura K, Nakagawa H, Nawata H (2000) Epidemiologic study of adrenal gland disorders in Japan. *Biomed Pharmacother* 54 Suppl 1: 164s–168s.
40. Alexandraki KI, Kaltsas GA, Isidori AM, Akker SA, Drake WM, *et al.* (2009) The prevalence and characteristic features of cyclicity and variability in Cushing's disease. *Eur J Endocrinol* 160: 1011–1018.
41. Nowak E, Vogel F, Albani A, Braun L, Rubinstein G, *et al.* (2023) Diagnostic challenges in cyclic Cushing's syndrome: a systematic review. *Lancet Diabetes Endocrinol* 11: 593–606.
42. Steffensen C, Pereira AM, Dekkers OM, Jørgensen JO (2016) Diagnosis of Endocrine Disease: Prevalence of hypercortisolism in type 2 diabetes patients: a systematic review and meta-analysis. *Eur J Endocrinol* 175: R247–R253.
43. Anderson GH Jr, Blakeman N, Streeten DH (1994) The effect of age on prevalence of secondary forms of hypertension in 4429 consecutively referred patients. *J Hypertens* 12: 609–615.
44. Omura M, Saito J, Yamaguchi K, Kakuta Y, Nishikawa T (2004) Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan. *Hypertens Res* 27: 193–202.
45. Ness-Abramof R, Nabriski D, Apovian CM, Niven M, Weiss E, *et al.* (2002) Overnight dexamethasone suppression test: a reliable screen for Cushing's syndrome in the obese. *Obes Res* 10: 1217–1221.
46. Tsujimoto Y, Shichi H, Fukuoka H, Yamamoto M, Sato I, *et al.* (2021) Tumor shrinkage by metyrapone in Cushing disease exhibiting glucocorticoid-induced positive feedback. *J Endocr Soc* 5: bvab055.
47. Rubinstein G, Osswald A, Hoster E, Losa M, Elenkova A, *et al.* (2020) Time to diagnosis in Cushing's syndrome: a meta–analysis based on 5367 patients. *J Clin Endocrinol Metab* 105: dgz136.
48. Kreitschmann-Andermahr I, Psaras T, Tsiogka M, Starz D, Kleist B, *et al.* (2015) From first symptoms to final diagnosis of Cushing's disease: experiences of 176 patients. *Eur J Endocrinol* 172: 285–289.
49. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, *et al.* (2008) The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 93: 1526–1540.
50. Braun LT, Vogel F, Zopp S, Seiter TM, Rubinstein G, *et al.* (2022) Whom should we screen for Cushing syndrome? The endocrine society practice guideline recommendations 2008 revisited. *J Clin Endocrinol Metab* 107: e3723–e3730.
51. Motomura Y, Urai S, Bando H, Yamamoto M, Suzuki M, *et al.* (2024) Diagnostic dilemma in Cushing's syndrome: discrepancy between patient-reported and physician-assessed manifestations. *Endocrine* 86: 417–427.
52. Stratakis CA (2016) Skin manifestations of Cushing's syndrome. *Rev Endocr Metab Disord* 17: 283–286.
53. Erden F, Borlu M, Simsek Y, Kelestemur HF (2019)

Differences in skin lesions of endogenous and exogenous Cushing's patients. *Postepy Dermatol Alergol* 36: 272–275.
54. Fleseriu M, Auchus R, Bancos I, Ben-Shlomo A, Bertherat J, *et al.* (2021) Consensus on diagnosis and management of Cushing's disease: a guideline update. *Lancet Diabetes Endocrinol* 9: 847–875.
55. Kageyama K, Oki Y, Sakihara S, Nigawara T, Terui K, *et al.* (2013) Evaluation of the diagnostic criteria for Cushing's disease in Japan. *Endocr J* 60: 127–135.
56. Findling JW, Raff H (2017) Diagnosis of endocrine disease: differentiation of pathologic/neoplastic hypercortisolism (Cushing's syndrome) from physiologic/non-neoplastic hypercortisolism (formerly known as pseudo-Cushing's syndrome). *Eur J Endocrinol* 176: R205–R216.
57. Scaroni C, Albiger NM, Palmieri S, Iacuaniello D, Graziadio C, *et al.* (2020) Approach to patients with pseudo-Cushing's states. *Endocr Connect* 9: R1–R13.
58. Brien TG (1981) Human corticosteroid binding globulin. *Clin Endocrinol (Oxf)* 14: 193–212.
59. Bando H, Kanie K, Yamamoto M, Fukuoka H (2023) High cortisol concentration without cushingoid appearance. *Am J Med* 136: e43–e44.
60. Hwang S, Tatsi C, Kuehn HS, Niemela JE, Stoddard J, *et al.* (2022) Cushing syndrome and glucocorticoids: T-cell lymphopenia, apoptosis, and rescue by IL-21. *J Allergy Clin Immunol* 149: 302–314.
61. Van Zaane B, Nur E, Squizzato A, Dekkers OM, Twickler MT, *et al.* (2009) Hypercoagulable state in Cushing's syndrome: a systematic review. *J Clin Endocrinol Metab* 94: 2743–2750.
62. Fukuoka H, Takeuchi T, Matsumoto R, Bando H, Suda K, *et al.* (2014) D-dimer as a significant marker of deep vein thrombosis in patients with subclinical or overt Cushing's syndrome. *Endocr J* 61: 1003–1010.
63. Putignano P, Kaltsas GA, Korbonits M, Jenkins PJ, Monson JP, *et al.* (2000) Alterations in serum protein levels in patients with Cushing's syndrome before and after successful treatment. *J Clin Endocrinol Metab* 85: 3309–3312.
64. Ceccato F, Barbot M, Mondin A, Boscaro M, Fleseriu M, *et al.* (2023) Dynamic testing for differential diagnosis of ACTH-dependent Cushing syndrome: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 108: e178–e188.
65. Suda T, Kageyama K, Nigawara T, Sakihara S (2009) Evaluation of diagnostic tests for ACTH-dependent Cushing's syndrome. *Endocr J* 56: 469–476.
66. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, *et al.* (2016) Complications of Cushing's syndrome: state of the art. *Lancet Diabetes Endocrinol* 4: 611–629.
67. Nieman LK, Biller BM, Findling JW, Murad MH, Newell-Price J, *et al.* (2015) Treatment of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 100: 2807–2831.
68. Lugat A, Lasolle H, François M, Benhenda N, Bricaire L, *et al.* (2023) Pneumocystis pneumonia in patients with Cushing's syndrome: a French multicenter retrospective study. *Ann Endocrinol (Paris)* 84: 37–44.
69. Varlamov EV, Langlois F, Vila G, Fleseriu M (2021) Management of endocrine disease: cardiovascular risk assessment, thromboembolism, and infection prevention in Cushing's syndrome: a practical approach. *Eur J Endocrinol* 184: R207–R224.
70. Stuijver DJ, van Zaane B, Feelders RA, Debeij J, Cannegieter SC, *et al.* (2011) Incidence of venous thromboembolism in patients with Cushing's syndrome: a multicenter cohort study. *J Clin Endocrinol Metab* 96: 3525–3532.
71. Beuschlein F, Else T, Bancos I, Hahner S, Hamidi O, *et al.* (2024) European Society of Endocrinology and Endocrine Society Joint Clinical Guideline: diagnosis and therapy of glucocorticoid-induced adrenal insufficiency. *J Clin Endocrinol Metab* 109: 1657–1683.
72. Valassi E, Franz H, Brue T, Feelders RA, Netea-Maier R, *et al.* (2018) Preoperative medical treatment in Cushing's syndrome: frequency of use and its impact on postoperative assessment: data from ERCUSYN. *Eur J Endocrinol* 178: 399–409.
73. Andela CD, van Haalen FM, Ragnarsson O, Papakokkinou E, Johannsson G, *et al.* (2015) Mechanisms in endocrinology: Cushing's syndrome causes irreversible effects on the human brain: a systematic review of structural and functional magnetic resonance imaging studies. *Eur J Endocrinol* 173: R1–R14.
74. Papakokkinou E, Ragnarsson O (2023) Functional brain alterations in Cushing's syndrome. *Front Endocrinol (Lausanne)* 14: 1163482.
75. Pupier E, Santos A, Etchamendy N, Lavielle A, Ferriere A, *et al.* (2022) Impaired quality of life, but not cognition, is linked to a history of chronic hypercortisolism in patients with Cushing's disease in remission. *Front Endocrinol (Lausanne)* 13: 934347.
76. Castinetti F, Nieman LK, Reincke M, Newell-Price J (2021) Approach to the patient treated with steroidogenesis inhibitors. *J Clin Endocrinol Metab* 106: 2114–2123.
77. Newell-Price J, Pivonello R, Tabarin A, Fleseriu M, Witek P, *et al.* (2020) Use of late-night salivary cortisol to monitor response to medical treatment in Cushing's disease. *Eur J Endocrinol* 182: 207–217.
78. Yoshida K, Fukuoka H, Odake Y, Nakajima S, Tachibana M, *et al.* (2017) Multiple salivary cortisol measurements are a useful tool to optimize metyrapone treatment in patients with Cushing's syndromes treatment: case presentations. *Front Endocrinol (Lausanne)* 8: 375.
79. Fleseriu M, Biller BMK (2022) Treatment of Cushing's syndrome with osilodrostat: practical applications of recent studies with case examples. *Pituitary* 25: 795–809.