

New diagnostic criteria of adrenal subclinical Cushing's syndrome: opinion from the Japan Endocrine Society

Toshihiko Yanase¹⁾, Yutaka Oki²⁾, Takuyuki Katabami³⁾, Michio Otsuki⁴⁾, Kazunori Kageyama⁵⁾, Tomoaki Tanaka⁶⁾, Hisaya Kawate^{7),8)}, Makito Tanabe¹⁾, Masaru Doi⁹⁾, Yuko Akehi¹⁾ and Takamasa Ichijo¹⁰⁾

¹⁾ Department of Endocrinology and Diabetes Mellitus, Faculty of Medicine, Fukuoka University, Fukuoka 814-0180, Japan

²⁾ Department of Community and Family Medicine, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan

³⁾ Division of Metabolism and Endocrinology, Department of Internal Medicine, St. Marianna University School of Medicine Yokohama City Seibu Hospital, Yokohama 241-0811, Japan

⁴⁾ Department of Metabolic Medicine, Osaka University Graduate School of Medicine, Suita 565-0871, Japan

⁵⁾ Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine, Hirosaki 036-8562, Japan

⁶⁾ Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

⁷⁾ Department of Medicine and Bioregulatory Sciences, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

⁸⁾ Department of Nutritional Sciences, Nakamura Gakuen University, Fukuoka 814-0198, Japan

⁹⁾ Doi Clinic, Kumamoto 861-5255, Japan

¹⁰⁾ Department of Diabetes and Endocrinology, Saiseikai Yokohamashi Tobu Hospital, Yokohama 230-0012, Japan

Abstract. New diagnostic criteria and the treatment policy for adrenal subclinical Cushing's syndrome (SCS) are proposed on behalf of the Japan Endocrine Society. The Japanese version has been published, and the essential contents are presented in this English-language version. The current diagnostic criteria for SCS have elicited two main problems: (i) the relatively low reliability of a low range of serum cortisol essential for the diagnosis by an overnight 1-mg dexamethasone suppression test (DST); (ii) different cutoff values for serum cortisol after a 1-mg DST compared with those of other countries. Thus, new criteria are needed. In the new criteria, three hierarchical cortisol cutoff values, 5.0, 3.0 and 1.8 µg/dL, after a 1-mg DST are presented. Serum cortisol ≥5 µg/dL after a 1-mg DST alone is considered sufficient to judge autonomous cortisol secretion for the diagnosis of SCS, and the current criterion based on serum cortisol ≥3 µg/dL after a 1-mg DST can continue to be used. Clinical evidence suggests that serum cortisol ≥1.8–2.9 µg/dL after a 1-mg DST is not always normal, so cases who meet the cutoff value as well as a basal adrenocorticotrophic hormone (ACTH) level <10 pg/mL (or poor ACTH response to corticotropin-releasing hormone (CRH)) and nocturnal serum cortisol ≥5 µg/dL are proposed to have SCS. We suggest surgery if cases show serum cortisol ≥5 µg/dL after a 1-mg DST (or are disheartened by treatment-resistant problems) or suspicious cases of adrenal cancer according to tumor imaging.

Key words: Subclinical Cushing's syndrome, Adrenal tumor, Cortisol, Adrenocorticotrophic hormone, Dexamethasone suppression test

Proposal of New Diagnostic Criteria of SCS and Their Background

The current diagnostic criteria of SCS were formulated by the Ministry of Health, Labour and Welfare (MHLW)

Submitted Nov. 2, 2017; Accepted Feb. 28, 2018 as EJ17-0456

Released online in J-STAGE as advance publication Mar. 23, 2018

Correspondence to: Toshihiko Yanase, Department of Endocrinology and Diabetes Mellitus, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.

E-mail: tyanase@fukuoka-u.ac.jp

Adrenal Study Group in 1996 [1]. Since then, the diagnostic criteria have made considerable contributions to the understanding and pathology of SCS. This is evident from a national epidemiologic survey by the MHLW which showed that the number of SCS cases increased rapidly from 290 cases in the survey period 1992–1996 [2] to 1,829 cases in 2003–2007 [3].

SCS lacks the clinical signs of Cushing's syndrome (CS), so SCS is often detected by further examination of adrenal incidentalomas. SCS is hidden within "lifestyle diseases" such as obesity, diabetes mellitus, and hyper-

tension [3-5] and a high prevalence of such complications has been reported in a nationwide survey of SCS [3]. SCS is also related to cardiovascular disease [6], bone loss [7, 8] and a reduction in bone quality when evaluated by the Spinal Density Index [9]. However, with changes in the measurement system (from radioimmunoassay to enzyme immunoassay) of cortisol in blood, four main discussions have arisen: (i) the relatively low reliability of a low range of cortisol concentrations ($<3 \mu\text{g/dL}$) essential for the diagnosis after an overnight 1-mg DST [10]; (ii) the inconsistency of the screening criteria of SCS with those proposed by the American Endocrine Society (serum cortisol $\geq 1.8 \mu\text{g/dL}$ after a 1-mg DST) [11] or other countries [1]; (iii) the “globalization” of diagnostic criteria; (iv) the necessity of a 8-mg DST for the SCS diagnosis [12]. Thus, new diagnostic criteria for adrenal SCS have become an important agenda for the Japan Endocrine Society (JES).

The current task force was organized officially in 2012 and multicenter collaborative research was done to collect evidence for a revision. Based on the results of the research and peer reviews of articles focusing on SCS, the final new diagnostic criteria for adrenal SCS and the treatment policy were proposed. These evidence-based diagnostic criteria and the treatment policy for adrenal SCS were reviewed by a JES subcommittee and approved. These documents were also approved by Research Committee on Disorders of Adrenal Hormones from the MHLW, Japan and Japan Hormonal Steroid Association. The Japanese version has been published and the English-language version has been described here. The new diagnostic criteria for adrenal SCS and the treatment policy is shown in Table 1 and Table 2, respectively. An algorithm of the new diagnostic criteria is summarized in Fig. 1.

Current Status of SCS Diagnostic Criteria in Europe and the USA

Various clinical practice guidelines for adrenal SCS have been proposed in Europe and the USA, and a summary table from Shen and coworkers [13] has been modified here (Table 3). In Table 3, a part of clinical practice guideline for adrenal incidentaloma from European Society of Endocrinology (ESE) in collaboration with the European Network for the Study of Adrenal Tumors (ENSAT) [14] has been also cited. In all guidelines, a 1-mg DST has been proposed as the first screening test for the diagnosis of adrenal SCS. The cutoff

value of cortisol varies depending on the guideline, but it is $1.8 \mu\text{g/dL}$ or $5 \mu\text{g/dL}$ after a 1-mg DST.

Italian guidelines published in 2011 suggested that SCS is unlikely if serum cortisol $<1.8 \mu\text{g/dL}$ after a 1-mg DST and likely if it is $\geq 5 \mu\text{g/dL}$ after a 1-mg DST, but that the diagnosis cannot be denied if it is between $1.8 \mu\text{g/dL}$ and $5 \mu\text{g/dL}$ [15]. Another research team from Italy tried to predict the complications of hypertension, type-2 diabetes mellitus, and vertebral fracture using three cutoff values of cortisol after a 1-mg DST: 1.8, 3 and $5 \mu\text{g/dL}$; among them, $3 \mu\text{g/dL}$ was reported to be the best for prediction of these complications with respect to sensitivity and specificity [6]. A guideline of ESE and ENSAT [14] also followed the algorithm of above Italian guidelines [15] as to the diagnosis of SCS by 1 mg DST.

Eller-Vainicher and colleagues reported that improvement of metabolic conditions (weight, blood pressure, glucose and cholesterol levels) could be obtained by surgery with a sensitivity of 65.2% and specificity of 68.8% for SCS patients who satisfied two or more of the following conditions: (i) serum cortisol $>3 \mu\text{g/dL}$ after a 1-mg DST; (ii) urinary free cortisol (UFC) $>70 \mu\text{g/24-h}$; (iii) basal ACTH $<10 \text{ pg/mL}$ [16]. These three conditions have been termed the “UFC-ACTH-DST criteria”. A recent report from Italy [17] stated that adrenalectomy in patients with SCS who have serum cortisol $>5 \mu\text{g/dL}$ after a 1-mg DST or two or more of the UFC-ACTH-DST criteria have a reduced risk of osteoporosis and vertebral-body fractures.

In Japan, Akehi *et al.* reported that serum cortisol $\geq 1.8 \mu\text{g/dL}$ after a 1-mg DST can help to confirm the diagnosis of SCS when if two criteria are met: (i) basal ACTH $<10 \text{ pg/mL}$ (or poor plasma ACTH response to CRH); (ii) serum cortisol $\geq 5 \mu\text{g/dL}$ at 21:00–23:00 h [18]. Under this condition, the complication of impaired glucose tolerance was detected with good sensitivity and specificity [18].

Only guidelines from Endocrine Society (USA) [11] have recommended late-night measurement of salivary cortisol as a primary screening test (Table 3). Adoption of confirmatory tests to prove subtle (but autonomous) cortisol secretion, such as high-dose DST, adrenal scintigraphy, and measurements of UFC/24-h, late-night serum or salivary cortisol, basal ACTH, or DHEA-S, has not been uniform in each guideline.

Table 1 New diagnostic criteria for adrenal subclinical Cushing's syndrome (SCS)

1. Presence of an adrenal mass (adrenal incidentaloma)
2. Lack of characteristic features of Cushing's syndrome (CS)¹⁾
3. Laboratory data:
 - 3.1. Normal basal serum cortisol levels²⁾
 - 3.2. Autonomic cortisol secretion confirmed by an overnight 1-mg dexamethasone suppression test (DST)^{3),4),5)}
 - 3.3. Low plasma levels of adrenocorticotrophic hormone (ACTH) in the early morning⁶⁾
 - 3.4. No diurnal changes in serum cortisol levels⁷⁾
 - 3.5. Unilateral uptake on adrenal scintigraphy⁸⁾
 - 3.6. Low serum levels of dehydroepiandrosterone sulfate (DHEA-S)⁹⁾
 - 3.7. Transient adrenal insufficiency or atrophy of the attached normal adrenal cortex after removal of the adrenal tumor¹⁰⁾

The diagnosis is defined based on the presence of 1, 2, 3.1 (essential) plus the following conditions of (1) or (2) or (3).

- (1) Serum cortisol after a 1-mg DST ≥ 5 $\mu\text{g/dL}$ (3.2)
- (2) Serum cortisol after a 1-mg DST ≥ 3 $\mu\text{g/dL}$ (3.2) plus at least one of 3.3–3.6 or the presence of 3.7
- (3) Serum cortisol after a 1-mg DST ≥ 1.8 $\mu\text{g/dL}$ (3.2) plus 3.3 and 3.4 or the presence of 3.7

Note 1): Hypertension and general obesity (physical status) and glucose intolerance, osteoporosis and dyslipidemia (laboratory examinations) are not regarded as characteristic conditions of CS.

Note 2): Basal serum cortisol levels should be measured in the fasting condition at rest in the early morning, at least twice. Reproducible hypercortisolemia excludes the possibility of SCS. The normal range of serum cortisol is judged according to the reference range of each assay kit.

Note 3): An overnight 1-mg DST should be carried out. Serum cortisol ≥ 1.8 $\mu\text{g/dL}$ after a 1-mg DST is considered to be not completely normal or to take into account the possibility of a functioning adrenal tumor or that of nonfunctioning adrenal tumor possessing clinical significance.

Note 4): A high dose (4–8 mg) DST is not necessary for the confirmatory diagnosis. However, if it is necessary for the differential diagnosis of pathogenesis, we suggest that it should be done.

Note 5): Low serum levels of cortisol vary by around 10%, so serum cortisol ≈ 3 $\mu\text{g/dL}$ varies with a difference of around 0.3 $\mu\text{g/dL}$. Then, we recommend a comprehensive diagnosis by considering the number of positive criteria.

Note 6): If the basal ACTH level in the early morning is <10 pg/mL , then it is desirable to take more than one additional measurement. There can be a poor response of ACTH to the ACTH-stimulating test (less than 1.5-fold of the basal ACTH level). Be aware and cautious that the plasma level of ACTH is not always low when biologically inactive ACTH is secreted.

Note 7): Serum cortisol at 21.00–24.00 is ≥ 5 $\mu\text{g/dL}$.

Note 8): Quantitative evaluation using adrenal scintigraphy is desirable because suppressed intake in the contralateral intact adrenal gland is correlated with autonomous cortisol secretion.

Note 9): A DHEA-S level lower than the age- and sex-matched reference level.

Note 10): Preoperatively, sufficient informed consent, including the possibility of a nonfunctioning adenoma, is necessary.

Table 2 Recommendation of the treatment policy for adrenal SCS

In cases with SCS diagnosed by the condition of (1) (serum cortisol ≥ 5 $\mu\text{g/dL}$ after a 1-mg DST), and accompanying treatment-resistant complications (hypertension, general obesity, glucose intolerance, decrease in bone mineral density, dyslipidemia), we recommend unilateral adrenalectomy. In other cases, we suggest tumor resection or careful follow-up with reference to the number of positive criteria and the presence/absence of complications.

(Supplementary comments)

1) If the tumor diameter is ≥ 3 cm, or if the tumor diameter tends to increase even if it is <3 cm upon follow-up, or if the possibility of adrenal cancer cannot be excluded with reference to imaging findings, we recommend surgery.

2) Always remember that glucocorticoid supplementation may be needed in some cases after removal of an adrenal tumor showing SCS.

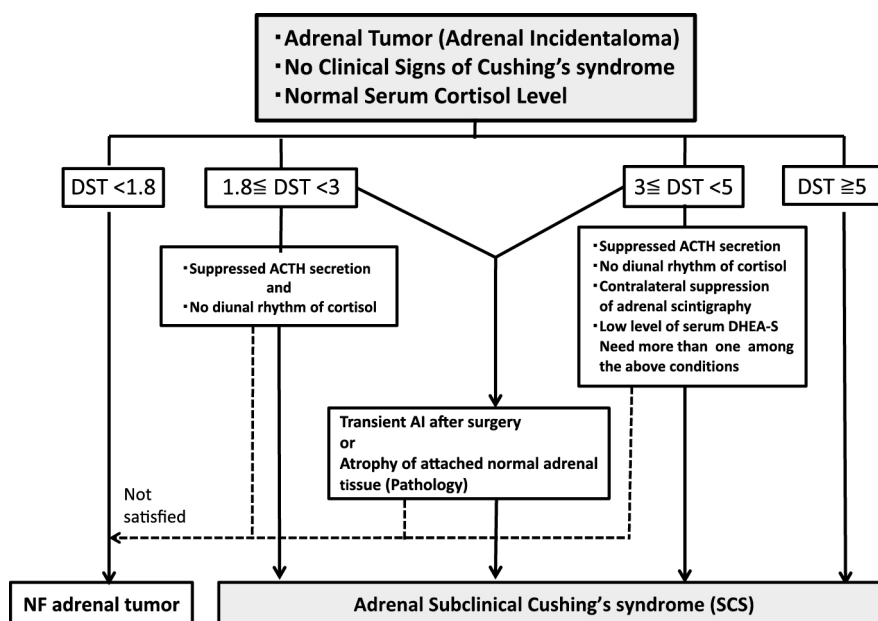


Fig. 1 Diagnostic algorithm of SCS.

Please refer to the text and Table 1. AI, adrenal insufficiency; CS, Cushing's syndrome; DST, dexamethasone suppression test. The number in parenthesis is the serum cortisol level ($\mu\text{g}/\text{dL}$) after a 1-mg DST.

Suppression of ACTH secretion, plasma ACTH in the early morning <10 pg/mL or poor response of plasma ACTH to a CRH loading test (<1.5 fold); No diurnal rhythm of serum cortisol, serum cortisol ≥ 5 $\mu\text{g}/\text{dL}$ at 21.00–24.00; NF, non-functioning adrenal tumor

Summary of the Results from Multicenter Collaborative Research by Working Groups (WGs)

Here, to explain the basis for our proposal for new diagnostic criteria for SCS, we summarize the results of research (unpublished data).

We conducted a retrospective cross-sectional study under the approval of the clinical research review committees/ethics committees of Hirosaki University, Chiba University, Saint Marianna Medical University, Hamamatsu Medical University, Osaka University, Kyushu University and Fukuoka University. We included only cases with an adrenal tumor. With regard to the SCS diagnosis in this retrospective cross-sectional study, the diagnostic criteria proposed in 1996 [1] were strictly adopted and data were reviewed. Finally, 530 cases (SCS, suspected SCS, and nonfunctioning adrenal tumors) were assessed. The study cohort comprised 270 men and 260 women (mean age, 60.1 ± 11.1 years).

In this multicenter collaborative research, the assay kits adopted for measurement of blood concentrations of cortisol differed among facilities. A total of 294 cases

were assessed using the ECRIA method (Roche Diagnostics), 112 cases with the CLEIA method from Beckman Coulter, and 124 cases with the CLEIA method from Siemens. Kuwa *et al.* have developed a calibration formula for standardization of different kits for measurement of serum levels of cortisol using the certified serum reference material (NMIJ CRM 6401) based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) [19]. Using this formula, standardization of the serum cortisol values after a 1-mg DST and at night was done, and we investigated whether the diagnosis of SCS might be affected before and after calibration. As a result, when serum cortisol was ≥ 3 $\mu\text{g}/\text{dL}$ after a 1-mg DST was used, the diagnosis of 11 out of 92 SCS cases was changed to "non-SCS", and all of 189 non-SCS cases remained non-SCS after calibration. After all, diagnostic concordance rate before and after calibration was 96.1% in the case of serum cortisol ≥ 3 $\mu\text{g}/\text{dL}$ after a 1-mg DST and 97.7% in the case of serum cortisol was ≥ 1.8 $\mu\text{g}/\text{dL}$ after a 1-mg DST. So, it was considered that the actual diagnosis did not change dramatically if the serum cortisol was ≥ 3 $\mu\text{g}/\text{dL}$ or ≥ 1.8 $\mu\text{g}/\text{dL}$ after a 1-mg DST [20]. Conversely, the result must be interpreted

Table 3 Diagnostic criteria for SCS in Europe and the USA

Organization	NIH	ES	AACE/AAES	FSE	IACE	ESE/ENSAT
Diagnosis						
<The first screening test>						
1-mg overnight DST	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
Cutoff point	>5.0 µg/dL	>1.8 µg/dL	>5.0 µg/dL	>1.8 µg/dL	<1.8 µg/dL: exclude >5.0 µg/dL: consider 1.8–5.0 µg/dL: indeterminate	<1.8 µg/dL: exclude >5.0 µg/dL: autonomous 1.8–5.0 µg/dL: possible
Late-night salivary cortisol	NM	Recommended	NR	NR	NR	NR
Cutoff point		>145 ng/dL				
<The second screening test>						
24-h urine free cortisol	NM	NR	NR	Recommended	Recommended	Recommended (in 'autonomous' case)
Late-night serum cortisol	NM	NR	NM	Recommended	Recommended	NM
Late-night salivary cortisol	NM	NR	NR	Recommended	NR	Recommended (in 'autonomous' case)
High-dose 2-day DST	NM	NM	NM	NM	NR	Recommended (in 'autonomous' case)
<The confirmatory test>						
Low-dose 2-day DST	NM	NR	Recommended	NM	NR	NM
Cutoff point			NM			
<Evaluation of autonomous cortisol secretion from an adrenal adenoma>						
ACTH	NM	Recommended	Recommended	Recommended	Recommended	Recommended
Cutoff point		Suppressed	Low or suppressed	NM	Low or suppressed	Low or suppressed
DHEAS	NM	Recommended	Recommended	NR	NR	NM
Cutoff point		Suppressed	Low			
Adrenal scintigraphy	NR	NM	NM	Recommended	NR	NM

Table was cited from reference 13 and partly extracted and modified. ESE/ENSAT guideline content was cited from reference 14.

NIH, National Institutes of Health; ES, Endocrine Society; AACE/AAES, American Association of Clinical Endocrinologists/American Association of Endocrine Surgeons; FSE, French Society of Endocrinology; IACE, Italian Association of Clinical Endocrinologists; ESE/ENSAT, European Society of Endocrinology/European Network for the Study of Adrenal Tumors; NM, not mentioned; NR, not recommended.

carefully because the variance in the measurement of serum cortisol values is around 10% [19]. In 2016, the cortisol measurement kit manufactured by Roche (the most widely used kit in Japan) was changed to “Cortisol II™”. In a study using 200 serum specimens, the correlation between Cortisol II™ (y axis) and the previous cortisol kit (x axis) was found to be $y = 0.7912x + 0.3201$, $r = 0.9913$. At low concentrations ($<3 \mu\text{g/dL}$) in blood, the correlation of cortisol values between the two kits was $y = 0.9011x - 0.0001$, $r = 0.937$ [21]. The cortisol level using Cortisol II™ was 10–20% lower than that using the old kit, but the cortisol value obtained using Cortisol II™ was almost identical to that obtained by LC-MS/MS. The cortisol value obtained with Cortisol II™ was close to the standardized value measured using the old kit. From these findings, in a practical sense, it was

considered that the cortisol value of Cortisol II™ did not need to be recalibrated using the certified serum reference material, *e.g.* NMJ CRM 6401 at the end-user level.

A total of 530 cases with adrenal tumors were classified into 363 cases with non-SCS, 2 cases with overt CS, 133 cases with definite SCS, and 32 cases with suspected SCS based on the diagnostic criteria from 1996 [1]. The suspected cases of SCS were those who satisfied the criterion of serum cortisol $\geq 3 \mu\text{g/dL}$ after a 1-mg DST, but other findings relevant for the diagnosis of SCS were lacking. According to the criteria set by the Japanese Society of Hypertension, the Japan Diabetes Society and Japan Atherosclerosis Society, respectively, the complications of high blood pressure, impaired glucose tolerance and hypercholesterolemia were defined, and the cortisol values after a 1-mg DST were compared based

on the presence or absence of these complications. As a result, overall the presence or absence of each complication did not affect the mean cortisol value after a 1-mg DST. However, if the target was restricted to only the non-SCS group, the cortisol value after a 1-mg DST was significantly higher in the group with hypertension than that in the group with normotension (1.50 ± 0.81 vs. 1.30 ± 0.76 , $p = 0.025$). In the non-SCS group, serum cortisol after a 1-mg DST showed a positive correlation only with hypertension ($r = 0.12$, $p = 0.026$). When serum cortisol after a 1-mg DST was stratified by increments of $1.0 \mu\text{g/dL}$, the coincidental occurrence of hypertension and impaired glucose tolerance was more frequently observed in the $2\text{--}2.99 \mu\text{g/dL}$ group than in the $0\text{--}0.99 \mu\text{g/dL}$ group. These results suggested that the threshold for the onset of complications of adrenal tumors may be serum cortisol of $2\text{--}3 \mu\text{g/dL}$ after a 1-mg DST. However, in SCS cases and suspected SCS cases, the incidence of hypertension and impaired glucose tolerance was not affected by the cortisol level after a 1-mg DST or even by stratification of the cortisol level.

Evidence for the Proposal of New Diagnostic Criteria for SCS Including the Results of Multicenter Collaborative Research

Katabami *et al.* [22] investigated the adoption of 0.5 mg or 1 mg for the DST. They measured the blood concentration of dexamethasone (DEX) in SCS cases using LC-MS/MS. Inter-individual differences in the blood concentration of DEX were large, but intra-individual variations of the concentration were small. The blood concentration of DEX after loading with 1 mg of DEX was about twice that after loading with 0.5 mg of DEX. Also, the blood concentration of DEX was not associated with body weight, body surface area or the body mass index [22]. From these findings, it was concluded that a 1-mg DST was more effective than a 0.5-mg DST in terms of reducing the risk of false-positive results in the DST due to insufficient suppression of ACTH.

A confirmatory test using a 8-mg DST was adopted in the diagnostic criteria for SCS proposed in 1996 [1] and serum cortisol $\geq 1 \mu\text{g/dL}$ was considered to demonstrate SCS. However, autonomous cortisol secretion in SCS is not as strong as that observed in overt CS. There have been contradictory scenarios in which serum cortisol was $< 3 \mu\text{g/dL}$ after a 1-mg DST, but more than $1\text{--}3 \mu\text{g/dL}$ after a 8-mg DST [12], leading to confusion in the diag-

nosis of SCS. Hence, we did not adopt a high-dose DST in the new criteria for SCS. It is rational to undertake a high-dose DST if the differential diagnosis of disease type of SCS is needed or if the extent of autonomy of cortisol secretion must be examined.

With regard to the basis of the diagnostic criteria for SCS (serum cortisol level after a 1-mg DST), three hierarchical cortisol cutoff levels, 1.8, 3.0 and $5 \mu\text{g/dL}$, are presented in the new criteria. The cutoff level of $3.0 \mu\text{g/dL}$ used in the current criterion is included, thereby lowering the risk of confusion. The major reasons for presenting these three cutoff values are discussed below.

First, multicenter collaborative research has suggested that patients with serum cortisol $\geq 1.8 \mu\text{g/dL}$ after a 1-mg DST may not be completely healthy, and may carry the risk of complications. Some guidelines outside Japan have adopted serum cortisol $\geq 1.8 \mu\text{g/dL}$ after a 1-mg DST as the first screening for SCS (Table 3), so international consistency was considered for the new criteria. Second, to avoid confusion in Japan due to the change in the cutoff value for cortisol in serum after a 1-mg DST, the current diagnostic criterion of $\geq 3.0 \mu\text{g/dL}$ after a 1-mg DST has been retained. We also considered that some diagnostic criteria from Italy are very close to this standard [6, 16, 17]. As a diagnostic criterion of SCS, it was also considered that most guidelines adopt serum cortisol $\geq 5 \mu\text{g/dL}$ after a 1-mg DST [13, 14] because the autonomy of cortisol secretion in patients who meet this condition is relatively strong. In this multicenter collaborative research, almost all of the respective criteria, such as basal ACTH $< 10 \text{ pg/mL}$ and/or nocturnal cortisol at $21.00\text{--}23.00 \text{ h} \geq 5 \mu\text{g/dL}$, and a low level of DHEA-S, are satisfied in almost all cases who have serum cortisol $\geq 5 \mu\text{g/dL}$ after a 1-mg DST, suggesting that serum cortisol $\geq 5 \mu\text{g/dL}$ after a 1-mg DST alone is sufficient to make a diagnosis of SCS. In addition, serum cortisol $\geq 5 \mu\text{g/dL}$ after a 1-mg DST is important for the decision of resection of an adrenal tumor (see below).

If a cutoff value for serum cortisol $\geq 1.8 \mu\text{g/dL}$ after a 1-mg DST is set, then a clearer definition of a nonfunctional adrenal tumor is warranted. According to Akehi and colleagues [18], when using a 1-mg DST, most (but not all) cases with serum cortisol $1.8\text{--}3.0 \mu\text{g/dL}$ have a nonfunctioning tumor, but some of such patients with a tumor have SCS [18]. These patients should be distinguished correctly. That is, for serum cortisol $\geq 1.8 \mu\text{g/dL}$ after a 1-mg DST, if ACTH in the early morning is $< 10 \text{ pg/mL}$ (if the ACTH level is $\geq 10 \text{ pg/mL}$, no or low response of ACTH to CRH is needed) and the nocturnal

cortisol level at 21.00–23:00 h is ≥ 5 $\mu\text{g/dL}$, then the complications of glucose intolerance can be detected with high sensitivity (85.7%), specificity (72.7%) and accuracy (77.8%). Thus, the criteria are very useful for the diagnosis of SCS and show equivalent diagnostic confidence with that of current criteria [18].

Even with the results of multicenter collaboration, serum cortisol ≥ 5 $\mu\text{g/dL}$ at 21.00–23:00 h and ACTH < 10 pg/mL showed a higher correlation with the serum cortisol level after a 1-mg DST compared with a low DHEA-S level in blood and urinary free cortisol ≥ 70 $\mu\text{g/day}$. Thus, these two parameters were considered to be more superior indicators reflecting autonomic secretion of cortisol. Serum cortisol levels in normal individuals decline slowly from morning to evening, further decrease from late night to midnight and then, build up overnight to peak in the early morning [23]. Then, we slightly extended the time of measurement of cortisol from 21.00–23:00 h to 21.00–24:00 h because the extension boost the convenience with little risk of false positive diagnosis of SCS. From the data shown above, serum cortisol ≥ 1.8 $\mu\text{g/dL}$ and < 3 $\mu\text{g/dL}$ after a 1-mg DST, ACTH < 10 pg/mL in the early morning (if the ACTH level is ≥ 10 pg/mL , no or low response of ACTH to CRH is needed) and serum cortisol ≥ 5 $\mu\text{g/dL}$ at 21.00–24:00 h can be defined as one of the criteria of SCS.

Improvement of glucose intolerance and hypertension has been reported to be higher in cases with a nonfunctional adrenal tumor that has been resected compared with non-operated cases [4, 24–26]. Even in nonfunctional tumors, hormone production that slightly exceeds normal levels may increase the risk of complications. This finding seems to be compatible with the result observed in our collaborative study that complications increased if serum cortisol was 2–3 $\mu\text{g/dL}$ after a 1-mg DST. Indeed, in a report from Italy, patients with adrenal adenomas with serum cortisol > 50 nmol/L (equivalent to 1.8 $\mu\text{g/dL}$) showed an increased risk of complications (type-2 diabetes mellitus, dyslipidemia, central obesity, osteoporosis, vertebral fractures) [6].

If ACTH with low bioactivity is secreted, even in cases with ACTH deficiency, the blood ACTH level may not necessarily be lowered in some assay kits. For example, Esclucial (Roche Diagnostics) is a monoclonal antibody that specifically recognizes the C-terminal of ACTH, but some large ACTH molecules may not be recognized. Conversely, in the E-test TOSOH II (Tosoh Corporation), blood levels of ACTH can be increased. The antibody is a polyclonal antibody against C-terminal

ACTH, so any large ACTH molecule can be recognized. Thus, it is very important to interpret the measurement results based on the characteristics of the measurement system [27].

Atrophic finding of a normal adrenal gland adjacent to an adenoma after surgery is a diagnostic criterion because this scenario demonstrates autonomic cortisol secretion and ACTH suppression. The atrophic cortex attached to cortisol producing adenoma is characterized by reduced thickness of the one or more of the cortical layers due to a decrease in cell size or a loss of cells. The zona fasciculata and reticularis are more often affected than the zona glomerulosa [28]. Adrenal scintigraphy can be used to confirm the functional suppression of healthy adrenal glands in preoperative imaging. Katabami *et al.* showed that reduced uptake in the contralateral healthy adrenal gland (rather than increased uptake in the tumor) is correlated with autonomous secretion of cortisol from the tumor as assessed by a 1-mg DST, and the laterality ratio for detecting SCS was 3.07 [29]. In adrenal scintigraphy, one must not judge the difference between left and right glands qualitatively but instead evaluate the decline in contralateral adrenal uptake quantitatively.

The usefulness of salivary cortisol in the diagnosis of SCS has been studied in Japan. However, its specificity for the diagnosis of SCS has been reported to be low if it is used for differentiating SCS from nonfunctioning adrenal tumors [30]. Except for the guideline from Endocrine Society (USA), all non-Japanese guidelines have not adopted salivary cortisol as a screening test [13–15]. In Japan, the diagnosis of adrenal-gland disease using salivary cortisol has not been verified (at least in part) because the measurement is not covered by medical insurance and is limited to medical research. Hence, measurement of salivary cortisol was not incorporated into the new diagnostic criteria for SCS.

Treatment Guidelines for SCS

A prognostic survey of SCS in surgical cases has been conducted in several institutional studies. However, there are no results of a deterioration in blood pressure or glucose tolerance upon surgery; instead improvement (or at least an unchanged result) has been reported [24, 31–34] (Table 4). A national epidemiologic survey conducted by the MHLW revealed that only 4% of patients with the complications of obesity, impaired glucose tolerance or hypertension had a worse surgical outcome, and mostly improved or unchanged outcomes were observed postop-

Table 4 Comparison of the prognosis of metabolic parameters in patients with SCS between surgical and non-surgical groups in several studies

Author (reference number)	Average observation period (years)	N	Ope (+)			N	Ope (-)		
			HT	DM	Dyslipidemia		HT	DM	Dyslipidemia
			Improve/ Aggravate	Improve/ Aggravate	Improve/ Aggravate		Improve/ Aggravate	Improve/ Aggravate	Improve/ Aggravate
Tsuiki <i>et al.</i> , 2008 [31]	OP 1.2 OP (-) 2.3	10	83/0 (%)	29/0 (%)	67/0 (%)	12	0/25 (%)	0/17 (%)	0/0 (%)
Toniato <i>et al.</i> , 2009 [32]	7.7	23	67/0 (%)	63/0 (%)	38/0 (%)	22	0/33 (%)	0/25 (%)	0/44 (%)
Chiodini <i>et al.</i> , 2010 [24]	1.5	25	56/0 (%)	48/0 (%)	36/24 (%)	16	0/50 (%)	0/38 (%)	19/50 (%)
Akaza <i>et al.</i> , 2011 [33]	OP 2.3 OP (-) 3.3	8	63/0 (%)	50/0 (%)	13/0 (%)	8	0/38 (%)	0/0 (%)	0/38 (%)
Kawate <i>et al.</i> , 2014 [34]	4.3	10	71/0 (%)	75/0 (%)	17/0 (%)	12	17/50 (%)	40/40 (%)	44/0 (%)

HT, Hypertension; DM, Diabetes mellitus; OP, operation (adrenalectomy)

eratively [3]. Hence, there seem to be few problems after resection of an adrenal tumor but, in real clinical situations, judgments regarding surgery and follow-up in asymptomatic cases may be troublesome. Therefore, if serum cortisol ≥ 5 $\mu\text{g/dL}$ after a 1-mg DST (which is considered to indicate high autonomous cortisol productivity in a functional sense), we suggest surgery, especially if treatment-resistant clinical problems such as hypertension, systemic obesity, impaired glucose tolerance, osteoporosis or dyslipidemia are present. In other cases, follow-up observation or surgery might be decided by taking into account the number of positive findings in the diagnostic criteria for SCS and the number of potential complications.

It is of note that even in SCS cases, atrophy of the healthy side of the adrenal gland may be observed to some extent, and it may be necessary to start glucocorticoid (GC) replacement after surgery in a similar manner to that in CS. Such cases indicate high autonomous cortisol productivity and especially cases showing serum cortisol ≥ 5 $\mu\text{g/dL}$ after a 1-mg DST should be careful. In the case of operated Cushing's syndrome due to an adrenal adenoma, GC replacement usually started with 15–20 mg Cortril® can be gradually tapered along with the degree of symptoms of adrenal insufficiency and ACTH values. The replacement period has been reported to be average 1 year and 8 months. In the case of SCS, the same protocol is recommended, but the replacement period may be shorter than that of CS because of the relatively weaker suppression of hypothalamic-pituitary adrenal (HPA) axis [35].

Considering the possibility of adrenal cancer is important. The current recommendation is that a tumor diame-

ter of ≥ 5 cm is an indication for tumor resection. However, according to guideline for management of adrenal tumors in Japan [36] (compiled mainly by the Japanese Urological Association in March 2015), the tumor diameter for consideration of surgery is >3 cm. According to our guideline, resection of an adrenal tumor should be considered if the tumor diameter is ≥ 3 cm and adrenal cancer cannot be excluded completely. According to a nationwide survey of adrenal incidentalomas, adrenal cancer was observed in $\approx 4\%$ of adrenal tumors with a diameter ≥ 3 cm [37]. In addition, it has been reported that adrenal cancer can be excluded with a probability of 99.7% if the tumor diameter is <3 cm. We then considered that a cutoff tumor diameter of 3 cm could be recommended for resection [37]. Moreover, in the long-term follow-up of adrenal tumors, change from a nonfunctioning to functioning tumor should be considered carefully. Interestingly, a non-functioning tumor with a diameter >3 cm tends to change into a functioning tumor [38]. Prognostic analyses of SCS based on a nationwide survey revealed that the frequency of hypertensive complications in SCS was significantly higher (odds ratio: 2.28 times) in adrenal tumors with a diameter ≥ 3.5 cm than in those with a diameter <3.5 cm [39]. This finding was also considered for the proposal of a cutoff value of 3 cm in our guideline. However, in previous studies from other countries, a cutoff of a 4 cm in adrenal size by computed tomography (CT) *etc.* has been reported to be the most reliable way to diagnose malignancy (or non-adenomatous lesions) but with very low specificity [40–42]. The cutoff a 4 cm is also recommended for the indication of adrenalectomy in several guidelines of adrenal incidentaloma [13–15, 43, 44]. So,

further investigation may be needed for the proposal of a cutoff value of the diameter for adrenalectomy.

Conflicts of Interest

In the proposal of the diagnostic criteria of adrenal SCS, the conflict of interest in the WG was that, in the study of cortisol measurement reagents, YO (Hamamatsu School of Medicine) undertook contract research with Roche Diagnostics and obtained research funding from them. Otherwise, there is no conflict of interest to declare.

Acknowledgements

We thank Dr. K. Kuwa (National Metrology Institute

of Japan, National Institute of Advanced Industrial Science and Technology) for teaching us about the standardization of cortisol assays by kits. We thank Arshad Makhdum, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Funding

This study was supported by Health and Labour Sciences Research Grants, Research on Intractable Diseases, Research Committee on Disorders of Adrenal Hormones from the Ministry of Health, Labour, and Welfare, Japan (16769897).

References

1. Nawata H, Demura H, Suda T, Takayanagi R (1996) Adrenal preclinical Cushing's syndrome. Annual report of the Ministry of Health and Welfare "Disorder of Adrenal Hormones" Research Committee, Japan 1995: 223–226 (In Japanese).
2. Nawata H, Takayanagi R, Nakagawa H, Miura K (1999) Annual report of the Ministry of Health and Welfare "Disorder of Adrenal Hormones" Research Committee, Japan 1998: 11–55 (In Japanese).
3. Yanase T, Fujieda K, Kajieda H, Tanahashi Y, Suzuki S (2011) A nationwide epidemiological study of Addison's disease and subclinical Cushing's disease. Annual report of Intractable Disease Research Grant of Ministry of Health, Labour and Welfare, Japan "Research on Adrenal Hormone Disorders" 2010: 117–124 (In Japanese).
4. Rossi R, Tauchmanova L, Luciano A, Di Martino M, Battista C, *et al.* (2000) Subclinical Cushing's syndrome in patients with adrenal incidentaloma: clinical and biochemical features. *J Clin Endocrinol Metab* 85: 1440–1448.
5. Tauchmanova L, Rossi R, Biondi B, Pulcrano M, Nuzzo V, *et al.* (2002) Patients with subclinical Cushing's syndrome due to adrenal adenoma have increased cardiovascular risk. *J Clin Endocrinol Metab* 87: 4872–4878.
6. Morelli V, Masserini B, Salcuni AS, Eller-Vainicher C, Savoca C, *et al.* (2010) Subclinical hypercortisolism: correlation between biochemical diagnostic criteria and clinical aspects. *Clin Endocrinol (Oxf)* 73: 161–166.
7. Torlontano M, Chiodini I, Pileri M, Guglielmi G, Cammisia M, *et al.* (1999) Altered bone mass and turnover in female patients with adrenal incidentaloma: the effect of subclinical hypercortisolism. *J Clin Endocrinol Metab* 84: 2381–2385.
8. Chiodini I, Guglielmi G, Battista C, Carnevale V, Torlontano M, *et al.* (2004) Spinal volumetric bone mineral density and vertebral fractures in female patients with adrenal incidentalomas: the effects of subclinical hypercortisolism and gonadal status. *J Clin Endocrinol Metab* 89: 2237–2241.
9. Chiodini I, Morelli V, Masserini B, Salcuni AS, Eller-Vainicher C, *et al.* (2009) Bone mineral density, prevalence of vertebral fractures, and bone quality in patients with adrenal incidentalomas with and without subclinical hypercortisolism: an Italian multicenter study. *J Clin Endocrinol Metab* 94: 3207–3214.
10. Odagiri E, Naruse M, Terasaki K, Yamaguchi N, Jibiki K, *et al.* (2004) The diagnostic standard of preclinical Cushing's syndrome: evaluation of the dexamethasone suppression test using various cortisol kits. *Endocr J* 51: 295–302.
11. Nieman LK, Biller BMK, 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.
12. Katabami T, Obi R, Shirai N, Naito S, Saito N (2005) Discrepancies in results of low-and high-dose dexamethasone suppression tests for diagnosing preclinical Cushing's syndrome. *Endocr J* 52: 463–469.
13. Shen J, Sun M, Zhou B, Yan JP (2014) Nonconformity in the clinical practice guidelines for subclinical Cushing's syndrome: which guidelines are trustworthy? *Eur J Endocrinol* 171: 421–431.
14. Fassnacht M, Arlt W, Bancos I, Dralle H, Newell-Price J, *et al.* (2016) Management of adrenal incidentalomas: European Society of Endocrinology Clinical Practice Guideline in collaboration with the European Network for the Study of Adrenal Tumors. *Eur J Endocrinol* 175: G1–

- G34.
15. Terzolo M, Stigliano A, Chiodini I, Loli P, Furlani L, *et al.* (2011) Italian Association of Clinical Endocrinologists. AME position statement on adrenal incidentaloma. *Eur J Endocrinol* 164: 851–870.
 16. Eller-Vainicher C, Morelli V, Salcuni AS, Battista C, Torlontano M, *et al.* (2010) Accuracy of several parameters of hypothalamic-pituitary-adrenal axis activity in predicting before surgery the metabolic effects of the removal of an adrenal incidentaloma. *Eur J Endocrinol* 163: 925–935.
 17. Salcuni AS, Morelli V, Vainicher CE, Palmieri S, Cairolì E, *et al.* (2016) Adrenalectomy reduces the risk of vertebral fractures in patients with monolateral adrenal incidentalomas and subclinical hypercortisolism. *Eur J Endocrinol* 174: 261–269.
 18. Akehi Y, Kawate H, Murase K, Nagaishi R, Nomiyama T, *et al.* (2013) Proposed diagnostic criteria for subclinical Cushing's syndrome associated with adrenal incidentaloma. *Endocr J* 60: 903–912.
 19. Kuwa K (2016) Standardization of serum cortisol measurement in low concentration range. *ACTH RELATED PEPTIDES* 26: 44–53 (in Japanese).
 20. Tanabe M, Kageyama K, Tanaka T, Katabami T, Oki Y, *et al.* (2016) Reevaluation of diagnosis of adrenal subclinical Cushing's syndrome in relation to the standardization in the assay of low range of cortisol concentration. Annual report of Intractable Disease Research Grant of Ministry of Health, Labour and Welfare, Japan "Research on Adrenal Hormone Disorders" 2015: 92–93 (In Japanese).
 21. Kakizawa K, Okawa Y, Ohishi T, Yamashita M, Sasaki S, *et al.* (2016) Fundamental evaluation of cortisol assay kit "cortisol II". *Jpn J Med Pharm Sci* 73: 71–76 (In Japanese).
 22. Sasaki Y, Katabami T, Asai S, Fukuda H, Tanaka Y (2017) In the overnight dexamethasone suppression test, 1.0 mg loading is superior to 0.5 mg loading for diagnosing subclinical adrenal Cushing's syndrome based on plasma dexamethasone levels determined using liquid chromatography-tandem mass spectrometry. *Endocr J* 64: 833–842.
 23. Debono M, Ghobadi C, Rostami-Hodjegan A, Huatan H, Campbell MJ, *et al.* (2009) Modified-release hydrocortisone to provide circadian cortisol profiles. *J Clin Endocrinol Metab* 94: 1548–1554.
 24. Chiodini I, Morelli V, Salcuni AS, Eller-Vainicher C, Torlontano M, *et al.* (2010) Beneficial metabolic effects of prompt surgical treatment in patients with an adrenal incidentaloma causing biochemical hypercortisolism. *J Clin Endocrinol Metab* 95: 2736–2745.
 25. Midorikawa S, Sanada H, Hashimoto S, Suzuki T, Watanabe T (2001) The improvement of insulin resistance in patients with adrenal incidentaloma by surgical resection. *Clin Endocrinol (Oxf)* 54: 797–804.
 26. Emral R, Uysal AR, Asik M, Gullu S, Corapcioglu D, *et al.* (2013) Prevalence of subclinical Cushing's syndrome in 70 patients with adrenal incidentaloma: clinical, biochemical and surgical outcomes. *Endocr J* 50: 399–408.
 27. Oki Y (2011) Pitfall in the evaluation of plasma ACTH. *ACTH RELATED PEPTIDES* 22: 9–10 (In Japanese).
 28. Scarpelli M, Algaba F, Kirkali Z, Van Poppel H (2004) Handling and pathology reporting of adrenal gland specimens. *Eur Urol* 45: 722–729.
 29. Katabami T, Ishii S, Obi R, Asai S, Tanaka Y (2016) Contralateral adrenal suppression on adrenocortical scintigraphy provides good evidence showing subclinical cortisol overproduction from unilateral adenomas. *Endocr J* 63: 1123–1132.
 30. Tateishi Y, Kouyama R, Mihara M, Doi M, Yoshimoto T, *et al.* (2012) Evaluation of salivary cortisol measurements for the diagnosis of subclinical Cushing's syndrome. *Endocr J* 59: 283–289.
 31. Tsuiki M, Tanabe A, Takagi S, Naruse M, Takano K (2008) Cardiovascular risks and their long-term clinical outcome in patients with subclinical Cushing's syndrome. *Endocr J* 55: 737–745.
 32. Toniato A, Merante-Boschin I, Opocher G, Pelizzo MR, Schiavi F, *et al.* (2009) Surgical versus conservative management for subclinical Cushing syndrome in adrenal incidentalomas: a prospective randomized study. *Ann Surg* 249: 388–391.
 33. Akaza I, Yoshimoto T, Iwashima F, Nakayama C, Doi M, *et al.* (2011) Clinical outcome of subclinical Cushing's syndrome after surgical and conservative treatment. *Hypertens Res* 34: 1111–1115.
 34. Kawate H, Kohno M, Matsuda Y, Akehi Y, Tanabe M, *et al.* (2014) Long-term study of subclinical Cushing's syndrome shows high prevalence of extra-adrenal malignancy in patients with functioning bilateral adrenal tumors. *Endocr J* 61: 1205–1212.
 35. Yanase T, Tajima T, Katabami T, Iwasaki Y, Tanahashi Y, *et al.* (2016) Diagnosis and treatment of adrenal insufficiency including adrenal crisis: a Japan Endocrine Society clinical practice guideline [Opinion]. *Endocr J* 63: 765–784.
 36. Consensus of Practice in Adrenal tumor (The third edition, March 2015) edited by The Japanese Urological Association, The Japanese Society of Pathology, The Japan Radiological Society, The Japan Endocrine Society and Japan Association of Endocrine Surgeons (Kanehara) 1–123, 2015 (In Japanese)
 37. Ichijyo T, Ueshiba H (2005) Epidemiological survey of adrenal tumors in serial five years in Japan (final report). Annual report of Intractable Disease Research Grant of Ministry of Health, Labour and Welfare, Japan "Research on Adrenal Hormone Disorders" 2004: 121–129 (In Japanese).

- nese).
38. Barzon L, Scaroni C, Sonino N, Fallo F, Paoletta A, *et al.* (1999) Risk factors and long-term follow-up of adrenal incidentalomas. *J Clin Endocrinol Metab* 84: 520–526.
 39. Miyake Y, Tanaka K, Nishikawa T, Naruse M, Takayanagi R, *et al.* (2013) A nation wide secondary epidemiological study (Final Report) Annual report of Intractable Disease Research Grant of Ministry of Health, Labour and Welfare, Japan “Research on Adrenal Hormone Disorders” 2012: 23–38 (In Japanese).
 40. Mansmann G, Lau J, Balk E, Rothberg M, Miyachi Y, *et al.* (2004) The clinically inapparent adrenal mass: update in diagnosis and management. *Endocr Rev* 25: 309–340.
 41. Mantero F, Terzolo M, Arnaldi G, Osella G, Masini AM, *et al.* (2000) A survey on adrenal incidentaloma in Italy. Study group on adrenal tumors of the Italian Society of Endocrinology. *J Clin Endocrinol Metab* 85: 637–644.
 42. Young WF Jr (2007) Clinical practice. The incidentally discovered adrenal mass. *N Engl J Med* 356: 601–610.
 43. Grumbach MM, Biller BM, Braunstein GD, Campbell KK, Carney JA, *et al.* (2003) Management of the clinically inapparent adrenal mass (“incidentaloma”). *Ann Intern Med* 138: 424–429.
 44. Tabarin A, Bardet S, Bertherat J, Dupas B, Chabre O, *et al.* (2008) Exploration and management of adrenal incidentalomas. French Society of Endocrinology Consensus. V; French Society of Endocrinology Consensus. *Ann Endocrinol (Paris)* 69: 487–500.