

REVIEW

Evaluation of the diagnostic criteria for Cushing's disease in Japan

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Abstract. Adrenocorticotrophic hormone (ACTH)-dependent Cushing's syndrome is caused by an ACTH-producing tumor, as is the case with Cushing's disease and ectopic ACTH syndrome (EAS). Diagnosis and differential diagnosis of Cushing's disease from EAS in ACTH-dependent Cushing's syndrome are thus challenging problems in clinical endocrinology. The diagnostic criteria for Cushing's disease in Japan, established by the working group of the Japan Ministry of Health, Labour and Welfare, were originally reported in 2003 and revised in 2007 and 2010. In addition, criteria for subclinical Cushing's disease were established in Japan in 2010. In this review, we evaluate the usefulness and accuracy of the most recent diagnostic criteria. Previous data suggest that as an initial test of Cushing's syndrome, 0.5 mg dexamethasone is more sensitive than 1 mg in the overnight dexamethasone suppression test (DST). Here, we recommend 0.5 mg plus a plasma cortisol cut-off level of 3 µg/dL as a suitable low-dose overnight DST for screening of all cases of ACTH-dependent Cushing's syndrome in Japan. Recently, standardization of cortisol measurements by the ID-LC/MS/MS method using seven assay kits with standard plasma material containing synthetic hydrocortisone-d4 was carried out in Japan. The resulting relative standard deviation was within 10%. The cut-off value remains valid even after standardization of plasma cortisol measurements. Although the recent diagnostic criteria achieve higher diagnostic specificity, care should be taken since data for Cushing's disease partially overlaps with some cases of EAS. Overall, therefore, this review suggests that the accuracy of each diagnostic test should be considered.

Key words: ACTH, Cortisol, Cushing's disease, Pituitary, Ectopic ACTH syndrome

CUSHING'S SYNDROME results from chronic glucocorticoid excess with accompanying clinically characteristic signs and symptoms [1]. Adrenocorticotrophic hormone (ACTH)-dependent Cushing's syndrome includes Cushing's disease and ectopic ACTH syndrome (EAS). However, while Cushing's disease is primarily caused by a pituitary ACTH-secreting tumor, EAS is considered to result from extra-pituitary ACTH-secreting tumors such as bronchial carcinoid, thymic carcinoid and pheochromocytoma [2]. Differential diagnosis of Cushing's disease from EAS in ACTH-dependent Cushing's syndrome is thus a challenging problem in clinical endocrinology.

Diagnostic criteria for Cushing's disease were established by the working group of the Ministry of Health, Labour, and Welfare, Japan [3, 4]. Diagnostic criteria were first reported in 2003 then in 2007 and 2010 (Table 1). In the most recent guidelines, the following endocrinological findings are considered diagnostic criteria: 1. a Cushingoid appearance; 2. basal levels of ACTH and cortisol showing (1) normal-high plasma ACTH and cortisol levels and (2) high levels of free cortisol in the urine; 3. screening tests showing (1) incomplete suppression of plasma cortisol levels (> 5 µg/dL) in a low-dose (0.5 mg) overnight dexamethasone suppression test (DST), (2) high plasma cortisol levels (> 5 µg/dL) during night-time sleep, and (3) response of plasma ACTH levels to the desmopressin (DDAVP) test; and (4) high salivary cortisol levels (> 1.5, compared with the mean level) during night-time sleep. 4. Moreover, differential diagnosis of Cushing's disease from EAS includes (1) a normal or exaggerated

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Table 1 Revised diagnostic criteria for diagnosis of Cushing's disease according to the Ministry of Health, Labour, and Welfare, Japan (2010).

1. Cushingoid appearance
(1) Typical features
Moon face: Central obesity or dorsocervical fat pad (buffalo hump): Purple striae:
Thin skin and easy bruising: Proximal myopathy
In children, a decrease in weight gain velocity with obesity
(2) Atypical features
Hypertension: Menstrual abnormalities: Acne: Hirsutism: Peripheral edema:
Impaired glucose tolerance: Osteoporosis: Pigmentation: Mental abnormalities
Presence of more than one typical or atypical feature.
2. Basal levels of ACTH and cortisol
(1) Normal-high plasma ACTH and cortisol levels
(2) High levels of urinary free cortisol
2-(1) is necessary.
Perform screening tests when 1 and 2 are positive.
3. Screening tests
(1) Incomplete suppression of plasma cortisol levels with a low-dose (0.5 mg) overnight dexamethasone suppression test (DST)
(2) High plasma cortisol levels (> 5 µg/dL) during night-time sleep
(3) Response of plasma ACTH levels to the desmopressin (DDAVP) test (>50% increase in plasma ACTH levels after test)
(4) High salivary cortisol levels (> 1.5, compared with the mean level of each institute) during night-time sleep
Perform differential diagnosis when (1) and one other test are positive.
4. Differential diagnosis of Cushing's disease from EAS
(1) Normal or exaggerated response of plasma ACTH levels to the human CRH test (>50% increase in plasma ACTH levels after test)
(2) Suppression of plasma cortisol levels (less than half, compared with the basal level) with a high-dose (8 mg) overnight DST
(3) The presence of a pituitary adenoma in MRI
(4) Positive results in a selective sinus sampling test

Diagnostic Criteria
Reliable cases: Meet 1, 2, 3, and 4 (1), (2), (3), and (4).
Almost reliable cases: Meet 1, 2, 3, and 4 (1), (2), and (3).
Suspected cases: Meet 1, 2, and 3.

response of plasma ACTH levels in the human corticotropin-releasing hormone (hCRH) test; (2) suppression of plasma cortisol levels (less than half, compared with the basal level) in high-dose (8 mg) overnight DST; (3) the presence of a pituitary adenoma in magnetic resonance imaging (MRI); and (4) positive results in a selective venous sampling test.

In addition, diagnostic criteria for subclinical Cushing's disease were established in Japan in 2010. The following endocrinological findings are considered diagnostic criteria: (1) the presence of a pituitary adenoma on MRI; (2) normal-high plasma ACTH and normal cortisol levels in the morning; and (3) absence

of a typical Cushingoid appearance. Screening and confirmatory tests for subclinical Cushing's disease are the same as those for Cushing's disease except for the 3.0 µg/dL cut-off of plasma cortisol level by 0.5 mg DST in subclinical Cushing's disease.

In this review, we evaluate the usefulness and accuracy of these recent diagnostic criteria of Cushing's disease in Japan, referring to our previous extensive data.

Presence of a Cushingoid appearance

Cushing's disease is usually recognized because of a Cushingoid appearance, characterized by a moon face,

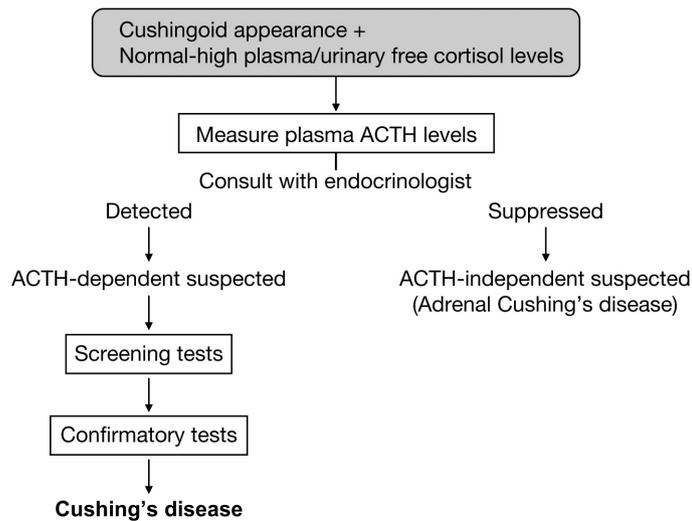


Fig. 1 Algorithm for testing patients suspected of having Cushing's disease

central obesity, dorsocervical fat pad (buffalo hump), purple striae, thin skin, easy bruising, and proximal myopathy. Moreover, in children a decrease in weight gain velocity is also observed. In general, patients will also have a number of atypical features triggered by cortisol such as hypertension, menstrual abnormalities, acne, hirsutism, peripheral edema, impaired glucose tolerance, diabetes, osteoporosis, pigmentation, or mental abnormalities. The diagnostic guidelines require the presence of more than one typical and atypical feature, respectively. Patients who meet the biochemical criteria, but show no typical clinical features are diagnosed as subclinical Cushing's syndrome [5, 6].

Basal levels of ACTH and cortisol

Plasma cortisol values vary among nonRIA cortisol assay kits. Recently, standardization of cortisol measurements with seven assay kits using standard plasma material containing synthetic hydrocortisone-d4 (NMIJ CRM 6007-a) and the ID-LC/MS/MS method was carried out in Japan [7, 8]. The resulting relative standard deviation was set within 10% in Japan, but not in other countries. Analysis of plasma cortisol levels and excretion of urinary free cortisol are commonly used as initial steps in the diagnosis of Cushing's syndrome. Simultaneous evaluation of ACTH and cortisol levels in the blood is important for determining ACTH-dependent Cushing's syndrome [4]. In the morning, levels of plasma ACTH and cortisol are often not elevated, and can fall within a normal range in some patients.

Urinary free cortisol levels show high to normal levels in ACTH-dependent Cushing's syndrome [4]. High to normal levels of plasma ACTH and cortisol are indispensable for diagnosing Cushing's disease.

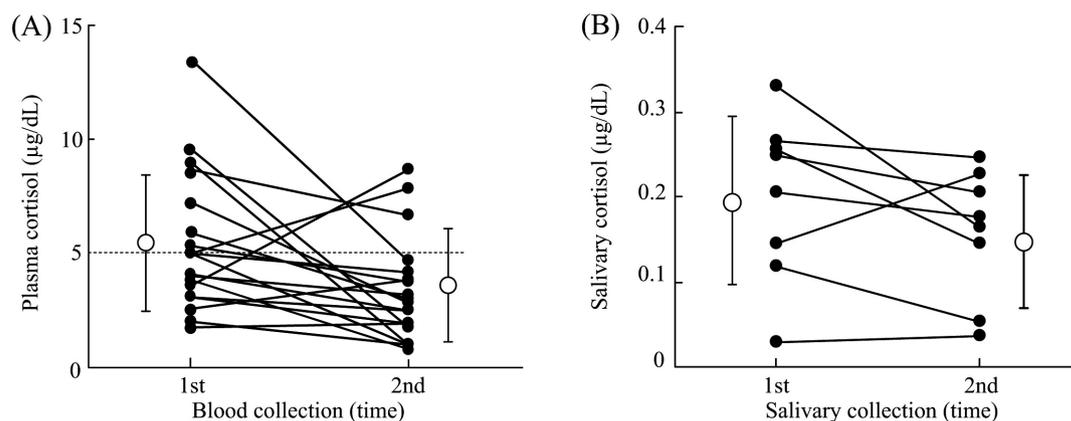
We propose an algorithm for testing patients suspected of having Cushing's disease (Fig. 1). When Cushing's syndrome is suspected, and normal-high plasma and/or urinary-free cortisol levels are observed, plasma ACTH levels should also be measured. At this point, a generalist should consult with an endocrinologist before proceeding with the screening tests.

Screening tests

When laboratory data suggest ACTH-dependent hypercortisolism, screening tests are recommended to determine autonomic or abnormal secretion of ACTH. The following endocrinological findings are considered indicative of ACTH-dependent Cushing's syndrome in screening tests for Cushing's disease.

(1) Low-dose dexamethasone suppression test (DST)

Low-dose DST is indispensable in screening for Cushing's disease. Briefly, dexamethasone (0.5 mg) is administered *per os* at 23:00. Blood samples are then taken between 08:00 and 09:00 the next morning, and plasma cortisol levels determined. The present Japanese clinical guidelines for the diagnosis of Cushing's disease indicate a cut-off plasma cortisol level for overt Cushing's disease of 5 $\mu\text{g}/\text{dL}$, while that for subclinical Cushing's diseases is 3 $\mu\text{g}/\text{dL}$. After correcting stan-



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Fig. 2 Reproducibility of late-night plasma and salivary cortisol levels

Blood (A) and salivary (B) collection was performed at 23:00 twice within three days of study entry (first time) then one week later (second time).

standard deviation to within 10%, our recent data show that the 5.0 µg/dL cut-off showed a sensitivity of 100% and specificity of 98% for the diagnosis of Cushing's disease [9]. On the other hand, for the diagnosis of subclinical Cushing's disease, the 3.0 µg/dL cut-off showed a sensitivity of 92% and specificity of 93%, while the 2.4 µg/dL cut-off showed a lower specificity (76%), and the 3.6 µg/dL cut-off showed a lower sensitivity (58%) [9]. Therefore, these cut-off values remain valid even after standardization of plasma cortisol measurements.

On the other hand, The Endocrine Society recommended that 1 mg DST should be used as a primary screening test for diagnosis of Cushing's syndrome [10]. The guideline also recommended the use of the more stringent cut-off value of 1.8 µg/dL for assessing post-dexamethasone serum cortisol level. However, cortisol values, especially at low levels, vary between cortisol assay kits. Our previous studies revealed that low cortisol levels are associated with poor measurement accuracy. For example, a measurement of 1.8 µg/dL \pm 2SD had a variation of \pm 1.36 µg/dL, even after standard deviation was corrected to within 10% [7, 8, 9]. The 1 mg DST is also used as a standard screening test of adrenal Cushing's syndrome in Japan; however, since East and Southeast Asians are generally leaner than Western populations, it has been suggested that this level may be too strong to suppress plasma cortisol in Japanese patients [11]. In addition, in patients with subclinical Cushing's syndrome, cortisol levels are usually within the normal range in the early morning, resulting in weak resistance to suppression by glucocorticoids. In our previous study, the cortisol cut-off

level with the greatest power was within a very narrow range when using 1 mg DST [11]. The 1.8 µg/dL cut-off showed a sensitivity of 75% and specificity of 87% for the diagnosis of Cushing's disease [9]. The data suggested that 0.5 mg was more sensitive than 1 mg DST for initial diagnosis of Cushing's syndrome.

(2) Plasma cortisol measurement during night-time sleep

Sampling plasma cortisol late at night can be limiting as well as stressful [12], but produce a high sensitivity. In 2007, the revised diagnostic criteria for Cushing's disease found high cortisol levels ($>$ 5 µg/dL) during night-time sleep to be indicative of ACTH-dependent Cushing's syndrome. Blood collection is performed quietly and quickly at 23:00 during night-time sleep twice within three days of study entry (first time) and again one week later (second time). We previously found plasma cortisol levels during night-time sleep to be significantly lower at the time of the second measurement than the first (Fig. 2), suggesting that measurements be performed twice.

Our previous results show that the sensitivity of the 2.5 µg/dL cut-off was slightly higher than that of the 5 µg/dL cut-off in subclinical Cushing's syndromes (Table 2). However, the 2.5 µg/dL cut-off makes it difficult to discriminate cases of subclinical Cushing's syndromes from normal subjects because of poor specificity. In fact, the 2.5 µg/dL cut-off shows a specificity of 31% in all cases of Cushing's syndrome, while the 5.0 µg/dL cut-off shows a specificity of 88%. Additionally, no significant differences were observed in the sensitivity of either cut-off value after standardization of cortisol

Table 2 Summary of the diagnostic tests for Cushing's syndrome.

	Etiology	Plasma cortisol		Salivary cortisol		Urinary cortisol	
		Cut-off level		Cut-off ratio		Cut-off level	
		2.5 µg/dL	5.0 µg/dL	1.0	1.5	18 µg/g cre	30 µg/g cre
Sensitivity (%)	Subclinical	100	90	100	100	100	75
	Overt	100	100	100	96	100	78
	Total	100	97	100	97	100	78
Specificity (%)		31	88	61	88	48	76

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Plasma cortisol: plasma cortisol levels during night-time sleeping.

Salivary cortisol: ratio of salivary cortisol to the mean level of each institute.

measurements, thus meeting the Japan criteria of a cut-off level of 5.0 µg/dL, which is appropriate for the diagnosis of overt and subclinical Cushing's syndrome.

(3) *Desmopressin (DDAVP) test*

Differential diagnosis of ACTH-dependent Cushing's syndrome using the DDAVP test [13, 14] relies on detection of an abnormal increase in vasopressin type 1b receptors in response to a vasopressin type 2 receptor agonist, DDAVP, as a result of ACTH release [13]. Briefly, a single dose (4 µg) of DDAVP (Kyowa Hakko Kirin Co., Tokyo, Japan) is intravenously injected under fasting conditions. Blood samples are taken before and 30, 60, 90, and 120 min after injection and plasma ACTH levels determined. A more than 50% increase in plasma ACTH level after test compared with the basal level is considered a significant response.

The DDAVP test is also used for the diagnosis and differential diagnosis of ACTH-dependent Cushing's syndrome from pseudo-Cushing's syndrome and normal subjects [13]. Plasma ACTH levels increase in response to DDAVP in Cushing's disease, but generally, there is only a slight response in normal or EAS cases [14]. Specifically, plasma ACTH levels were found to be significantly increased in 86% (19/22) of cases with Cushing's disease, especially those with microadenomas (90%), while in cases of EAS, 44% (4/9) responded to DDAVP. Other prior studies showed that 30-60% of cases of EAS responded to DDAVP [15, 16], while our previous results showed sensitivity of 86-100% in Cushing's disease [4, 13]. These results suggest that while the DDAVP test is a sensitive screening test for ACTH-dependent Cushing's syndrome, it does not discriminate Cushing's disease effectively from EAS.

(4) *Salivary cortisol levels during night-time sleep*

Cortisol is an important steroid in the regulation of the stress response [17]. It exists in both free (active) and protein-bound forms in the blood, but only in a free form in saliva [17]. Evans *et al.* first studied the feasibility of late-night salivary cortisol measurements for the clinical evaluation of endogenous hypercortisolism in the 1980s [18]. Since then, these measurements have been shown to offer high sensitivity and specificity in the diagnosis of Cushing's syndrome [19].

Salivary cortisol sampling is simple and relatively noninvasive, so may be more useful and less stressful than blood collection [18, 19]. Salivary collection is performed by placing a cotton pledget in the mouth and chewing for 1-2 min, in a recumbent position at 23:00 in Japan, whereas this is done at bedtime or between 23:00 and 24:00 in the United States. In our previous study, late-night plasma and salivary cortisol levels showed a positive correlation [20]. Indeed, salivary cortisol concentrations were shown to be directly proportional to serum unbound cortisol concentrations [21]. Our data, combined with a simple, stress-free, and non-invasive collection procedure, suggest that the salivary cortisol level is a more appropriate measure for the clinical assessment of adrenocortical function than the plasma cortisol level.

A salivary cortisol level of more than 0.4 µg/dL at night produces a sensitivity of 86% and specificity of 100% in all Cushing's syndromes, including subclinical adrenal Cushing's syndrome (sensitivity of 85%) and subclinical Cushing's disease. In our previous study, when either plasma cortisol levels (> 5 µg/dL) or salivary cortisol levels (> 0.4 µg/dL) were considered positive, a sensitivity of 100% and specificity of 82% were observed. However, the basal value or normal range can differ significantly between different institutes. The Endocrine Society Clinical Practice

Guideline recommends an enzyme-linked immunosorbent assay (ELISA) and the LC-MS/MS (RIA) assay as the best-validated methods for measuring salivary cortisol. However, inherent differences between assays complicate the process of determining optimal diagnostic criteria. For example, ELISA and RIA—both antibody-based techniques—have produced different results from the same sample. We found a significant correlation between the plasma cortisol levels and the ratio of salivary cortisol in individuals to the mean level of salivary cortisol in each institute. In a previous study involving multiple institutes, we found the salivary cortisol ratio at each institute to be more than 1.5 in all subjects with subclinical Cushing's syndrome and in 26 out of 27 cases of Cushing's syndrome [20]. A ratio cut-off of 1.5 showed a sensitivity of 97% (overt Cushing's syndrome, 96%; subclinical Cushing's syndrome, 100%) and specificity of 88% in all Cushing's syndrome cases (Table 2). Calculation of the cortisol ratio is therefore particularly useful in the screening of Cushing's syndrome, although standardization of salivary cortisol measurements is required in the future.

Differential diagnosis of Cushing's disease from EAS

Data for Cushing's disease has been known to overlap with some cases of EAS [2]. Therefore, distinguishing Cushing's diseases from EAS is of significant importance.

(1) Human corticotropin-releasing hormone (hCRH) test

The CRH test is well known for its use in diagnosis of Cushing's disease [16, 22]. Briefly, a single dose (100 µg) of hCRH (Mitsubishi Tanabe Pharma, Osaka, Japan) is intravenously injected under fasting conditions in the morning. Blood samples are taken before and 30, 60, 90, and 120 min after injection and then plasma ACTH levels determined. A greater than 50% increase in plasma ACTH level after test compared with the basal level is considered a significant response.

In a prior study, increments of 35% and 20% above baseline ACTH and cortisol levels, respectively, were shown to produce good results in the CRH test, indicating a positive response in Cushing's disease [23]. Kaye and Crapo suggested that the diagnostic criteria consistent with Cushing's disease consist of an increase of 20% from the basal level in peak cortisol, or an increase in peak ACTH in the blood of 50%, follow-

ing the administration of ovine CRH [24]. When used to examine ACTH responses in the differential diagnosis of Cushing's syndrome, these criteria showed a sensitivity of 86% and specificity of 95%, while the cortisol responses gave an improved sensitivity of 91% and similar specificity of 95% [24]. In our previous study, plasma ACTH levels increased more than 1.5-fold in response to hCRH in almost all cases of Cushing's disease, including 100% (62/62) and 73% (8/11) of cases with microadenomas and macroadenomas, respectively (Table 3). On the other hand, 27% (4/15) of cases with EAS also responded to hCRH (Table 3). Reimondo *et al.* reported that an ovine CRH-induced ACTH percentage increment of 50% produced sensitivity of 86% and specificity of 90% [25]. Taken together, these results suggest that the hCRH test is effective in distinguishing Cushing's diseases from EAS; however, definitive discrimination between Cushing's disease and EAS using the CRH test alone remains difficult.

(2) High-dose DST

Less than half the cortisol level, compared with the basal level, is suppressed by high-dose (8 mg) DST. After high-dose DST, morning plasma cortisol levels are suppressed in 89% (55/62) of Cushing's disease with microadenomas and 82% (60/73) of all Cushing's disease cases (Table 3). Our previous results are consistent with those of previous studies showing that the efficiency of this test in diagnosing Cushing's disease is almost 80% [4, 26]. In cases with EAS, high-dose dexamethasone suppresses 50% (3/6) of bronchial carcinoids and 0% (0/9) of other lung cancers (Table 3). Salgano *et al.* reported that a high dose of dexamethasone caused a high false positive rate in lung carcinoid tumors [2]. These results suggest that a high-dose dexamethasone test is useful for diagnosis of Cushing's disease, although caution is required in interpreting results produced by macroadenomas or Croke cell adenomas with Cushing's disease and in cases of bronchial carcinoids with EAS [27]. When both the CRH test and 8 mg DST are considered together, our study showed sensitivity of 81% and specificity of 60% in Cushing's disease cases [4]. Thus, the combination of these two tests is effective for distinguishing Cushing's diseases from EAS.

(3) MRI

MRI shows high specificity in diagnosis of Cushing's disease [28]. Both the CRH test and 8 mg DST achieve

Table 3 Summary of the diagnostic tests for ACTH-dependent Cushing's syndrome.

Etiology		DST			DDAVP test	CRH test
		No Suppression/Total (%)	Suppression/Total (%)		Responder/Total (%)	Responder/Total (%)
		0.5 mg	1 mg	8 mg		
Cushing's disease	Micro	62/62 (100)	59/62 (95)	55/62 (89)	19/21 (90)	62/62 (100)
	Macro	11/11 (100)	11/11 (100)	5/11 (45)	0/1 (0)	8/11 (73)
	Total	73/73 (100)	70/73 (96)	60/73 (82)	19/22 (86)	70/73 (96)
EAS	Br ca	6/6 (100)	6/6 (100)	3/6 (50)	1/3 (33)	3/5 (60)
	Others	9/9 (100)	9/9 (100)	0/9 (0)	3/6 (50)	1/10 (10)
	Total	15/15 (100)	15/15 (100)	3/15 (20)	4/9 (44)	4/15 (27)
Sensitivity	(%)	100	96.0	82.0	86.0	96.0
Specificity	(%)	0	0	80.0	55.6	73.3

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A plasma cortisol level of more than 5 µg/dL is considered a positive result (no suppression) in low-dose DST. Compared with the basal level, a plasma cortisol level of less than half is considered a suppressive effect with high-dose (8 mg) DST. DST, dexamethasone suppression test; DDAVP, desmopressin; CRH, corticotropin-releasing hormone; EAS, ectopic ACTH syndrome; Micro, pituitary microadenomas; Macro, pituitary macroadenomas; Br ca, bronchial carcinoids; Others, other lung cancers. Sensitivity and specificity represent the results for diagnosis of Cushing's disease from ACTH-dependent Cushing's syndrome.

higher specificity in diagnosis of Cushing's disease when combined with MRI [29, 30], showing 90% specificity when combined with the presence of a pituitary adenoma. The ability of MRI to detect pituitary ACTH-secreting adenomas in patients with Cushing's disease is, however, limited because the calculated accuracy in detecting a pituitary source of ACTH is reportedly around 60% with conventional MRI [31]. The ability to detect pituitary ACTH-secreting adenoma might be improved by using a 3 Tesla magnet, although small non-functioning adenoma can also be detected with this technique. In some cases of EAS, ¹⁸F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) would be helpful in detecting the localization [32].

(4) A selective sinus sampling test

Cavernous or inferior petrosal sinus sampling has been validated as a highly accurate investigative tool in the differential diagnosis of ACTH-dependent Cushing's syndrome [33]; however, it cannot be used to discriminate Cushing's disease from normal subjects. Gradients of sinus (central) to peripheral ACTH are calculated before and after stimulation with CRH. Cushing's disease is diagnosed when the basal gradient is greater than 2, or the gradient after stimulation is greater than 3 [34]. Administration of CRH to stimulate ACTH secretion during sampling is routinely used to elicit diagnostic gradients in Cushing's disease, and to improve the sensitivity of this procedure [34].

Considerations

(1) Differential diagnosis of Cushing's disease in pseudo-Cushing's syndrome

Depression, alcohol dependence, and other psychiatric disorders may cause over-activity of the hypothalamic-pituitary-adrenal (HPA) axis, which is called pseudo-Cushing's syndrome. Data for some cases of this disease are known to overlap with those of Cushing's disease. Attention should be paid to false positive results which are sometimes found in this condition. The dexamethasone-CRH test is often used in the United States to exclude false positive results [10], but this test is not popular in Japan.

The HPA axis is also activated in poorly controlled diabetes mellitus. High glucose levels may be involved in the regulation of the HPA axis, as high glucose levels lead to generation of mitochondrial oxygen radicals. This high-glucose-induced free radical generation may mediate activation of CRH and vasopressin neurons, thereby activating the HPA axis [35]. Therefore, blood glucose levels should be controlled in diabetes mellitus before evaluating these tests.

(2) Drugs affecting evaluation of the diagnosis of Cushing's disease

Some drugs may influence the evaluation of the diagnosis of Cushing's disease. Carbamazepine accelerates dexamethasone metabolism *via* induction of CYP3A4 activity and increases cortisol-binding globu-

lin (CBG) and then cortisol levels [36]. Consequently, DST causes a false positive result. Phenobarbital and rifampin also accelerate dexamethasone metabolism [37], and estrogen and mitotane can increase CBG [38]. Similarly, women taking an oral contraceptive pill may produce a false positive result with DST because of increased CBG levels. In contrast, other drugs, such as itraconazole and cimetidine, which impair dexamethasone metabolism *via* inhibition of CYP3A4 activity, may cause a false negative result.

Conclusion

In conclusion, the findings suggest that the recent diagnostic criteria for diagnosis of Cushing's disease in

Japan do indeed achieve a higher specificity. Despite the findings, however, it remains important that the usefulness and accuracy of each diagnostic tool be considered because of partial overlap of the data with some cases of EAS. The DDAVP test and measurements of salivary cortisol have not yet been approved for use under the National Health Insurance in Japan; however, approval is expected in the future.

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