Netherlands
The Journal of Medicine

ORIGINAL ARTICLE

Evaluation of Endocrine Tests B: screening for hypercortisolism

F. Holleman[1*], E. Endert[2], M.F. Prummel[1†], M. van Vessem-Timmermans[1], W.M. Wiersinga[1], E. Fliers[1]

[1]Department of Endocrinology and Metabolism, [2]Laboratory of Endocrinology and Radiochemistry, Department of Clinical Chemistry, Academic Medical Centre, Amsterdam, the Netherlands, tel.: +31 (0)20-566 59 54/566 91 11, fax: +31 (0)20-691 49 04, e-mail: f.holleman@amc.uva.nl, *corresponding author

ABSTRACT

Background: While reference values for 24-hour free urinary cortisol excretion and the overnight 1 mg dexamethasone-suppression test in the healthy population are available, cut-off values in patients clinically suspected of Cushing's syndrome have to be established.
Methods: This was a prospective follow-up study in one academic centre of 144 patients with clinical suspicion of Cushing's syndrome (group A) and 50 patients with adrenal incidentaloma (group B) who were referred for putative hypercortisolism between 1 January 1993 and 1 January 2003. The 24-hour urinary free cortisol and post-dexamethasone plasma cortisol were measured. Accurate diagnosis of (absence of) Cushing's syndrome was confirmed by histopathological data and long-term follow-up. Based on the data obtained in group A, sensitivity, specificity and receiver operating characteristic (ROC) curves were calculated.
Results: Complete follow-up was obtained in 86%, and partial follow-up was obtained in 8% of patients. Median follow-up was 36 (1 to 122) months. In group A, 17 patients were found to have Cushing's syndrome. In this group median 24-hour urinary free cortisol was 77 (<5 to 51458) nmol/24 hours and median post-dexamethasone plasma cortisol was <50 (<50 to 4900) nmol/l. Area under the ROC curve was 0.958 for 24-hour urinary free cortisol and 0.985 for post-dexamethasone plasma cortisol. Optimal cut-off values were 180 nmol/24 hours (sensitivity 94%, specificity 93%) and 95 nmol/l (sensitivity 100%, specificity 94%) respectively.
Conclusion: We established cut-off values for 24-hour free urinary cortisol excretion (180 nmol/24 hours) and for post-dexamethasone plasma cortisol (95 nmol/l) in the evaluation of patients referred for hypercortisolism.

KEYWORDS

Adrenal incidentaloma, cortisoluria, Cushing, dexamethasone-suppression test, screening

INTRODUCTION

The laboratory investigation of patients clinically suspected of having Cushing's syndrome is usually divided into two distinct phases. The first diagnostic phase tries to establish the presence of hypercortisolism, which is the hallmark of Cushing's syndrome. In the second phase, the cause of the hypercortisolism is established.
The 24-hour excretion of urinary free cortisol and the overnight 1 mg dexamethasone-suppression test are widely accepted as screening tests.[1-3] We previously established reference values for 24-hour excretion of urinary free cortisol and the 1 mg dexamethasone-suppression test.[4]
It should be realised that the reference values established in healthy controls are not necessarily of discriminative value in the patients referred for evaluation. For instance, obese subjects are more likely to be screened for Cushing's syndrome than lean patients, and obesity is associated with disturbances in cortisol metabolism mimicking Cushing's syndrome.[5] Thus, it is also necessary to estab-

lish cut-off values within the patient group that is referred because of clinical suspicion of Cushing's syndrome.
We prospectively studied the data of all patients referred to our hospital with suspected Cushing's syndrome over a ten-year period. Referral was based either on clinical characteristics or on incidental findings of imaging studies.

MATERIALS AND METHODS

Patients

In this study, data from all patients who underwent endocrine evaluation for Cushing's syndrome at the Department of Endocrinology of the Academic Medical Centre, Amsterdam between 1 January 1993 and 1 January 2003 were analysed. If a patient had repeated tests during the study period, only the first set of tests was used to evaluate the diagnostic accuracy.
A definite diagnosis of Cushing's syndrome was made on the basis of clinical data and histopathological data obtained by surgery. Patients referred because of an adrenal incidentaloma were analysed separately and were excluded from the formal analyses with regard to test characteristics.
Patients who had a clear, final diagnosis and patients who were still being seen in our hospital in 2003 were considered to have adequate follow-up. To obtain follow-up data for those patients who were no longer visiting our hospital, we contacted their general practitioners by telephone, asking specifically about a diagnosis of Cushing's syndrome, pituitary or adrenal surgery and chronic systemic steroid use before considering follow-up as negative. Patients who had moved some years after being tested but in whom Cushing's syndrome had not been diagnosed at their last GP visit were considered to have a partial follow-up.

The 24-hour excretion of urinary free cortisol

Patients were asked to collect two separate consecutive 24-hour urine samples. During this collection, the urine was to be kept refrigerated. Total urine volumes as well as concentrations of free cortisol and creatinine were measured. The mean urinary excretion of these two samples was used for analysis. If total creatinine excretion in the sample with the highest creatinine excretion was more than 150% of the creatinine excretion of the other sample, both samples were excluded, but if only one 24-hour sample was obtained this was used for analysis.

Overnight 1 mg dexamethasone-suppression test

This test was performed one day after the second 24-hour urine collection. On day 1, a blood sample was taken at 08.30 hours by venipuncture. At 23.00, 1 mg of dexamethasone was given orally. On day 2, a second blood sample was taken at 08.30 by venipuncture. Both samples were taken in the postabsorptive phase, in seated position and after 30 minutes rest. In both samples concentrations of ACTH and cortisol were measured.

Analytical methods

Measurement of adrenocorticotrophic hormone (ACTH) and (urinary) cortisol was carried out in duplicate.
Initially, plasma cortisol was measured by fluorescence polarisation assay on a TDX analyser (Abbott Laboratories, Chicago, IL, USA). After five years we switched to a luminescence immunoassay on an Immulite I analyser (DPC, Los Angeles, CA, USA). Long-term reproducibility was monitored by comparing with the laboratory results in the Dutch National External Quality Assessment Scheme (NEQAS) for Ligand Assay of Hormones (LWBA). The Abbott cortisol assay consistently measured 15% higher than the all trimmed mean, while the DPC assay equalled this value during the study period. Hence, the Abbott cortisol concentrations were divided by 1.15 to convert to the DPC cortisol values. Both assays had an interassay variation coefficient of 9% at the level of 200 nmol/l and 5% at the level of 850 nmol/l. Detection limit for both assays was 50 nmol/l.
Urinary cortisol was measured by an in-house high performance liquid chromatography (HPLC) method. Long-term reproducibility was monitored by comparing with the laboratory results in the United Kingdom NEQAS. A consistent negative difference of 30 to 40% was seen compared with the (immunoassay) all trimmed mean. Internal quality control samples, used during periods of two years, showed an interassay variation of 10.5% at levels of 49 and 126 nmol/l. Detection limit was 5 nmol/l.
In 1993 and 1994 ACTH was measured by immunoradiometric (IRMA) assay and after this period by immunoluminometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). Because ACTH is not covered by the LWBA, long-term reproducibility was monitored by internal quality control samples. New kit lot numbers were compared with quality control samples that were used in the old kit lot numbers. No significant changes were seen during the study period. The interassay variation was 7.5% at levels of 30 and 320 ng/l. Detection limit was 1 ng/l.

Statistical analysis

Values below the detection limits of the assays were included in the analyses as having a value of 50% of the detection limit. Variables were tested for normality using P-P plots. For normally distributed variables, means, standard deviation and T tests were used. For other variables medians and nonparametric tests were used. ROC curves were created to establish the optimal cut-off values for urinary free cortisol levels and post-dexamethasone cortisol levels. Statistical analysis was performed using SPSS for Windows version 11.5.0.

RESULTS

A total of 194 patients was evaluated, 49 men and 145 women. Of these patients, 144 were referred because of clinical suspicion of Cushing's syndrome (group A), whereas the other 50 were tested because of an incidental adrenal mass (group B). Follow-up was complete for 86% of all these patients. Partial follow-up was obtained for 8% of patients, whereas in 6% no follow-up was obtained. None of the 100 patients who were followed-up by a contact with the general practitioner developed Cushing's syndrome. Median duration of follow-up was 36 (1 to 122) months.

The patient characteristics are presented in *table 1*. Patients in group A were significantly younger and had a higher body mass index than those in group B. The proportion of women was higher in group A than in group B, as was the proportion of women on oral contraceptives. Cushing's syndrome was diagnosed in 17 patients (12%) in group A. Of these, eight patients had an ACTH-producing pituitary adenoma, five an adrenal adenoma/adenocarcinoma and four an ectopic ACTH-producing tumour. Within group A, patients with Cushing's syndrome had a significantly higher diastolic blood pressure than those without.

On the basis of our historical cut-off values we classified five patients in group B as Cushing's syndrome. Three patients in group B had an increased plasma cortisol (>140 nmol/l) after dexamethasone and in two of these an adenoma was removed surgically; in the other the diagnosis of bilateral adrenal hyperplasia was made and the patient is still being followed up. Two patients had a urinary free cortisol of over 145 nmol/24 hours; in one of these the diagnosis of an adenoma was made and in the other an adenocarcinoma.

In 14 patients another final diagnosis (e.g. phaeochromocytoma or adrenal metastasis) was made.

Urinary free cortisol

The 24-hour urinary free cortisol was measured in 142 patients in group A, and in 46 patients in group B. Data for urinary free cortisol (mean of both samples) are presented in *figure 1*. Median urinary free cortisol was 77 (range <5 to 51458) nmol/24 hours in group A and 73 (range <5 to 304) nmol/24 hours in group B. In group A (but not in group B) the difference in urinary free cortisol between those without and those with Cushing's disease was significant (median 68 and 609 nmol/24 hours, respectively, p<0.001 Mann-Whitney U test).

Within group A, there was a nonsignificant difference in urinary free cortisol between women using and those not using oral contraceptives (median 92 and 69 nmol/24 hours, respectively, p=0.10). After exclusion of those in whom Cushing's syndrome had been diagnosed, this difference became significant (median 88 *vs* 58 nmol/24 hours, p=0.016).

Overnight 1 mg dexamethasone-suppression test

Test results were available for all patients. Median baseline cortisol was 440 (range <50 to 4400) nmol/l in group A and 415 (range 100 to 730) nmol/l in group B. Median baseline ACTH was 25 (range <1 to 310) ng/l in group A and 15 (range <1 to 210) ng/l in group B.

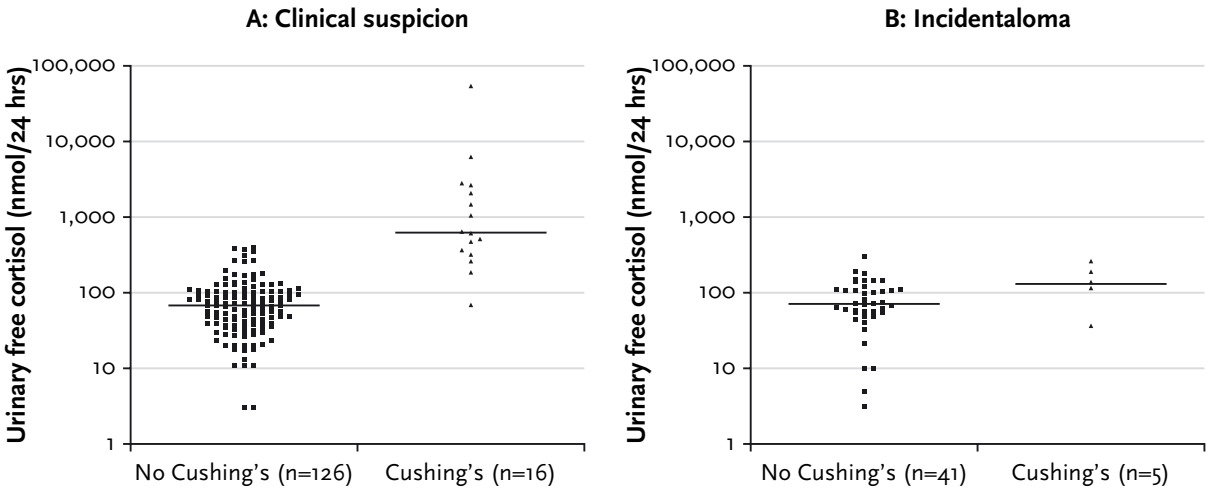
Data for post-dexamethasone cortisol are presented in *figure 2*. Median post-dexamethasone cortisol was <50 (range <50 to 4900) nmol/l in group A and 55 (range <50 to 720) nmol/l in group B. In group A (but not in group B) the difference in post-dexamethasone cortisol between those without and those with Cushing's syndrome was significant (median <50 and 890 nmol/l, respectively, p<0.001 Mann-Whitney U test).

Table 1 *Clinical characteristics of all patients evaluated for hypercortisolism*

	Clinical suspicion			Incidentaloma		
	All	Cushing's	No Cushing's	All	Cushing's	No Cushing's
N	144	17	127	50	5	45
Sex (m/f)	31/113[&]	4/13	27/100	18/32[&]	2/3	16/29
Males (%)	22%	24%	21%	45%	40%	36%
Age	40 ± 13[$]	40 ± 12	40 ± 13	59 ± 13[$]	59 ± 13	59 ±13
Systolic BP	150 ± 25	159 ± 23	148 ± 25	152 ± 23	153 ± 17	152 ± 24
Diastolic BP	92 ± 15	102 ± 17[#]	91 ± 15[#]	90 ± 12	91 ± 10	90 ± 12
BMI	30 ± 7[*]	28 ± 5	31 ± 7	28 ± 7[*]	28 ± 4	28 ± 7
Oral contraceptive users	30[&]	2	28	2[&]	0	2

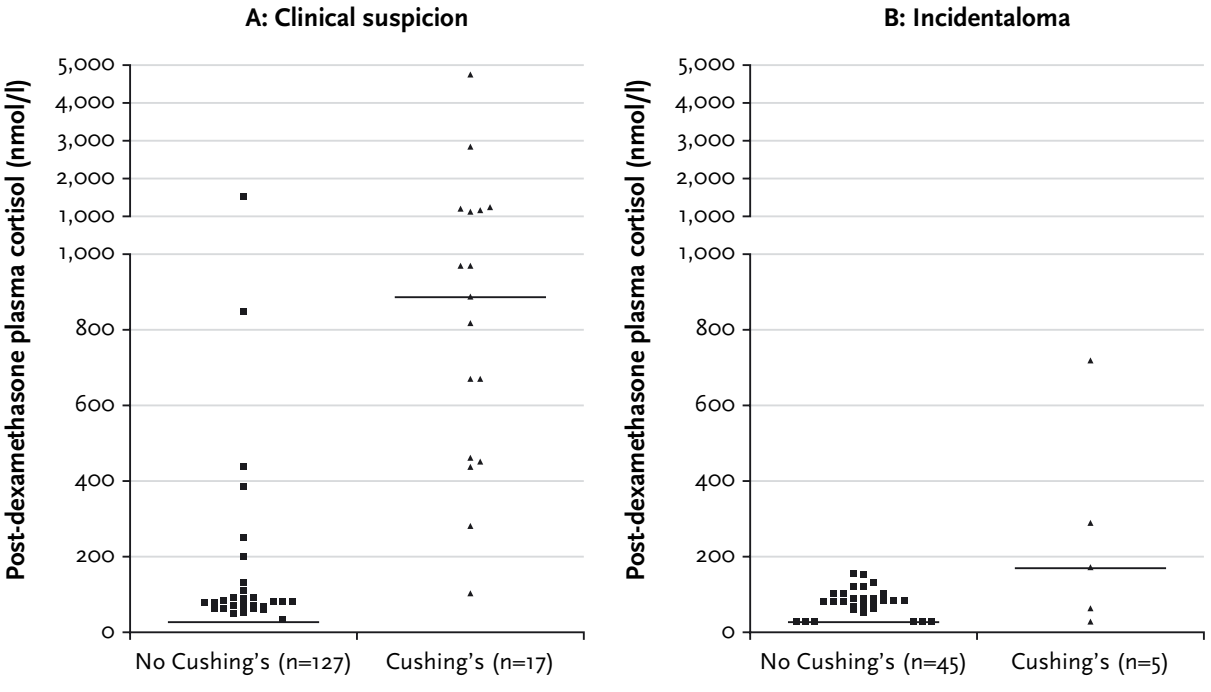
[*]p≤0.05 clinical suspicion *vs* incidentaloma (independent samples T test); [$]p<0.001 clinical suspicion *vs* incidentaloma (independent samples T test); [#]p<0.01 Cushing's *vs* no Cushing's (independent samples T test); [&]p≤0.05 clinical suspicion *vs* incidentaloma (χ^2 test). BP = blood pressure; BMI = body mass index.

Figure 1 *24-hour urinary free cortisol (nmol/24 hours) in patients with clinical suspicion of hypercortisolism (A) and in patients with adrenal incidentaloma (B)*



Please note the logarithmic nature of the scale. The line represents the median of the distribution.

Figure 2 *Plasma cortisol levels (nmol/l) after 1 mg dexamethasone in patients with clinical suspicion of hypercortisolism (A) and patients with adrenal incidentaloma (B)*



The line represents the median of the distribution.

Within group A, there was a significant difference in post-dexamethasone cortisol between women using and those not using oral contraceptives (median 60 and <50 nmol/l, respectively, p=0.014). This difference remained after exclusion of those patients with Cushing's syndrome (median 55 *vs* <50 nmol/l, p<0.001).

Test characteristics

ROC curves for 24-hour urinary free cortisol and post-dexamethasone cortisol in patients with clinical suspicion of Cushing's syndrome are shown in *figure 3*. The areas under the ROC curve were 0.958 for 24-hour urinary free cortisol and 0.985 for post-dexamethasone cortisol.
Cut-off values and corresponding sensitivities and specificities are shown in *table 2*. When women who were using oral contraceptives were excluded from the analysis, cut-off levels for urinary free cortisol and post-dexamethasone cortisol remained the same, with little change in sensitivity or specificity (data not shown).

DISCUSSION

Even though testing for Cushing's syndrome has a long history, controversies persist about the optimal screening procedure and the test cut-off levels to be used.
In the last few years there have been several promising reports about the use of salivary cortisol measurements.[6-8] Likewise, a midnight cortisol has been used as an alternative screening test.[8,9] In our hospital the overnight dexamethasone-suppression test and 24-hour urinary free cortisol have been used for over a decade, with prospective recording of patient data and a long follow-up period. Therefore, we decided to study all patients referred to our

Figure 3 *ROC curves for 24-hour urinary free cortisol (A) and post-dexamethasone cortisol levels (B) in patients with clinical suspicion of hypercortisolism*

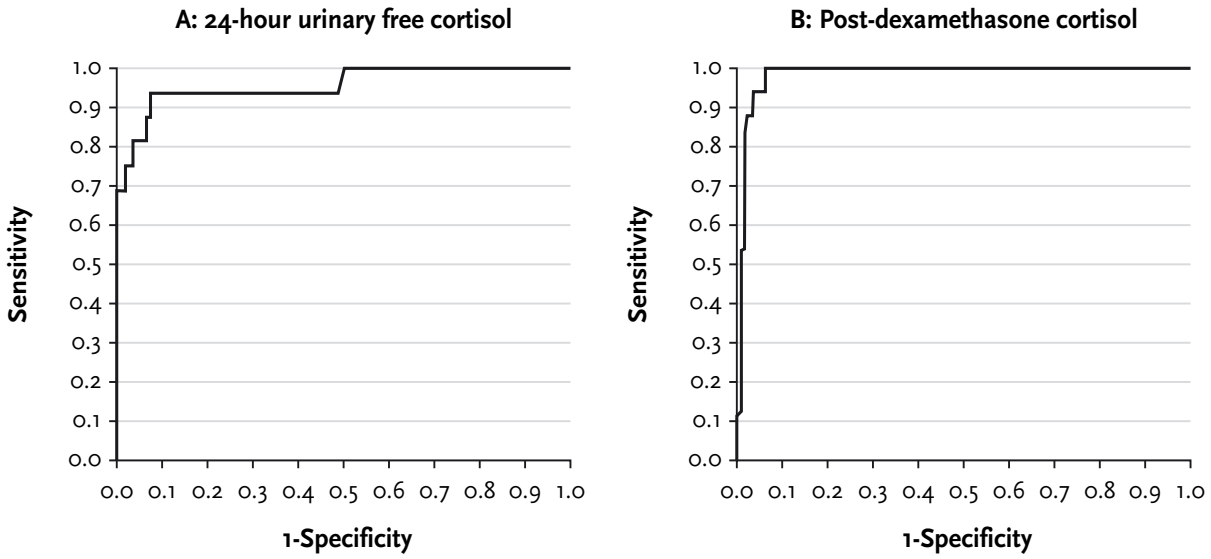


Table 2 *Cut-off values for 24-hour urinary free cortisol and post-dexamethasone plasma cortisol in patients with clinical suspicion of hypercortisolism*

	100% sensitivity	100% specificity	Optimal*
24-hour urinary free cortisol (nmol/24 hours)	68 (specificity 50%)	427 (sensitivity 69%)	180 (sensitivity 94%, specificity 93%)
Post-dexamethasone plasma cortisol (nmol/l)	95 (specificity 94%)	2225 (sensitivity 12%)	95 (sensitivity 100%, specificity 94%)

*The optimal cut-off value is the value that yields the highest sensitivity and specificity.

department for evaluation of putative hypercortisolism, in order to obtain optimal cut-off values for 24-hour urinary free cortisol and for the 1 mg dexamethasone-suppression test. There were two main reasons for referral: clinically suspected Cushing's syndrome and adrenal incidentaloma. While descriptive data are presented, we decided not to perform a formal analysis of the test characteristics in patients with an incidental adrenal mass for two reasons. Firstly, in these patients the test results form an integral part of the decision to operate and hence in the diagnosis, which makes it difficult to establish a 'gold standard'. At a recent National Institutes of Health (NIH) consensus conference a similar comment was made about the elusive nature of the diagnosis 'subclinical hypercortisolism'.[10] Secondly, only five patients were ultimately labelled as having Cushing's syndrome, too small a number to obtain reliable ROC curves.

Not surprisingly, in the group referred because of clinical suspicion of Cushing's syndrome, the range of values for urinary free cortisol and post-dexamethasone cortisol exceeded the reference range previously established in healthy volunteers.[4] While the optimal cut-off level for urinary free cortisol at 180 nmol/24 hours was above the upper normal limit of 145 nmol/24 hours, the 100% sensitivity level was much lower at 68 nmol/24 hours. This was associated with a very low specificity.

For post-dexamethasone cortisol the optimal level and the 100% sensitivity level were the same at 95 nmol/l, which was considerably lower than the upper limit of normal of 230 nmol/l previously established. This again underlines the fact that reference values established in healthy volunteers can not be equated to cut-off values for specific diagnostic groups.

The 100% sensitivity we found at 95 nmol/l compares favourably with data from Findling *et al.* who reported false-negative rates of 18% at a 135 nmol/l cut-off level and 8% at a 54 nmol/l cut-off level in a large series of patients with Cushing's syndrome.[11] While this may in part be explained by the fact that all measurements in our series were performed in one laboratory under standardised conditions, the number of patients with Cushing's disease in our study was limited and so some caution seems warranted in too rigorously interpreting our results.

Given the low prevalence of disease (12%) in the population studied for hypercortisolism, the dexamethasone-suppression test seems superior to 24-hour urinary free cortisol as a screening tool, with a greater area under the ROC curve and a far higher specificity at the 100% sensitivity level.

REFERENCES

1. Kaye TB, Crapo L. The Cushing Syndrome: an update on diagnostic tests. Ann Intern Med 1990;112:434-44.
2. Raff H, Findling JW. A physiologic approach to diagnosis of the Cushing syndrome. Ann Intern Med 2003;138:980-91.
3. Wood PJ, Barth JH, Freedman DB, Perry L, Sheridan B. Evidence for the low dose dexamethasone-suppression test to screen for Cushing's syndrome – recommendations for a protocol for biochemistry laboratories. Ann Clin Biochem 1997;34:222-29.
4. Bos Kuil MJJ, Endert E, Fliers E, Prummel MF, Romijn JA, Wiersinga WM. Establishment of reference values for endocrine tests. I: Cushing's syndrome. Neth J Med 1998;53:153-63.
5. Putignano P, Bertolini M, Losa M, Cavagnini F. Screening for Cushing's syndrome in obese women with and without polycystic ovary syndrome. J Endocrinol Invest 2003;26:539-44.
6. Gafni RI, Papanicolaou DA, Nieman LK. Nighttime salivary cortisol measurement as a simple, noninvasive, outpatient screening test for Cushing's syndrome in children and adolescents. J Pediatr 2000;137:30-35.
7. Castro M, Elias LLK, Elias PCL, Moreira AC. A dose-response study of salivary cortisol after dexamethasone-suppression test in Cushing's disease and its potential use in the differential diagnosis of Cushing's syndrome. Clin Endocrinol 2003;59:800-05.
8. Putignano P, Toja P, Dubini A, Giraldi FP, Corsello SM, Cavagnini F. Midnight salivary cortisol versus urinary free and midnight serum cortisol as screening tests for Cushing'syndrome. J Clin Endocrinol Metab 2003;88:4153-57.
9. Papanicolaou DA, Yanovski JA, Cutler GB, Chrousos GP, Nieman LK. A single midnight serum cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab 1998;83, 1163-67.
10. Grumbach M, Biller BMK, Braunstein GD et al. Management of the clinically inapparent adrenal mass ("incidentaloma"). Ann Intern Med 2003;138:424-29.
11. Findling JW, Raff H, Aron DC. The low-dose dexamethasone-suppression test: a reevaluation in patients with Cushing's syndrome. J Clin Endocrinol Metab 2004;89:1222-26.