

# Discrepancies in Results of Low- and High-dose Dexamethasone Suppression Tests for Diagnosing Preclinical Cushing's Syndrome

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**Abstract.** According to the diagnostic criteria for adrenal preclinical Cushing's syndrome (PreCS) established by a group headed by the Ministry of Health, Labor and Welfare (MHLW), low- and high-dose dexamethasone suppression tests (DSTs) must be performed to prove autonomous cortisol secretion, *i.e.*,  $\geq 3$   $\mu\text{g}/\text{dL}$  serum cortisol following 1-mg DST administration, and  $\geq 1$   $\mu\text{g}/\text{dL}$  serum cortisol following 8-mg DST administration. However, discrepancies have been documented in the results of low- and high-dose DSTs. We therefore investigated the validity of the DST for diagnosing PreCS by performing 1-mg and 8-mg DSTs in 39 patients with adrenal incidentaloma, but no characteristic Cushingoid symptoms. In about half of these patients (20/39, 51.3%), high-dose DST was positive but low-dose was negative, and one or more of the other abnormalities of hypothalamus-pituitary-adrenal axis dysfunction was seen in 75% of these patients. Furthermore, no significant difference in incidence of glucose intolerance and hypertension was noted in patients with positive high-dose DST and negative low-dose DST compared with patients with positive low- and high-dose DST. Under the current MHLW diagnostic criteria, patients with positive high-dose DST and negative low-dose DST are not diagnosed with PreCS, but some of these patients should be. Discrepancies in the results of low- and high-dose DSTs appear attributable to the current cutoff values, and further investigations are necessary to resolve these discrepancies.

*Key words:* Adrenal incidentaloma, Dexamethasone suppression test, Preclinical Cushing's syndrome, Diagnostic methods  
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**DIAGNOSTIC** methods for adrenal preclinical Cushing's syndrome (PreCS) have not been firmly established internationally [1–3]. However, the diagnostic criteria set forth by a Research Group of the Ministry of Health, Labor and Welfare (MHLW) for adrenal PreCS are generally used in Japan [4]. These diagnostic criteria include: presence of adrenal incidentaloma; a lack of characteristic features for Cushing's syndrome; normal basal serum cortisol levels; and autonomous cortisol secretion as assessed using low- and high-dose dexamethasone suppression tests (DSTs):  $\geq 3$   $\mu\text{g}/\text{dL}$  serum cortisol following 1-mg DST administration,

and  $\geq 1$   $\mu\text{g}/\text{dL}$  serum cortisol following 8-mg DST administration, respectively. These findings are essential for diagnosis of PreCS.

The diagnostic criteria were established to provide reliable evaluation of PreCS by standardizing diagnostic methods between medical institutions, but problems have been reported concerning discrepancies and reproducibility of DST results, including at our medical center [5–7]. The present study was conducted to investigate these problems, including the validity of using the DST in diagnostic criteria.

## Materials and Methods

### Patients

Subjects comprised 39 patients with adrenal incidentaloma without the characteristic clinical manifes-

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tations of Cushing's syndrome, who were hospitalized at our medical center between April 1999 and March 2003. Basal serum cortisol levels, including repeat testing, were normal in all patients. Patients with confirmed or suspected adrenomedullary tumor, cysts, metastatic lesions or primary aldosteronism based on endocrine testing, imaging studies, or histopathologic examination of resected tumors were excluded. Informed consent to participate in the study was obtained from each patient and the study was approved by the local ethical committee.

#### *Evaluation method*

Low-dose (1 mg) and high-dose (8 mg) DST were performed in each patient. Patients were classified as the accordance group (group A) if results on both DST doses met the diagnostic criteria set forth by the MHLW Research Group (serum cortisol levels  $\geq 3$   $\mu\text{g}/\text{dL}$  after 1 mg dexamethasone; serum cortisol levels  $\geq 1$   $\mu\text{g}/\text{dL}$  after 8 mg of dexamethasone); as the discrepancy group (group D) if results on either dose of DST met the diagnostic criteria; and as the normal group (group N) if results for neither of the DST doses met the diagnostic criteria. In each group, 6 types of studies were performed to evaluate hypothalamus-pituitary-adrenal (HPA) axis function, as per the diagnostic criteria of the MHLW Research Group: basal serum cortisol levels; autonomous cortisol secretion; cortisol diurnal rhythm; ACTH suppression; serum dehydroepiandrosterone sulfate (DHEA-S) levels; and  $^{131}\text{I}$ -6 $\beta$ -iodomethyl-19-norcholest-5(10)-en-3 $\beta$ -ol adrenal scintigraphy. Rates of glucose intolerance, hypertension and obesity were also compared between groups. One patient was excluded from glucose intolerance analysis, as the patient had no past history of diabetes and a 75-g oral glucose tolerance test (OGTT) could not be performed.

#### *Dexamethasone suppression test*

Blood samples were collected at 0800 h before DST, then dexamethasone 1 mg was administered orally at 2300 h on the same day. The following day, blood samples were collected at 0800 h, then 8 mg of dexamethasone was administered orally at 2300 h. On day 3, blood samples were again collected at 0800 h to complete the study.

#### *Evaluation parameters*

- 1) Loss of cortisol diurnal rhythm: Serum cortisol  $> 5$   $\mu\text{g}/\text{dL}$  from 2200 h to 0000 h.
- 2) ACTH suppression: Basal plasma ACTH level  $< 10$   $\text{pg}/\text{mL}$ , or after CRH stimulation test (100  $\mu\text{g}$ , i.v.), a peak plasma ACTH level of  $< 30$   $\text{pg}/\text{mL}$  or  $< 1.5$  times baseline value.
- 3) Low serum DHEA-S: Below normal limits for age and sex.
- 4) Adrenal scintigraphy: Increased uptake of isotope on side of tumor and suppressed uptake on contralateral side.
- 5) Complications of obesity (body mass index (BMI)  $\geq 25$ ), glucose intolerance (glucose intolerance defined as need for drug therapy or impaired glucose tolerance (IGT) or diabetic pattern on a 75-g OGTT) or hypertension (need for drug therapy or systolic blood pressure  $\geq 140$  mm Hg or diastolic blood pressure  $\geq 90$  mmHg).

#### *Hormone measurements*

Blood samples were immediately centrifuged at 4°C, and then stored at  $-80^\circ\text{C}$  until assay. Commercially available assay kits were used for hormone measurements: DHEA-S: radioimmunoassay (RIA) kits from Diagnostic Product Co. (Los Angeles, CA, USA); and ACTH: immunoradiometric assay kits from Mitsubishi Chemical Co. (Tokyo, Japan). In the 14 patients who underwent thorough inpatient tests before March 2001, levels of serum cortisol were measured using a RIA kit (Dade Behring, Stillwater, MN, USA). In the remaining 25 patients, levels of serum cortisol were measured using another RIA kit (Immunotech, Marseilles, France). With this change in assay kit, the reference values for serum cortisol were also changed (up to March 2001: 5.6–21.3  $\mu\text{g}/\text{dL}$ ; after April 2001: 4.0–23.3  $\mu\text{g}/\text{dL}$ ).

#### *Statistical analysis*

Results are shown as means  $\pm$  standard error. Student's *t*-test or  $\chi^2$ -test was used for inter-group comparisons. Values of  $p < 0.05$  were considered statistically significant.

## Results

### Classification based on DST

Fig. 1 shows the distribution of serum cortisol levels after DST. Classification according to DST results revealed 15 patients in group A, 20 patients in group D, and 4 patients in group N. The results of low-dose DST thus agreed with those of high-dose DST in only 19 of the 39 patients (48.7%). Breakdown of DST classifications for the 14 Dade Behring RIA Kit patients was as follows: group A,  $n = 6$ ; group D,  $n = 6$ ; and group N,  $n = 2$ . Breakdown of DST classifications for the 25 Immunotech RIA kit patients was as follows: group A,  $n = 9$ ; group D,  $n = 14$ ; and group N,  $n = 2$ . In group D no patients displayed serum cortisol levels suppressed under 1-mg DST alone.

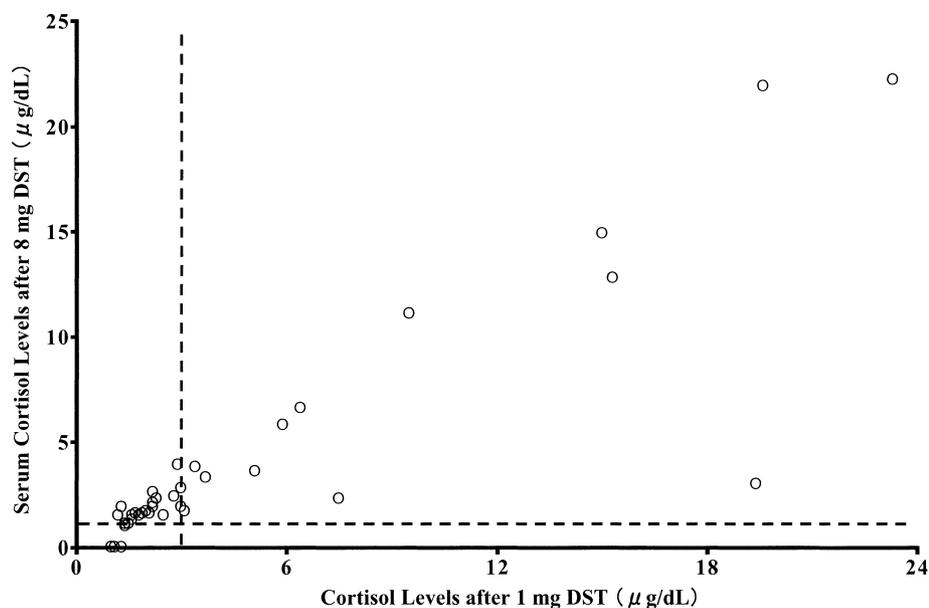
### Serum cortisol levels before and after DST in groups A and D

Basal serum cortisol levels did not significantly differ between groups A and D (Fig. 2, left). However, serum cortisol levels in group A were significantly higher after 1-mg DST (Fig. 2, middle) and 8-mg DST (Fig. 2, right). The same results were applicable to both cortisol assay kits.

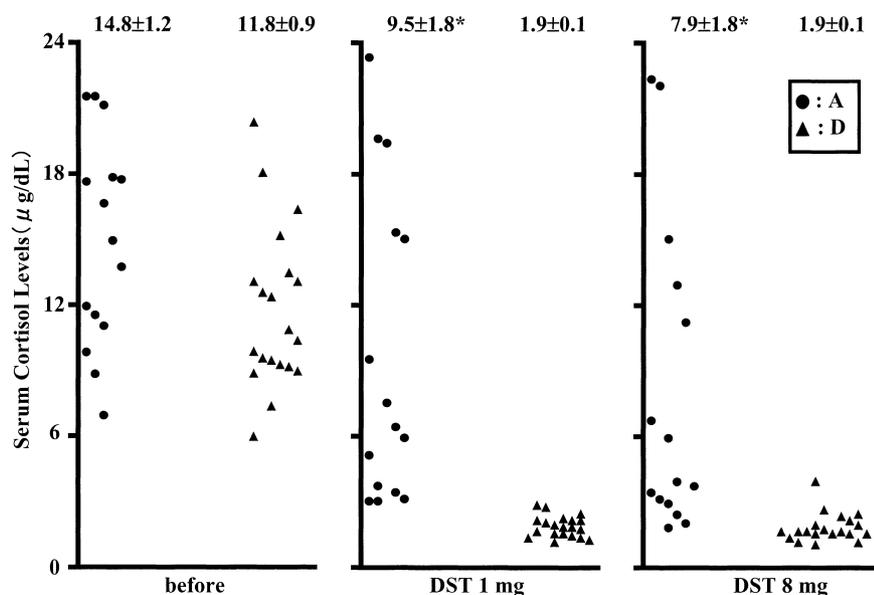
### Evaluation of HPA axis function in groups A and D

On testing of HPA axis function, including loss of cortisol diurnal rhythm, ACTH suppression, low serum DHEA-S, and significant uptake by the tumor side and suppressed uptake by the contralateral side on adrenal scintigraphy, rate of positive findings for each test parameter tended to be higher in group A than in group D (Fig. 3). Differences were significant for loss of cortisol diurnal rhythm and laterality of uptake on adrenal scintigraphy. Fig. 4 depicts the number of positive findings from functional investigations relevant to the HPA axis, excluding DST, and the corresponding number of patients. Positive rate for all endocrine tests was higher in group A than in group D. However, the number of positive findings in functional tests varied in both groups. In group D, some dysfunction was seen in 15 patients (75%), and at least 2 abnormalities were confirmed in 7 of the 15 patients. An isolated abnormal DST was not present in any patient in group A, compared with 5 patients in group D (Fig. 4).

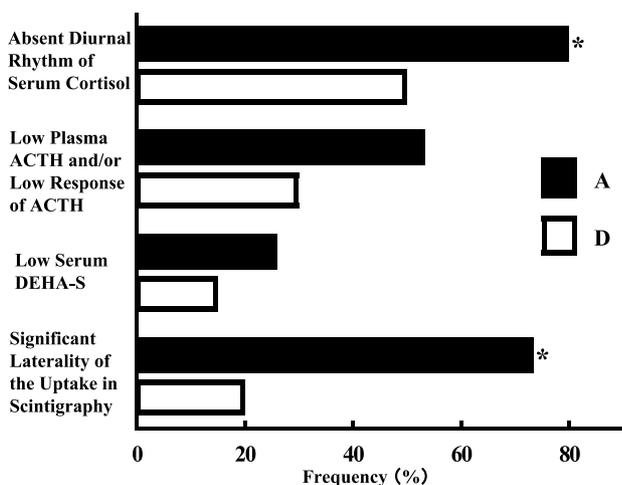
A diagnosis of PreCS based on the diagnostic criteria set forth by the MHLW Research Group requires, in addition to the 4 criteria mentioned previously, at least the following findings: loss of cortisol diurnal rhythm; ACTH suppression; low serum DHEA-S; or increased uptake of isotope on the side of the tumor and suppressed uptake on the contralateral side on adrenal



**Fig. 1.** Distribution of serum cortisol levels after dexamethasone suppression test (DST) in each patient. Vertical broken lines represent the cutoff value of 3 µg/dL for 1-mg DST, and horizontal broken lines indicate the cutoff value of 1 µg/dL for 8-mg DST.



**Fig. 2.** Comparison of serum cortisol levels before and after DST in patients with accordance (group A) and discrepancy (group D) in results of 1- and 8-mg DSTs. Numerical data for each group represent mean  $\pm$  SEM; \* $p < 0.05$  vs. group D.

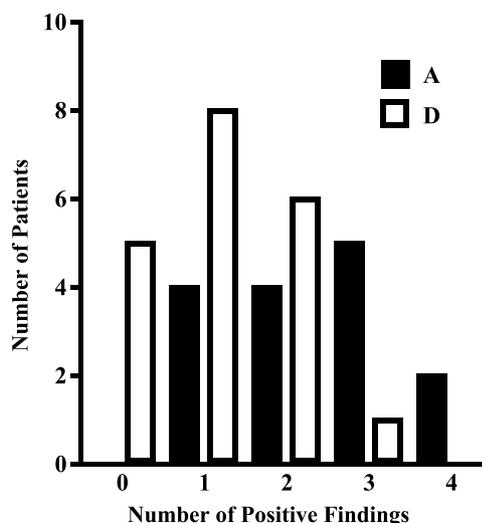


**Fig. 3.** Frequency of positive findings for each test of HPA axis function in patients with accordance (group A) and discrepancy (group D) in results of 1- and 8-mg DSTs. \* $p < 0.05$  vs. group D.

scintigraphy. Of the 39 patients in our study, 15 patients (38.5%) were diagnosed with PreCS.

*Clinical characteristics and complications in groups A and D (Table 1)*

The total number of positive findings for endocrine function tests, excluding DST, was significantly higher in group A, but no significant differences in age, gen-



**Fig. 4.** Number of positive findings (and corresponding number of patients) on testing of HPA axis function (loss of cortisol diurnal rhythm, ACTH suppression, low serum DHEA-S and significant laterality of the uptake in adrenal scintigraphy) in patients with accordance (group A) and discrepancy (group D) in results of 1- and 8-mg DSTs.

der or BMI were noted between groups. Rates of glucose intolerance and hypertension tended to be higher in group A, but no significant differences between groups were noted.

Group N (Table 2)

In group N, basal cortisol levels and cortisol levels after 1-mg DST were lower than in groups A or D, but patients with loss of cortisol diurnal rhythm or low serum DHEA-S were also encountered. Hypertension was present in 1 patient, and impaired glucose tolerance was noted in 3 patients.

**Table 1.** Clinical characteristics and metabolic complications in patients with accordance (group A) and discrepancy (group D) in results of 1- and 8-mg DSTs

	A (n = 15)	D (n = 20)
Number of Positive Findings	2.3 ± 0.3*	1.2 ± 0.2
Age (yr)	60.0 ± 3.3	62.4 ± 2.1
Gender (M/F)	6/9	11/9
BMI (kg/m <sup>2</sup> )	25.0 ± 1.4	24.5 ± 1.1
(Obesity)	(5/15)	(8/20)
HT	12/15 (80.0%)	12/20 (60.0%)
DM (IGT)	9 (2)/14 (78.6%)	6 (5)/20 (55.0%)

\*p<0.05 vs. group D. BMI: body mass index, HT: hypertension, DM: diabetes mellitus, IGT: impaired glucose tolerance.

Isolated abnormality of DST (Table 3)

Under the current diagnostic criteria, patients who exhibit isolated insufficient suppression of cortisol production by DST are not diagnosed with PreCS. Since this may represent a group differing from groups A or D, cortisol levels were compared between the 5 patients with isolated insufficient suppression of cortisol production on DST, and 15 patients in group D, after excluding 5 of 20 patients (Table 3). Compared to the 15 group D patients, levels of serum cortisol before and after DST in these 5 patients tended to be lower, but no significant difference was identified (levels of serum cortisol were 12.6 ± 1.0 µg/dL before DST, 2.0 ± 0.1 µg/dL after 1-mg DST, and 2.0 ± 0.1 µg/dL after 8-mg DST for the 15 group D patients). Hypertension was present in 2 patients, while impaired glucose tolerance was present in 1 patient.

**Discussion**

According to the research group headed by the MHLW, diagnosis of PreCS requires a positive reac-

**Table 2.** Summary of 4 patients with normal results from dexamethasone suppression tests

Case	Age	Gender	BMI (kg/m <sup>2</sup> )	Cortisol Levels (µg/dl)		Positive Finding	HT	IGT/DM
				Basal	DST 1 mg			
1	60	F	26.3	4.7	1.3	Absent Diurnal Rhythm of Cortisol	(+)	IGT
2	72	F	30.7	5.0	1.0	Low DHEA-S	(-)	IGT
3	56	M	23.2	7.6	1.3	(-)	(-)	IGT
4	73	M	20.0	11.9	1.1	(-)	(-)	(-)
			65.3 ± 4.3	22.6 ± 1.6	7.3 ± 1.7*	1.2 ± 0.1*		

\*p<0.05 vs. groups A and D. BMI: body mass index, HT: hypertension, DM: diabetes mellitus, IGT: impaired glucose tolerance.

**Table 3.** Summary of 5 patients with isolated insufficient suppression on dexamethasone suppression test

Case	Age (yr)	Gender	BMI (kg/m <sup>2</sup> )	Cortisol Levels (µg/dl)			HT	IGT/DM
				Basal	DST 1	DST 8		
1	54	F	35.0	9.6	1.2	1.6	(+)	IGT
2	71	F	19.4	9.9	1.5	1.2	(-)	(-)
3	56	F	22.5	7.4	1.8	1.6	(+)	(-)
4	43	M	17.9	9.3	1.6	1.6	(-)	(-)
5	65	M	24.4	9.0	1.9	1.7	(-)	(-)
			57.8 ± 4.8	23.3 ± 3.1	9.0 ± 0.4	1.6 ± 0.1	1.5 ± 0.1	

Mean ± SEM. DST 1: DST 1-mg, DST 8: DST 8-mg, BMI: body mass index, HT: hypertension, DM: diabetes mellitus, IGT: impaired glucose tolerance.

tion to low- (1-mg) and high-dose (8-mg) DSTs, with  $\geq 3$   $\mu\text{g/dL}$  serum cortisol following 1-mg DST administration, and  $\geq 1$   $\mu\text{g/dL}$  serum cortisol following 8-mg DST administration. Patients thus cannot be diagnosed with PreCS if they test positive to only one of the 2 tests. However, discrepancies and a potential lack of reproducibility in DST results have been reported including at our medical center [5–7]. This study was conducted to investigate these problems, including the validity of using DST for these diagnostic criteria. In the present study, an accordance of results between the 1- and 8-mg DSTs was achieved in only 19 of the 39 patients with adrenal incidentalomas. This suggests that encountering such patients in actual clinical practice is more common than might be expected.

We evaluated whether patients who only met the diagnostic criteria for the 8-mg DST (group D) should be managed differently than those patients meeting the diagnostic criteria for both 1- and 8-mg DSTs (group A). Degree of cortisol production from tumor and rate of complications were compared between groups A and D. Comparison between groups showed that serum cortisol levels after 1- and 8-mg DST were significantly higher in group A. Evaluation of HPA axis function, other than the DST, also indicated a higher rate of abnormalities in group A. Autonomous cortisol production was greater in group A. However, some dysfunction was seen in 15 group D patients (75.0%), and at least 2 abnormalities were confirmed in 7 of the 15 patients. Furthermore, comparison between groups from the standpoint of metabolic complications indicated no significant differences in the frequencies of hypertension, glucose intolerance, or obesity. Even in group D, more than half of the patients displayed hypertension and/or glucose intolerance. These findings suggest that some patients with positive high-dose DST and negative low-dose DST should be diagnosed with PreCS.

When a discrepancy was noted in the results of low- and high-dose DSTs, high-dose DST was positive and low-dose DST was negative in all cases. This suggests that the problem may lie in the cutoff values for the DST. In other words, discrepancies between results for the 2-dose DST could be minimized by lowering the cutoff value for 1-mg DST or elevating the cutoff value for 8-mg DST. Optimal cutoff values for serum cortisol need to be established in future after studying more patients.

In the present study, 15 of the 39 patients with inci-

dentalomas were diagnosed with PreCS. This figure is clearly higher than reported previously [8, 9]. The reason for this may be that the present study included more patients with relatively high cortisol production from tumor, as adrenal incidentalomas were discovered during therapy and observation for hypertension, glucose intolerance or obesity, which shows a close correlation to excessive cortisol levels.

Different cortisol assay kits reportedly yield different results [10]. In the present study, cortisol levels in the same blood sample were not measured using different cortisol assay kits. However, the cortisol assay kit used was changed after April 2001. Frequency of PreCS was 42.9% ( $n = 6$ ) for the 14 Dade Behring Kit patients and 36.0% ( $n = 9$ ) for the 25 Immunotech Kit patients, and accordance rates of low- and high-dose DSTs were 57.1% ( $n = 8$ ) and 44.0% ( $n = 11$ ), respectively. While no significant differences were identified, cases may exist in which a diagnosis for PreCS will turn on which assay kit was used in a certain ratio. The standardization of these cortisol assays is required.

The present finding also suggests some problems with the current diagnostic criteria of the MHLW Research Group. In 5 patients, cortisol secretion was not suppressed by either low- or high-dose DSTs, although other functional tests of the HPA axis were normal. False positive DST results may occur in patients with obesity, depression, alcoholism or in those who are taking drugs that increase dexamethasone clearance (*e.g.*, phenytoin, phenobarbital, rifampicin and troglitazone) [11, 12]. Among patients with an isolated abnormal DST, one was obese.

Normal DST results were noted in 2 patients, despite negative findings for functional tests relevant to the HPA axis. Each patient displayed 1 positive finding: loss of cortisol diurnal rhythm in one, and low serum DHEA-S in the other. The isolated case of loss in cortisol diurnal rhythm may have been due to the relatively relaxed criteria used, namely, a night-time serum cortisol of  $>5$   $\mu\text{g/dL}$ . If more stringent criteria of  $>7.5$   $\mu\text{g/dL}$  had been used, cortisol diurnal rhythm would have been considered normal. In addition to hyperadrenocorticism, other causes of low DHEA-S include anorexia nervosa, acute stress due to myocardial infarction or surgery, and use of oral contraceptives [13, 14]. None of these factors were involved in the above 2 patients.

In conclusion, using the diagnostic criteria established by the MHLW, level of serum cortisol was

above the cutoff value for high-dose DST, but was below the cutoff value for low-dose DST in almost 50% of patients. Under the current diagnostic criteria, these patients cannot be diagnosed with PreCS, but no significant differences in the frequency of metabolic complications were apparent between these patients and those with positive low- and high-dose DSTs. Some HPA axis dysfunction besides DST was seen in 75% of

patients with positive high-dose DST and negative low-dose DST. These findings suggest that some cases with positive results on high-dose DST and negative results for low-dose DST should be diagnosed with PreCS. Discrepancies in the results of low- and high-dose DSTs appear attributable to the cutoff values used, and further investigations are necessary to resolve these problems.

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