0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(9):4153–4157

doi: 10.1210/jc.2003-030312

Midnight Salivary Cortisol *Versus* Urinary Free and Midnight Serum Cortisol as Screening Tests for Cushing's Syndrome

PIETRO PUTIGNANO, PAOLA TOJA, ANTONELLA DUBINI, FRANCESCA PECORI GIRALDI, SALVATORE MARIA CORSELLO, AND FRANCESCO CAVAGNINI

Department of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca (P.P., P.T., A.D., F.P.G., F.C.), 20149 Milan, Italy; and Università Cattolica del Sacro Cuore, Policlinico A. Gemelli (S.M.C.), 00168 Rome, Italy

The diagnosis of Cushing's syndrome (CS) is often a challenge. Recently, the determination of late night salivary cortisol levels has been reported to be a sensitive and convenient screening test for CS. However, no studies have included a comparison with other screening tests in a setting more closely resembling clinical practice, *i.e.* few patients with CS to be distinguished from patients with pseudo-Cushing states (PC), including the large population of obese patients. The aim of this study was to compare the diagnostic performance of midnight salivary cortisol (MSC) measurement with that of midnight serum cortisol (MNC) and urinary free cortisol (UFC) in differentiating 41 patients with CS from 33 with PC, 199 with simple obesity, and 27 healthy normal weight volunteers. Three patients with CS had MSC levels lower than the cut-off point derived from receiver operator characteristic analysis (9.7 nmol/liter), yielding a sensitivity for this parameter of 92.7%. In the whole study population, no statistically significant differences in terms of sensitivity, specificity, diagnostic accuracy, and predictive values were observed among tests. In particular, the overall diagnostic accuracy for MSC (93%; 95% confidence interval, 90.1–95.9%) was similar to those of UFC (95.3%; 94.1–96.5%) and MNC (95.7%; 93.4–98%; both P = NS). The diagnostic performance of MSC was superimposable to that of MNC also within the area of overlap in UFC values (≤569 nmol/24 h) between CS and PC. In conclusion, MSC measurement can be recommended as a first-line test for CS in both low risk (simple obesity) and high-risk (*i.e.* PC) patients. Given its convenience, this procedure can be added to tests traditionally used for this purpose, such as UFC and MNC. (*J Clin Endocrinol Metab* 88: 4153–4157, 2003)

THE DIAGNOSIS OF Cushing's syndrome (CS) is based on the biochemical demonstration of elevated 24-h urinary free cortisol (UFC) levels associated with nondexamethasone (non-dex)-suppressible hypercortisolism and disruption of the serum cortisol circadian rhythm (1, 2). However, some of these features may also be encountered in disorders such as major depression, chronic alcoholism, type 2 diabetes mellitus, visceral obesity (1), and polycystic ovary syndrome (3–5), *i.e.* pseudo-Cushing (PC) states. Furthermore, the results of UFC and dex suppression studies may be misleading in patients who fail to correctly collect urine or take tablets, in those receiving drugs that induce cytochrome P450-related enzymes or with defective intestinal absorption of dex, and, finally, in patients with renal or hepatic failure (6, 7). A single midnight serum cortisol (MNC) reportedly has an excellent sensitivity for the diagnosis of CS, but its specificity remains uncertain due to the lack of adequate control groups, *i.e.* patients with PC states, and, indeed, false positive results have been described (6, 8, 9). Further, the test is burdened by costs of inpatient admission. In the attempt to improve on the suboptimal diagnostic performance of the above-mentioned tests and lower the costs of screening, other diagnostic procedures have been proposed. Along this line, promising results have been reported with the dex-CRH stimulation test (10) and the desmopressin test (11). However, although these tests may prove useful in many cases, their complexity argues against their use as first-line procedures to screen for CS. Conversely, there is increasing evidence (12–16) that late night salivary cortisol (MSC) determination may be a useful diagnostic tool given its diagnostic accuracy, convenience, and feasibility on an out-patient basis. However, only one study of MSC has included a comparison with the traditional tests (16), and none evaluated large populations of obese subjects, the patients most often suspected for CS.

To establish the diagnostic performance of MSC compared with other screening tests, we prospectively measured UFC, MNC, and MSC in normal weight, healthy volunteers (NS), in subjects with simple obesity (OB) and in patients evaluated for CS.

Subjects and Methods

Subjects

Three hundred subjects comprising 41 patients with CS, 33 with PC, 199 with OB, and 27 NS admitted at our institution between February 1994 and October 2000, participated in the study. The diagnosis of CS was based on clinical and laboratory findings (2, 11) and comprised 33 patients with Cushing's disease, six with cortisol-secreting adrenal tumor, one with ACTH-independent adrenocortical nodular hyperplasia, and one with ACTH-secreting pancreatic neuroendocrine tumor. The diagnosis of Cushing's disease was confirmed by pituitary pathology in 29 cases and by postoperative clinical and biochemical resolution of hypercortisolism in the remaining four.

Diagnosis of PC was established by the presence of Cushingoid signs, *e.g.* visceral obesity, buffalo hump, hirsutism, and purple striae, asso-

Abbreviations: AUC, Area under the curve; CS, Cushing's syndrome; dex, dexamethasone; MNC, midnight serum cortisol; MSC, midnight salivary cortisol; NS, normal subjects; OB, subjects with simple obesity; PC, pseudo-Cushing states; ROC, receiver operator characteristic; UFC, urinary free cortisol.

ciated with hypercortisolism (UFC range, 80.1–205.3 μg/24 h; 221–566.4 nmol/24 h) and in some cases lack of cortisol circadian rhythm and/or its suppression by low dose overnight dex. Of these patients, two had alcoholic PC, three had poorly controlled type 2 diabetes mellitus, 11 had major depression according to DSM IV criteria (17), seven had marked truncal obesity, and 10 had polycystic ovary syndrome according to standard clinical, hormonal, and ecographic criteria (18). Patients with PC displayed no clinical or biochemical progression toward overt CS on prolonged follow-up (mean, 27 months; range, 24–46 months). When appropriate, the diagnosis of CS was also excluded by means of a dex-CRH test (10) and in patients with ACTH-dependent hypercortisolism by an absent ACTH response to desmopressin (11). Normal weight volunteers were hospital nurses and attendants admitted to the hospital for 2 d for MNC determination. None of the NS and OB had a history or present evidence of major psychiatric disease or was taking medications known to interfere with the hypothalamic-pituitary-adrenal axis. All subjects gave informed consent to participate in the study, which was approved by the ethical committee of our institution.

Study design

Blood samples were collected at 0800 and 2400 h, 1 h after placement of an in-dwelling venous catheter. When asleep, subjects were awakened to collect saliva immediately before blood sampling. Between 2100 and 2400 h, patients and volunteers were fasting and resting in bed and were not engaged in any activity. Tests were performed in all study subjects at least 24 h after admission. Three consecutive 24-h urine collections for UFC measurement were performed for each subject. UFC values were calculated as the mean of the data from these specimens.

Assays

Saliva was collected in commercially available devices (Salivette) using a cotton swab chewed for 2–3 min and inserted into a double-chamber plastic test tube. Serum and salivary samples were centrifuged at 4 C and stored at −20 C until assayed. Serum and urinary cortisol were measured by RIA [Byk-Sangtec Diagnostica (Dietzenbach, Germany) for serum cortisol and Diagnostic Products (Los Angeles, CA) for urinary cortisol]. Salivary cortisol was measured with the same assay used for serum with the analyte volume increased from 25–250 μl. UFC was assayed after urine extraction with dichloromethane. The sensitivity of the methods was 50 ng/dl (1.4 nmol/liter) for salivary cortisol and 0.5 μg/dl (13.8 nmol/liter) for serum and urinary cortisol. Intra- and interassay coefficients of variations were 4.4 and 4.9% for serum cortisol, 4.5 and 5.8% for salivary cortisol, and 3.5 and 6.2% for UFC, respectively. All samples from a given subject were run in the same assay. Normal ranges in our laboratory are 5–25 μg/dl (138–689.8 nmol/liter) and 10–80 μg/24 h (27.6–220.7 nmol/24 h) for 0800 h serum cortisol and UFC, respectively. No reference range is given for MNC values.

Statistical analyses

Results are presented as the mean ± SE. Intergroup differences were evaluated by ANOVA (Fisher's *post hoc* test), whereas correlations between serum and salivary cortisol were performed by linear regression analysis. Sensitivity, specificity, diagnostic accuracy, and predictive values were calculated according to standard statistical methods (19). Sensitivity against 1-specificity was plotted at each level, and the area under the receiver operator characteristic (ROC) curve (AUC) of each test, an index of the probability of correctly identifying CS and the other groups of subjects, was compared by nonparametric Wilcoxon statistic (20). Ninety-five percent confidence intervals for AUC and other diagnostic parameters were calculated according to the formulae: $\pm 1.96 \times$ SE and $x \pm 1.96\sqrt{x(1-x)/n}$, respectively. The concordance of the diagnoses assigned by salivary and serum cortisol measurements was assessed by χ^2 statistic using cut-off criteria obtained from ROC analysis. Statistical analyses were performed with the commercially available software package (SPSS for Windows, version 10.0, SPSS, Inc., Chicago, IL). $P < 0.05$ was considered statistically significant.

TABLE 1. Characteristics of the study population

Subjects	Gender (M/F)	Age (yr) (range)	BMI (kg/m^2)	0800-h serum cortisol (μg/dl)	2400-h serum cortisol (μg/dl) (MNC)	0800-h salivary cortisol (μg/dl)	2400-h salivary cortisol (μg/dl) (MSC)	UFC (μg/24 h)
CS	7/34	35.4 (18–56)	30.1 ± 1.30^e	19.8 ± 1.16	20.6 ± 1.17	0.87 ± 0.11	0.97 ± 0.13	389.5 ± 54.91
PC	9/24	34.9 (19–76)	32.3 ± 1.77^e	17.4 ± 0.78	6.4 ± 0.69^a	0.67 ± 0.07	0.23 ± 0.02^a	110.4 ± 5.31^a
OB	26/173	39.2 (18–75)	39.2 ± 0.53	13.5 ± 0.40^{a,d}	3.6 ± 0.22^{a,d}	0.45 ± 0.02^{a,c}	0.20 ± 0.01^a	38.6 ± 1.39^{a,c}
NS	8/19	36.3 (19–61)	22.7 ± 0.54^{c,e}	13.5 ± 0.84^{a,b}	2.5 ± 0.27^{a,d}	0.53 ± 0.05^a	0.18 ± 0.02^a	50.4 ± 3.95^a

BMI, Body mass index.
$^aP < 0.0001$ *vs.* patients with CS; $^bP < 0.01$, $^cP < 0.005$, and $^dP < 0.0005$ *vs.* PC patients. $^eP < 0.0001$ *vs.* obese patients.
To convert serum and salivary cortisol micrograms per deciliter to SI units, multiply by 27.6. To convert UFC, multiply by 2.76.

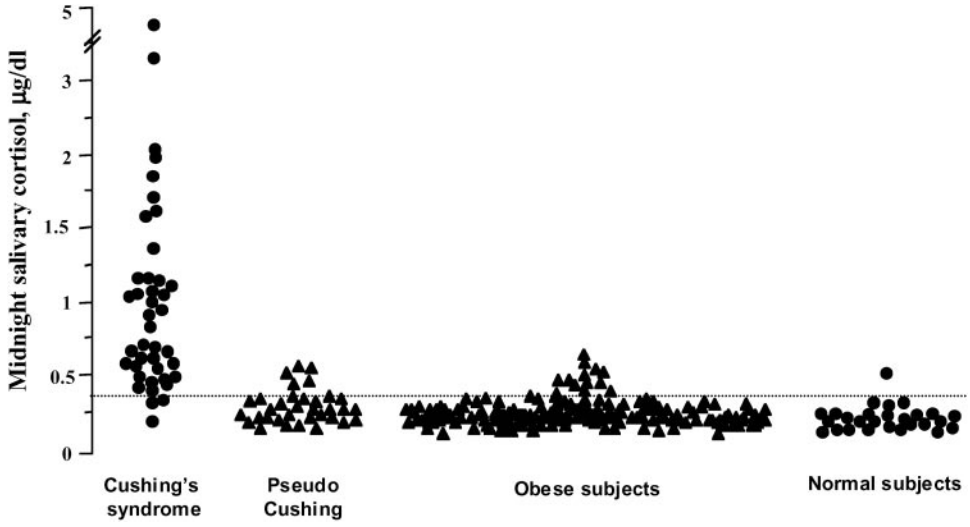

FIG. 1. MSC values in patients with CS and PC and in OB and NS. The *broken line* represents the cut-off value for the diagnosis of CS. To convert salivary cortisol micrograms per deciliter to Systeme International units, multiply by 27.6.

Results

Demographic and hormonal characteristics of patients are shown in Table 1. As expected, patients with CS, compared with PC, OB, and NS had higher UFC levels and higher serum and salivary cortisol levels at 0800 and 2400 h. In addition, patients with PC showed higher UFC and MNC concentrations and higher 0800 h cortisol values in serum and saliva, but similar MSC values compared with OB and NS (Table 1). There was no difference in cortisol levels between OB and NS (Table 1). In all subjects a positive correlation was found between serum and salivary concentrations at 0800 and 2400 h ($r = 0.67$; $P < 0.0001$). Further, UFC concentrations were correlated with cortisol levels in serum and saliva at 0800 h ($r = 0.51$; $P < 0.001$ and $r = 0.55$; $P = 0.0005$, respectively) and at 2400 h ($r = 0.43$; $P < 0.005$ and $r = 0.79$; $P < 0.0001$, respectively) in patients with CS, but not in the other groups. Individual MSC values are shown in Fig. 1.

Comparison among different screening tests

ROC curves were created to establish both the optimal threshold values for each test and their inherent diagnostic efficacy independently from specific cut-offs. In the whole study population no statistically significant differences in terms of sensitivity, specificity, diagnostic accuracy, or predictive values were observed among UFC, MNC, and MSC if cut-off values derived from ROC analysis were applied, *i.e.* 120 μg/24 h (331.1 nmol/24 h) for UFC, 12 μg/dl (331.1 nmol/liter) for MNC, and 0.35 μg/dl (9.7 nmol/liter) for MSC. However, the specificities of MNC and UFC appeared to be slightly, although not significantly, higher than that of MSC (Table 2). Accordingly, the AUCs relative to MNC and UFC were slightly greater than that of MSC (Fig. 2 and Table 2). In contrast, the specificity, positive predictive value, and diagnostic accuracy of UFC were lower when the upper limit of reference range (*i.e.* >80 μg/24 h; 221 nmol/24 h) was applied (Table 2). As interpretation of UFC values in clinical practice is currently based on reference range values, we used this lower cut-off for subsequent analyses.

The combination of MSC with UFC correctly identified all patients with CS and 209 of 259 control subjects yielding 80.7% specificity and 83.3% diagnostic accuracy. Similarly, MNC combined with UFC measurement allowed the identification of all patients with CS and 216 of 259 control subjects with 83.4% specificity and 85.7% diagnostic accuracy, statistically superimposable to MSC and UFC. Both MSC and MNC correctly identified the only patient with CS with normal UFC levels. Furthermore, MSC and MNC allowed us to rule out CS in 32 and 34 of 38 control subjects with elevated UFC concentrations, respectively. The concordance between diagnoses (having CS/not having CS) established by MSC and MNC was 92%. In detail, there were 271 (90.3%) correct and concordant diagnoses, 5 (1.7%) incorrect and concordant diagnoses, and 24 (8%) discordant diagnoses. Among the latter, MSC suggested the correct diagnosis in eight cases, and MNC did so in 16 cases (P = NS).

We also compared the diagnostic performance of MSC with MNC within the area of overlap in UFC values between CS and PC (*i.e.* UFC ≤206 μg/24 h; 569 nmol/24 h; 14 pa-

TABLE 2. Diagnostic performance of screening tests for CS

Criterion	Sensitivity (%)	Specificity (%)	+Predictive value	−Predictive value	Diagnostic accuracy (%)	AUC_{ROC}
UFC >80 μg/24 h[a]	97.6 (92.9–102.3)	85.3 (81.0–89.6)	0.51 (0.40–0.62)	0.99 (97.7–100.3)	87.0 (83.2–90.8)	0.986 ± 0.006 (0.975–0.997)
≥120 μg/24 h	90.2 (81.6–98.8)	96.1[d] (94.9–97.3)	0.79[c] (0.67–0.91)	0.98 (0.94–1)	95.3[d] (94.1–96.5)	
MNC ≥12.0 μg/dl	90.2 (81.6–98.8)	96.5[d] (95.4–97.6)	0.80[c] (0.69–0.91)	0.98 (0.90–1)	95.7[d] (93.4–98.0)	0.986 ± 0.007 (0.971–1)
MSC ≥0.35 μg/dl	92.7 (83.7–99.7)	93.1[c] (91.5–94.7)	0.68[b] (0.56–0.80)	0.99 (0.95–1)	93.0[c] (90.1–95.9)	0.967 ± 0.160 (0.935–0.999)

Individual cut-offs were derived from ROC analysis plotting patients with CS *vs.* all other study subjects. Numbers in *parentheses* correspond to 95% confidence intervals. To convert serum and salivary cortisol micrograms per deciliter to SI units multiply by 27.6. To convert UFC, multiply by 2.76.

[a] Upper limit of reference range; [b] $P = 0.055$; [c] $P < 0.05$; and [d] $P < 0.0005$ *vs.* UFC (>80 μg/24 h criterion).

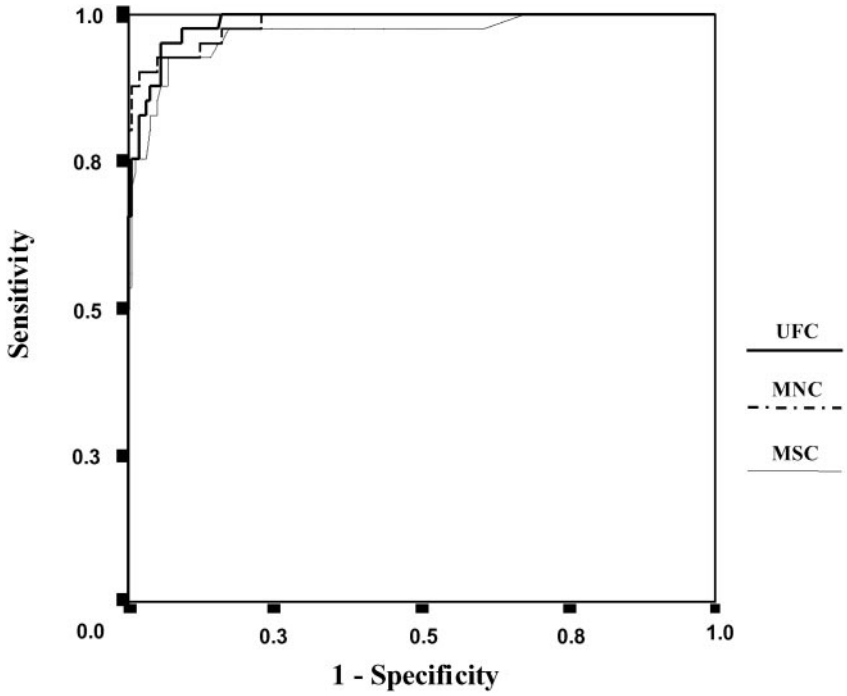

FIG. 2. ROC curves using UFC, MNC, and MSC as criteria for the diagnosis of CS.

TABLE 3. Diagnostic performance of different tests within the area of overlap in UFC values (≤206 μg/24 h) between CS (n = 14) and PC states (n = 33)

Criterion	Sensitivity (%)	Specificity (%)	+Predictive value	−Predictive value	Diagnostic accuracy (%)	AUC_{ROC}
MNC ≥12.0 μg/dl	85.7 (67.4–100)	90.9 (81.1–100)	0.8 (0.4–1)	0.94 (0.86–1)	89.4 (78.6–100)	0.959 ± 0.034 (0.893–1.025)
MSC ≥0.35 μg/dl	85.7 (67.4–100)	84.8 (72.6–97.0)	0.71 (0.49–0.93)	0.93 (0.84–1)	85.1 (74.9–95.3)	0.893 ± 0.053 (0.789–0.997)

Numbers in *parentheses* correspond to 95% confidence intervals. To convert serum and salivary cortisol micrograms per deciliter to SI units multiply by 27.6. All comparisons NS.

tients with CS and 33 with PC). Using the above-mentioned criteria, there were no differences in terms of sensitivity, specificity, diagnostic accuracy, predictive values, or AUC between MSC and MNC (Table 3).

Discussion

The present study has shown that MSC measurement compares favorably with MNC and UFC as a screening procedure for patients with suspected CS. Our results confirm and expand those of previous series (12, 16) showing that late night salivary cortisol is an accurate diagnostic tool. Interestingly, our study includes a group of healthy control subjects and a large cohort of patients with simple obesity, thus resembling a setting similar to clinical practice, *i.e.* few patients with CS to be distinguished from the large population of patients without endogenous hypercortisolism.

The measurement of cortisol in saliva has several advantages over its determination in serum. It is a stress-free diagnostic procedure, particularly useful when blood sampling is difficult (21) and venipuncture-induced cortisol elevation may occur and may represent a valid alternative to UFC in those patients who fail to properly collect urine over 24 h. In addition, it is an accurate index of free, biologically active, serum cortisol (22), unaffected by the estrogen milieu, which is known to increase corticosteroid-binding globulin levels. Lastly, saliva can be easily collected at home without medical assistance, and salivary samples can be stored at room temperature for several days and then mailed to the laboratory (23).

Thus, MSC appears to meet the requirements of a first-line test for the screening of hypercortisolism in large populations of patients at risk for CS, such as those affected by visceral obesity, poorly controlled type 2 diabetes mellitus, depression, and osteoporosis.

In our experience, using the 0.35 μg/dl (9.7 nmol/liter) cut-off obtained from ROC analysis, MSC yielded 93% sensitivity, comparable to the diagnostic detection rates of UFC and MNC. We also calculated the inherent diagnostic accuracy of each test, as assessed by the ROC AUC, which is independent of any specific threshold value. Both of these strategies confirmed a satisfactory diagnostic accuracy for MSC, which was superimposable to that of the other tests. Of note, the discriminative ability of MSC was also confirmed within the area of overlap in UFC values (≤569 nmol/24 h) between CS and PC.

We also observed a high degree of concordance between the diagnoses established by MSC and MNC; likewise, the accuracy of combined results of salivary and urinary cortisol approached that of the serum/urinary cortisol combination. All of these findings indicate that MSC, in addition to UFC, may represent a valid alternative to MNC in the screening for CS. However, as none of the available screening procedures

yields an absolute accuracy, more than one test should be performed to reliably detect all patients with CS.

In agreement with previous reports (12), UFC concentrations were correlated with salivary cortisol values at 0800 and 2400 h only in patients with CS. This is not surprising, as cortisol levels in saliva correlate with urinary cortisol excretion in excess of the corticosteroid-binding globulin saturation (~20 μg/dl; 552 nmol/liter). Thus, MSC may be used as an alternative to UFC to assess the degree of hypercortisolism in CS and possibly identify patients at greater risk for cortisol-induced complications (24).

As for the diagnostic performance of MSC, different assays for measurement of cortisol in saliva at bedtime have been used, and several cut-off points have been proposed, ranging from 0.13 μg/dl (3.6 nmol/liter) (12) to 0.28 μg/dl (7.7 nmol/liter) (13) and 0.55 μg/dl (15.2 nmol/liter) (16). Although the two former studies adopted lower cut-off values obtained from the distribution of salivary cortisol levels in normal subjects (*i.e.* 97.5th percentile value and subtraction of three times the intraassay CV from the morning value, respectively) (12, 13), Papanicolau and co-workers (16) computed the sensitivities of UFC, MNC, and MSC at 100% specificity for CS. Disappointingly, this latter approach may be affected by the presence of outliers among controls. In any case, despite the use of different criteria and cut-offs values, the sensitivity of MSC was 92–93% in both previous series (12, 13, 16) and in our study.

In conclusion, MSC can be recommended as a first-line diagnostic test for CS in both low risk (simple obesity) and high risk (PC states) patients. Given its convenience and diagnostic accuracy, this test may profitably be added to traditional screening procedures, such as UFC and MNC. Until widely acceptable thresholds are generated, however, each center should establish its own reference range and cut-off points.

Acknowledgments

We are grateful to Dr. Marco Besozzi (Istituto Auxologico Italiano) for his skillful statistical support.

Received February 24, 2003. Accepted May 19, 2003.

Address all correspondence and requests for reprints to: Prof. Francesco Cavagnini, Chair of Endocrinology, University of Milan, Istituto Scientifico Ospedale San Luca, Istituto Auxologico Italiano, Via Spagnoletto 3, 20149 Milan, Italy. E-mail: cavagnini@auxologico.it.

References

1. **Newell-Price J, Trainer P, Besser M, Grossman A** 1998 The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev 19:647–672
2. **Invitti C, Pecori Giraldi F, De Martin M, Cavagnini F, Study Group on the Hypothalamo-Pituitary-Adrenal Axis of the Italian Society of Endocrinology** 1999 Diagnosis and management of Cushing's syndrome: results of an Italian Multicentre Study. J Clin Endocrinol Metab 84:440–448
3. **Invitti C, Pecori Giraldi F, Dubini A, De Martin M, Cavagnini F** 1991 Increased urinary free cortisol and decreased serum corticosteroid-binding globulin in polycystic ovary syndrome. Acta Endocrinol (Copenh) 125:28–32
4. **Invitti C, De Martin M, Delitala G, Veldhuis JD, Cavagnini F** 1998 Altered morning and nighttime pulsatile corticotropin and cortisol release in polycystic ovary syndrome. Metabolism 47:143–148
5. **Putignano P, Bertolini M, Losa M, Cavagnini F** 2003 Screening for Cushing's syndrome in obese women with and without polycystic ovary syndrome. J Endocrinol Invest 26:539–544
6. **Newell-Price J, Trainer P, Perry L, Wass J, Grossman A, Besser M** 1995 A single sleeping midnight cortisol has 100% sensitivity for the diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf) 43:545–550
7. **Putignano P, Kaltsas GA, Satta MA, Grossman AB** 1998 The effects of anticonvulsant drugs on adrenal function. Horm Metab Res 30:389–397
8. **Papanicolaou DA, Yanovski JA, Cutler GB, Chrousos GB, Nieman LK** 1998 A single midnight serum cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab 83:1163–1167
9. **Pikkarainen L, Alfthan H, Markkanen H, Sane T** 2002 Midnight serum cortisol: comparison of healthy volunteers and hospitalised patients with Cushing's syndrome. Scand J Clin Lab Invest 62:357–360
10. **Yanovski JA, Cutler Jr GB, Chrousos GP, Nieman LK** 1993 Corticotropin-releasing hormone stimulation following low-dose dexamethasone administration. A new test to distinguish Cushing's syndrome from pseudo-Cushing's states. JAMA 269:2232–2238
11. **Moro M, Putignano P, Losa M, Invitti C, Maraschini C, Cavagnini F** 2000 the desmopressin test in the differential diagnosis between Cushing's disease and pseudo-Cushing states. J Clin Endocrinol Metab 85:3569–3574
12. **Raff H, Raff JL, Findling JW** 1998 Late-night salivary cortisol as a screening test for Cushing's syndrome. J Clin Endocrinol Metab 83:2681–2686
13. **Castro M, Elias PC, Quidute AR, Halah FP, Moreira AC** 1999 Out-patient screening for Cushing's syndrome: the sensitivity of the combination of circadian rhythm and overnight dexamethasone suppression salivary cortisol tests. J Clin Endocrinol Metab 84:878–882
14. **Martinelli CE Jr, Sader SL, Oliveira EB, Daneluzzi JC, Moreira AC** 1999 Salivary cortisol for screening of Cushing's syndrome in children. Clin Endocrinol (Oxf) 51:67–71
15. **Gafni RI, Papanicolau DA, Nieman LK** 2000 Nighttime salivary cortisol measurement as a simple, noninvasive, outpatient screening test for Cushing's syndrome in children and adolescents. J Pediatr 137:30–35
16. **Papanicolau DA, Mullen N, Kyrou I, Nieman LN** 2002 Nighttime salivary cortisol: a useful test for the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab 87:4515–4521
17. **American Psychiatric Association** 1994 Diagnostic and statistical manual of mental disorders, 4th Ed. Washington DC: American Psychiatric Press
18. **Zawadzki JK, Dunaif A** 1992 Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, Merriam GR, eds. Polycystic ovary syndrome. Boston: Blackwell; 377
19. **Barry HC, Ebell MH** 1977 Test characteristics and decision rules. Endocrinol Metab Clin North Am 26:45–65
20. **Hanley JA, McNeil BJ** 1982 The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 143:29–36
21. **Putignano P, Dubini A, Toja P, Invitti C, Bonfanti S, Redaelli G, Zappulli D, Cavagnini F** 2001 Salivary cortisol measurement in normal-weight, obese and anorexic women. Comparison with plasma cortisol. Eur J Endocrinol 145:165–171
22. **Riad-Fahmy D, Read GF, Walker RF, Griffiths K** 1982 Steroids in saliva for assessing endocrine function. Endocr Rev 3:367–395
23. **Chen YM, Cintron NM, Whitson PA** 1992 Long-term storage of salivary cortisol samples at room temperature. Clin Chem 38:304–305
24. **Pecori Giraldi F, Moro M, Cavagnini F, Study Group on the Hypothalamo-Pituitary-Adrenal Axis of the Italian Society of Endocrinology** 2003 Gender-related differences in the presentation and course of Cushing's disease. J Clin Endocrinol Metab 88:1554–1558