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Budget impact of using midnight salivary cortisol in the diagnosis of hypercortisolism

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ABSTRACT

Background: A single midnight serum cortisol (MSC) test has been reported to possess the best sensitivity and specificity for diagnosing Cushing's syndrome (CS). However, this test requires patient hospitalization, making it costly. This paper aims to compare the hospital budget impact and accuracy of using midnight salivary cortisol (MSVC), as opposed to MSC, in the diagnosis of hypercortisolism.
Methods: 77 patients with at least two high urinary free cortisol (UFC) values (>360 nmol/24 h) were selected from 611 patients with clinical symptoms of CS. The costs of the method to confirm the diagnosis of hypercortisolism was calculated comparing *Option A* using MSC (UFCx2, low-dose dexamethasone suppression test [LDDST]) that requires patient hospitalization versus *Option B* using MSVC (UFCx2, LDDST) in which the evaluation is done outside the Hospital. A budget impact analysis for one year was developed, and a sensitivity analysis in different scenarios was performed. Reproducibility and diagnostic performance of MSVC and MSC were also measured.
Results: Salivary cortisol is a sound analytical method for evaluating free serum cortisol due to its classification accuracy, good imprecision, linearity, and stability. AUC_{ROC} comparison between MSVC and MSC shows no significant differences. The substitution of the MSC for MSVC in our hospital could save between €16,762 and €132,804 in one year.
Conclusions: The use of MSVC in the diagnosis of hypercortisolism can result in a substantial decrease in the budget impact, without losing diagnosis accuracy and reliability, a significant advantage considering the current emphasis on reducing the financial burden of health care.

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1. Introduction

An increase in serum cortisol is the analytical feature indicative of endogenous hypercortisolism or Cushing's syndrome (CS). Aging populations and increasing obesity are complicating CS diagnosis due the similarity of certain characteristics among these conditions, thus requiring the detection or exclusion of hypercortisolism [1]. However, an optimal laboratory procedure to confirm the diagnosis of CS is not yet firmly established.

The 24-h urinary free cortisol (UFC) excretion has traditionally been used to screen for hypercortisolism, but improper sample collection and insufficient analytical specificity of the current immunoassays are drawbacks to the validity of this test as a diagnostic tool [2,3]. The overnight low-dose dexamethasone suppression test (LDDST) is widely used, but while it is reported to display high sensitivity, the specificity is less than optimal [4,5]. Furthermore, LDDST is prone to error (false-negatives or false-positives) in patients receiving drugs inducing cytochrome P450-related enzymes, and in patients with renal or hepatic failure [6].

According to consensus guidelines, when either discordance among first-line screening tests exists or variability within them is high, the use of midnight serum cortisol (MSC) test is appropriate [7,8]. A single MSC test has been reported to possess the best sensitivity and specificity for diagnosing CS [9,10]. Yet this procedure, that involves stress-free blood sampling, requires hospitalization of the patient and the placement of an i.v. catheter, making it expensive. Considering that health care costs are constantly on the rise it is essential to determine the most efficient and economical diagnostic methods in clinical settings.

The use of overnight salivary cortisol has recently been considered as a good test in the diagnosis of Cushing's syndrome [7]. Salivary cortisol, that reflects the biologically active unbound form of serum cortisol, is not influenced by alterations in protein binding. Furthermore, its concentration is not affected by salivary flow rate, and within a few minutes after changes in blood cortisol levels, equilibrium is quickly reestablished [11,12]. The midnight salivary cortisol (MSVC) measurement has proven to be a useful test for diagnosing hypercortisolism.

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Ninety-two percent to 100% sensitivity and 93% to 100% specificity have been reported for the diagnosis of CS [9,12–15] using a single midnight salivary cortisol measurement. In addition, the MSVC test possesses important advantages: it is an easy, non-invasive collection procedure with stability at room temperature for at least 5 days.

The aim of this study was to determine the economic impact of replacing the MSC for MSVC as a confirmatory test for the diagnosis of hypercortisolism. The cost analysis included three different scenarios depending on number of patients to be assessed with MSC. In order to provide reference range and cut-off criteria the accuracy of MSVC as compared to MSC was also analyzed.

2. Materials and methods

2.1. Patients

The study participants were patients under the suspicion of having CS and patients that were referred to our institution (tertiary universitary hospital) to manage proven CS. Patients recruitment took place during 2009. Patients' suspicion of CS was based on the presence of at least three of the following signs or symptoms: obesity, essential hypertension, impaired glucose tolerance or frank diabetes mellitus, mood disorders, irregular menses, buffalo hump, plethoric appearance and/or hirsutism. Hipercortisolism exclusion was based on two normal values of UFC and supression below 50 nmol/L after overnight 1 mg of dexamethasone. Patients referred for management of diagnosed CS were admitted in our hospital for central/peripheral source of ACTH diagnosis by bilateral inferior petrosal sinus sampling (BIPSS) or for undergoing transsphenoidal surgery or adrenalectomy. These patients had been diagnosed using standard criteria including elevated UFC, elevated MSVC or MSC, and lack of suppression after LDDST, plasma ACTH levels evaluation, high-dose dexamethasone suppression test, desmopressin [DDAVP] test, and MRI.

2.2. Study design

A midnight blood sampling for serum cortisol and a midnight sample of saliva were collected for cortisol quantification. In order to avoid stress prior to cortisol evaluation, an i.v. catheter was inserted in the forearm 2 h before the blood sampling. Saliva was collected by chewing a cylindrical cotton swab (Salivette, Sastedt, Germany [16]). Specimens were kept refrigerated at 2–8 °C until being sent to the laboratory. Saliva specimens were then centrifuged at 100 ×g for 10 min and the collected saliva was frozen at − 40 ºC until assayed. Serum samples were obtained by centrifuged blood specimens and assayed at the moment of delivery.

To validate the salivary cortisol measurements, imprecision and linearity studies were performed according to the protocols EP5-A2 and EP6-A of the Clinical and Laboratory Standard Institute (http://www.clsi.org). The imprecision was established using pooled saliva from Cushing's patients and healthy subjects (high, medium and low cortisol values) and quality-control material. The repeatability was determined by replicate measurements ($n=21$) in a single run, intermediate imprecision were obtained by analyzing quality-control material in duplicate over two runs per day for 21 days. Linearity was estimated for cortisol ranges from 0.5 to 100 nmol/L and evaluated by comparing the results from duplicate analyses of the cortisol samples with expected cortisol values.

Salivary cortisol stability was calculated from two salivette device samples obtained at the same time from 50 healthy subjects, one was assayed at the moment of sample delivery and one week later. To explore the normal values of salivary cortisol for our assay, we recruited 100 inpatients with non-toxic thyroid nodules waiting for surgery with normal hypothalamus-pituitary-adrenal axis.

2.3. Assays

Salivary cortisol samples were measured by electrochemiluminiscence immunoassay using the Elecsys E-170 automatic analyzer (Roche Diagnostic®, Basel Switzerland) [17]. The manufacturer reference range is from 0 to 11.9 nmol/L, with a repeatability of 1.5% and 6.1%, and an intermediate imprecision variation coefficient of 4.1% and 11.5%, for concentrations between 4.68 and 19.8 nmol/L, respectively. The results from our intra and inter-assay variation data are shown in results.

Serum cortisol (SC) and UFC were quantified by the same electrochemiluminiscence immunoassay used for salivary cortisol. Normal ranges for UFC and SC were 100–379 nmol/24 h and 171–536 nmol/L, respectively. The repeatability and intermediate imprecision variation coefficients were 1.5% and 1.7% and 1.8% and 2.8% for concentrations between 129 and 717 nmol/L, and 2.2% and 2.9% and 1.8% and 4.7% for concentrations between 617 and 1683 nmol/L, respectively.

2.4. Cost analysis

Cost analysis was done under the perspective of the hospital budget impact considering direct cost due to the need for two days of hospitalization to evaluate MSC, and compared the cost of using the new protocol which substitutes MSC for MSVC, that does not require hospitalization. The cost of the hospitalization and the cost of the different tests used to confirm hypercortisolism (UFC×2, MSC×1, LDDST×1) were used for this analysis. The unit cost was obtained from the regional list of price established by Andalusia Health Service for the year 2004 (http://www.juntadeandalucia.es). A budget impact analysis for the number of patients that took both tests in our hospital for one full year was developed.

A sensibility analysis was carried out by creating the following scenarios: a variability of tests prices in ± 20% (best–worst cases) and three scenarios for the budget impact analysis based on the rate of replacement between the tests (20, 50 and 100%).

2.5. Statistical analysis

Goodness of fit of a normal model to the data was assessed with the Kolmogorov–Smirnov test with the Dallal–Wilkinson–Lilliefors correction. Descriptive statistics were used to summarize the data. Rates and proportion were calculated for category data, and mean and ± SEM for continuous data; 95% confidence intervals (CI) are also provided. For assessing possible relationships between variables non-parametric tests were used.

The results of each test were compared with the definitive diagnosis. Univariate curves of the receiver operating characteristic (ROC) were calculated to define the best cut-off value with relevant sensitivity and specificity for each test. The quality of the test was expressed as the area under the ROC curve (AUC_{ROC}). The AUC_{ROC} for MSC and MSVC and positive and negative predictive values were calculated. Probability coefficients were also calculated. Comparisons between AUC_{ROC} were performed following Hanley and McNeil's method. Statistical analyses were performed using SPSS 15.0. For ROC calculus, the MedCalc package was used. $P<0.05$ was considered statistically significant.

3. Results

Of the 611 patients recruited consecutively (423 women and 188 men; mean age ± SEM, 44 ± 0.91 and 50 ± 1.35, respectively), 584 were referred for suspected hypercortisolism. Of these screened patients, in 534 hypercortisolism was excluded (two normal values of UFC and supression below 50 nmol/L after overnight 1 mg of dexamethasone), whereas the remaining 50 patients were selected to confirm or exclude hypercortisolism. Twenty-seven patients with proven CS (21 with ACTH dependent, and 6 with adrenal adenoma) were also included in the

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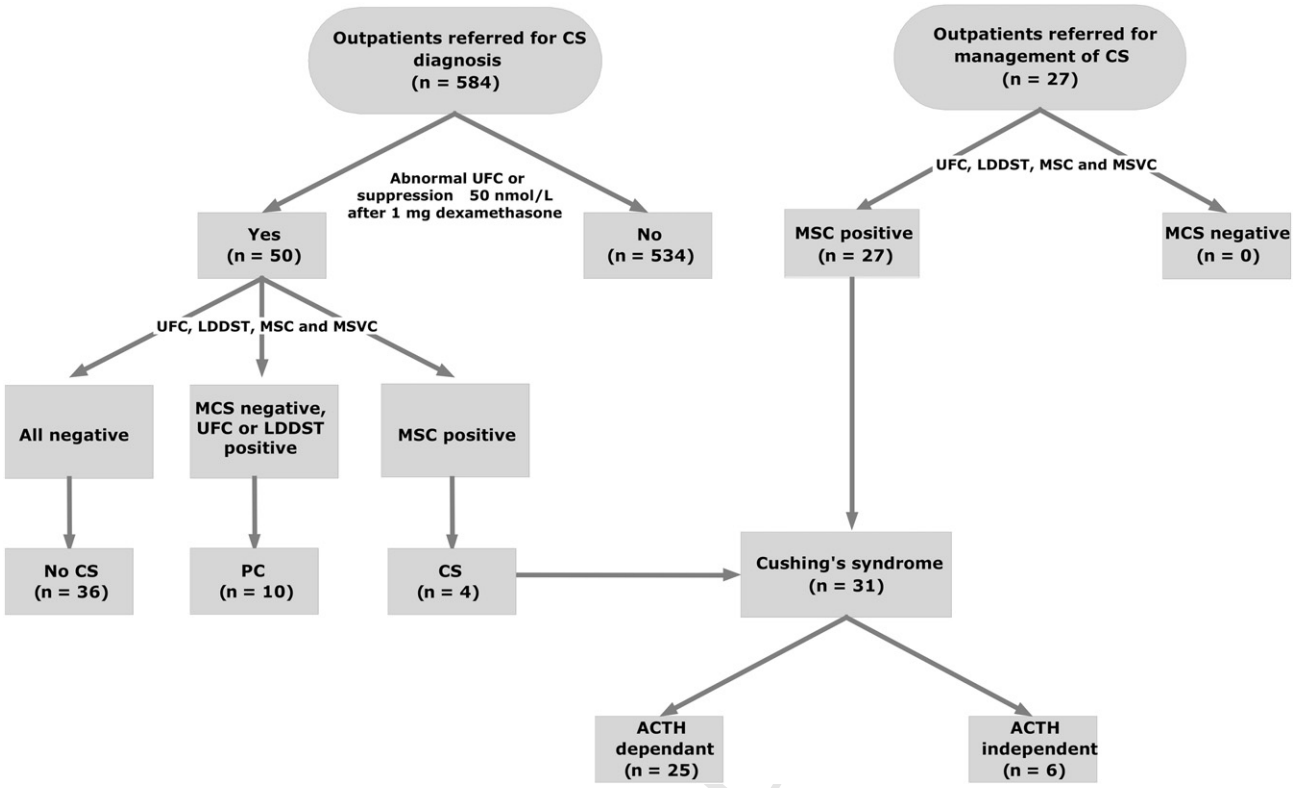

Fig. 1. Screening and confirmatory diagnosis in the two group of patients included in the study. Note, UFC: urinary free cortisol; CS: Cushing's syndrome; PC: pseudo-Cushing.

current study. This diagnosis was confirmed in our institution in all cases (Fig. 1).

Patients included in the current study (total $n = 77$) were admitted in our hospital in order to warrant adequate samples collection, handling and storage, for obtaining own reference ranges and define cut-off criteria of MSVC as compared to MSC. Thirty-six patients were considered healthy after hypercortisolism was excluded (normal UFC, normal MSC, and SC<50 nmol/L after LDDST), 10 were considered pseudo-Cushing (discordant UFC and LDDST, with normal MSC), and 31 (20 women and 11 men) were diagnosed of having CS (UFC, LDDST, MSVC and MSC positive). (See Table 1 for details). Written informed consent was obtained from all the patients. The protocol was approved by the Virgen del Rocio Hospital Ethics Committee.

3.1. Analytical validation of salivary cortisol

The imprecision results of the salivary cortisol measurements are summarized in Supplementary Data Table 1, The repeatability variation coefficients ranged from 1.26% to 6.75% for concentrations between 4.8 and 31.28 nmol/L and intermediate imprecision variation coefficients ranged from 3.76% to 8.3% for concentrations between and 2.9 and 31.29 nmol/L. Least-squared regression showed linear relationship fitted the data better than a nonlinear relationship over the interval between 0.5 and 100 nmol/L ($P<0.001$), with a regression's equation of $Y = -0.1519 + 1.2696X$, confidence interval intercept (−0.5873 0.2834) and slope (1.2618 1.2774), with no significant deviation from linearity ($P = 0.91$). The results of the stability of salivary cortisol expressed as mean ± SEM were 5.81 ± 0.9 nmol/L at delivery time and 5.96 ± 0.86/L one week later, no significant difference in these values was observed (*t*-test for repeated measures, $P<0.05$) (Supplementary Data Fig. 1).

3.2. Clinical validation of salivary cortisol

The normal values of MSVC in the inpatient control group (28 men and 72 women, aged 36.4 ± 1.4, with BMI of 25.15 ± 0.3), expressed as mean ± SEM were 2.5 ± 0.1 the minimum value was 0.5 nmol/L and the maximum value was 6.24 nmol/L. No significant differences for age and sex were found.

Table 1
Characteristics of 77 patients with suspected CS. Results of UFC, MSVC and MSC expressed as median and interquartile ranges.

	All patients	Hypercortisolism excluded	Pseudo-Cushing	Cushing confirmed**
N. of patients	77	36	10	31
Age (yr)	41 ± 14	37 ± 13	45 ± 17	41 ± 15
Female/male	58/19	31/5	7/3	20/11
BMI	30 ± 1.1	24 ± 1.0	36 ± 12	30 ± 7
UFC at diagnosis (nmol/d)*		258 (184–332)	424 (189–559)	819 (404–1749)
MSC (nmol/l)*		99 (82.5–117)	103 (45.3–165)	410 (322–518)
MSVC (nmol/l)*		2.6 (1.5–4.6)	6.0 (4.5–11)	19.3 (12.8–29.3)

BMI, body mass index result expressed as mean and SEM.
UFC*, urinary free cortisol evaluated during first day of inpatient; MSC, Midnight serum cortisol; MSVC, Midnight salivary cortisol.
**Cushing's disease 25 (confirmed adenoma with positive inmunostaining for ACTH). Adrenal adenomas 6.

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Table 2
Diagnostic performance of the four diagnostic tests in patients suspected of having CS.

	Sensitivity (%)	Specificity (%)	+Predictive value	−Predictive value	Diagnostic Accuracy (%)	AUC_{ROC}
MSVC	96.8 (83.8–99.4)	87 (74.3–93.9)	83.3 (68.1–92.1)	97.6 (87.4–99.6)	90.9 (82.4–95.5)	0.95 (0.74–0.98)
MSC	93.5 (79.3–98.2)	97.8 (88.7–99.6)	96.7 (83.3–99.4)	95.7 (85.8–98.8)	96.1 (89.2–98.7)	0.96 (0.76–0.98)
UFC	85,7 (65.4–95.0)	90.9 (76.4–96.9)	78.3 (58.1–90.3)	78.6 (52.4–92.4)	91.9 (71,3–95.8)	0.78 (0.63–0.93)
LDDST	90.9 (62.9–98.4)	81.8 (52.3–94.9)	83.3 (55.2–95.3)	90.0 (59.6–98.2)	86.4 (66.7–95.3)	0.79 (0.59–1.02)

Numbers in parentheses correspond to 95%.

Significant differences in UFC, LDDS, MSC and MSVC among non-hypercortisolism, pseudo-Cushing and confirmed hypercortisolism groups were observed (one-way ANOVA, $P<0.05$). At a cutoff level of 10 nmol/L MSVC, sensitivity was at 96.8% (83.8–99.4%) and specificity at 87% (74.3–93.9%), with positive and negative predictive values of 83.3% (68.1–92.1%) and 97.6% (87.4–99.6%) and positive and negative probability coefficients of 69.4% (49.8–83.8%) and 1.1% (0.1–8.8%). For MSC, with a 210 nmol/L cut-off level, sensitivity and specificity were at 93.5% (79.3–98.2%) and 97.8% (88.7–99.6%), with positive and negative predictive values of 96.7% (83.3–99.4%) and 95.7% (85.8–98.8%) and positive and negative probability coefficients of 92.9% (72.3–98.5%) and 2.0% (0.4–9.4%).

For CLU, with a 340 nmol/24 h cut off level, sensitivity and specificity were at 85.7% (65.4–95.0%) and 90.9% (76.4–96.9%), with positive and negative predictive values of 78.3% (58.1–90.3%) and 78.6% (52.4–92.4%) and positive and negative probability coefficients of 85.7% (65.4–95%) and 9.1% (3.1–23.6%). At a cut-off level of 52 nmol/L LDDST, sensitivity and specificity were at 90.9% (62.9–98.4%) and 81.8% (52.3–94.9%), with positive and negative predictive values of 85.3% (55.2–95.3%) and 90.09% (59.6–98.2%) and positive and negative probability coefficients of 85.3% (55.2–95.9%) and 10% (1.8–40.4%).

The diagnostic accuracy was 90.9% (82.4–95.5) and 96.1% (89.2–98.7) for MSVC and MSC respectively. Using the above-mentioned criteria, there were no differences in terms of sensitivity, specificity, predictive values and diagnostic accuracy (see Table 2). Individual MSVC and MSC values are shown in Fig. 2.

The AUC_{ROC} for MSC and MSVC were 0.969 (0.763–0.989) and 0.954 (0.740–0.989). Comparisons between the curves did not show statistically significant differences ($P<0.001$). The differences among AUC_{ROC} for MSC and for MSVC were 0.015 (−0,085 –0,115). (See ROC curves comparing the accuracy in Supplementary Data Fig. 2).

3.3. Costs and budget impact analysis

The cost of performing the hypercortisolism confirmation in patients with suspicion of CS is approximately €1472.90 with the algorithm that we used (Option A), rather than €51.79, which is the cost of the algorithm we are now evaluating (Option B) (Table 3).

In the sensibility analysis, a 20% of variation in the prices has been considered. Best-case is based on the highest price for option A (€1,767.48) and the lowest price for option B (€41.43), obtaining the potential maximum saving per patient (€1726.05). Worst-Case is based on the lowest price for option A (€1178.32) and the highest price for the option B (€62.15), obtaining the potential minimum saving (€1116.17) (Table 3). The current scenario shows the whole potential savings (from €21,885.09 to €109,425.27) depending on the number of patients that took each test (15 patients for partial replacement, 39 for half replacement). Budget impact of replacement of MSC for MSVC would yield a potential range of saving from €16,742.48, in the worst-case scenario to €132,905.70, in the best-case scenario.

4. Discussion

In this study, we have evaluated the impact budget and the efficacy in the diagnostic performance of MSVC as compared to MSC for hypercortisolism confirmation in patients suspected of having CS. Our data clearly show that the use of MSVC in the confirmatory diagnosis of hypercortisolism provides diagnostic accuracy levels above 90% and acceptable predictive values, similar to those obtained from the diagnosis with MSC, but with a potential cost saving up to €132,905.70 in a tertiary referral Hospital.

In the current practice, for the diagnostic approach for Cushing syndrome we use one of the different tests suggested by the Endocrine Society Clinical Practice guideline [7]. However, when the results are contradictory another test is recommended including dexamethasone-CRH test or midnight serum cortisol. Our study shows that the MSVC measure used in suitable conditions is a reliable parameter.

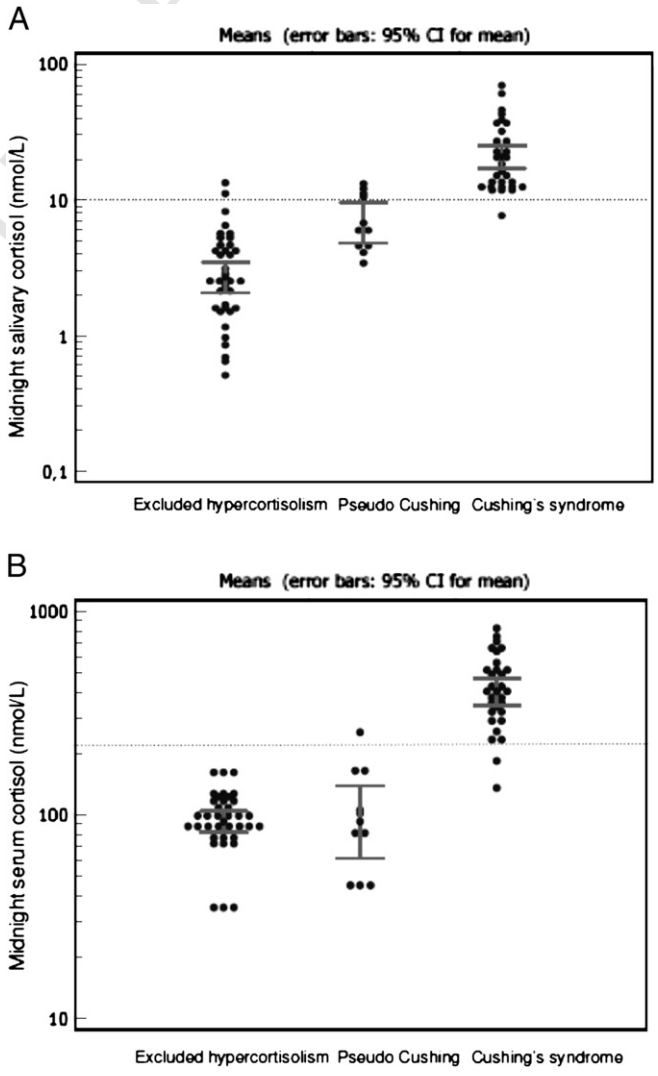

Fig. 2. Individual values of MSVC (A) and MSC (B) in non-hypercortisolism, pseudo-Cushing and Cushing's syndrome groups. The *dotted line* represents the cutoff level for diagnosis.

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Table 3
Cost analysis. Costs for each test and total cost for diagnosis are shown.

Cost analysis per patient (Euro)	Dexametasone	Consumable	Reactive	Personnel	Hospitalization cost	Total
MSC	0	1.14	2.3	0.87	1428.4	1432.71
MSVC	0	0.6	2.3	8.7	0	11.6
UFC	0	0.45	8.14	9.7	0	18.29
DSMC	0.3	0.14	2.3	0.87	0	3.61

Option A (UFC×2, MSC, LDDST): €1472.90 SA: ±20% (1178.32–1,767.48)
Option B (UFC×2, MSVC, LDDST): €51.79 SA: ±20% (41.43–62.15)
Best-case scenario, highest saving per patient (maximum difference) €1.726,05
Worst-case scenario, lowest saving per patient (minimal difference) €1.116,17

From analytical perspective the method showed an acceptable imprecision. According to Fraser et al. [18], objective goal for imprecision can be calculate based on the CV within-subject. They proposed a desirable performance for imprecision as 0.5×CV within-subject. The reported within-subject variation of salivary cortisol is 20.5% [19], it therefore can be concluded that our result of imprecision meet the criterion of desirable performance (CV<10.25%) at all concentrations. The assay linearity of the cortisol determinations in saliva, according to CLSI document EP6-A, showed that MSVC was linear up to 100 nmol/L.

In terms of its performance as a confirmation tool for CS diagnosis our results support previous findings in the literature [19–2] reporting an excellent discriminatory potential for differentiating patients with and without the disease. In our group, all patients but one with confirmed CS had an MSVC measurement over the cut-off level. For a threshold level of 10 nmol/L, the MSVC displayed 96.8% sensitivity and 87% specificity, results that reflect sensitivities and specificities reported in the literature.

Mostly, our data confirm that the MSVC test, used as part of the algorithm for hypercortisolism diagnosis especially when the initial tests results are contradictory, is as effective as the MSC, and far less expensive: Option A (UFC×2, MSC, LDDST): €1472.90; Option B (UFC×2, MSVC, LDDST): €51.79. Thus, important savings could be obtained if option B is implemented, a significant advantage considering the emphasis placed nowadays on reducing the financial burden of heath care. However, efficiency analysis is necessary before introducing new technologies. Appraisal for saving ensuring safety and efficacy test are seldom observed in health technology assessment.

The incidence of CS had been estimated at approximately 1 per 250,000 inhabitants [27]. However, the major problem is not the progressive increase of incidence [28–30], but rather the need for screening in high risk populations. If we consider that (1) obesity affects over 30% of the population in developed countries, (2) other indications are increasingly prevalent, and (3) diabetes is estimated to affect 200 million people by the year 2020, then it becomes apparent that cortisol assessment will be a common analytical parameter in these clinical settings. At present, the populations in which hypercortisolism screening might be justified includes patients with CS phenotype, subclinical CS, poorly-monitored diabetes, obesity, osteoporosis, hypertension and patients diagnosed with adrenal incidentaloma [28,31–35]. The results of the initial tests on these populations may be discordant and further evaluation to confirm or exclude the diagnosis is recommended.

Although we have evaluated the accuracy of MSVC as compared to MSC in inpatient conditions, we consider that our results can be also applied to outpatient conditions. Our own results and other recent studies have demonstrated that if the MSVC measurement is taken under suitable conditions [36], using a high sensitivity and specificity test derived from ROC analysis [10,37,38], its diagnostic performance does not differ between inpatient and outpatient conditions [15,20].

In conclusion, our data shows that MSVC is a sound measure with high reproducibility, effectiveness, accuracy and low cost. Given its simple use and relative cheapness, MSVC could be convenient to use it in an outpatient setting for the diagnosis of CS, instead of inpatient MSC.

5. Uncited references

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.cca.2011.08.013.

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Supplementary Data

Tables

Table 1. Analytical assessment and imprecision study. Within-run and total imprecision results expressed as coefficient variation and standard deviation for a) a mixture of low, medium and high cortisol concentrations in saliva, b) controls with low C. low) and high (C.high) concentrations of cortisol.

	Low	Medium	High	C Low	C High
Intraassay precision n=21					
Mean cortisol	**4.88**	**14.9**	**29.41**	**3.33**	**31.28**
SD	**0.25**	**0.32**	**0.37**	**0.22**	**0.95**
CV %	**5.12**	**2.14**	**1.26**	**6.75**	**3.03**
	C Low		**C High**		
Interaasay imprecision n=45					
Mean cortisol	**2.9**		**31.24**		
SD	**0.24**		**1.17**		
CV %	**8.33**		**3.76**		

Figures

Figure 1. A : Analytical assessment, linearity study and regression analysis result. Y axis shows expected cortisol values and X axis represents obtained cortisol values after analysis duplication of 6 samples obtained from two saliva mixtures of 0.5 (mixture 1) and 100 nmol/L (mixture 2). Sample 1: 100% of mixture 1, 0% mixture 2. Sample 2: 75% of mixture 1 and 25% of mixture 2. Sample 3: 50% of mixture 1, 50% mixture 2. Sample 4: 25% of mixture 1, 75% mixture 2. Sample 5: 0% of mixture 1, 100% mixture 2. Sample 6: 0% of mixture 1, 0% mixture. **B**: Stability Study. Regression analysis shows relationship between results obtained when the sample of cortisol in saliva (mmol/L) is taken (X axis) vs. results obtained after one week at room temperature (Y axis).

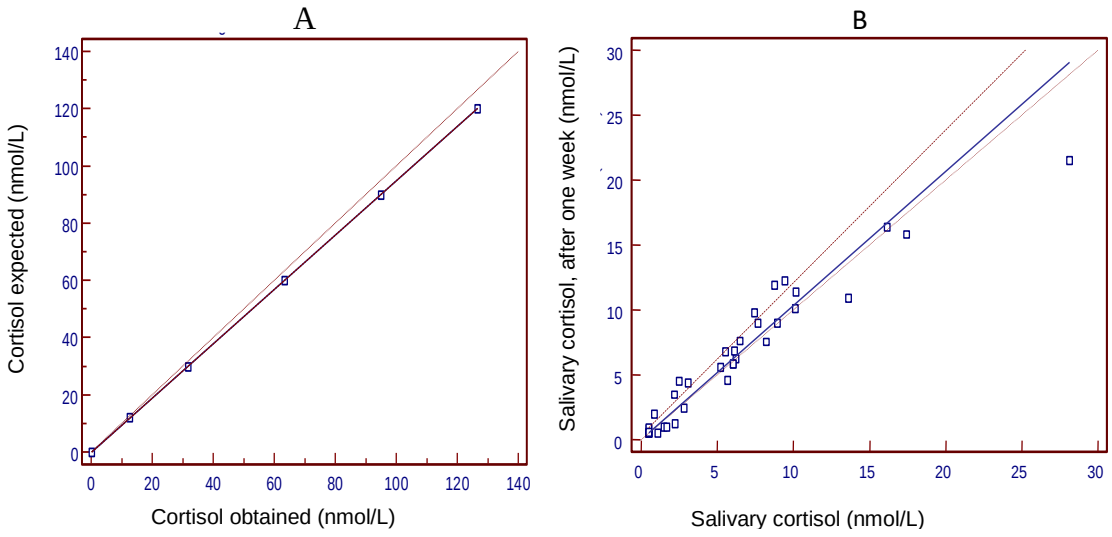
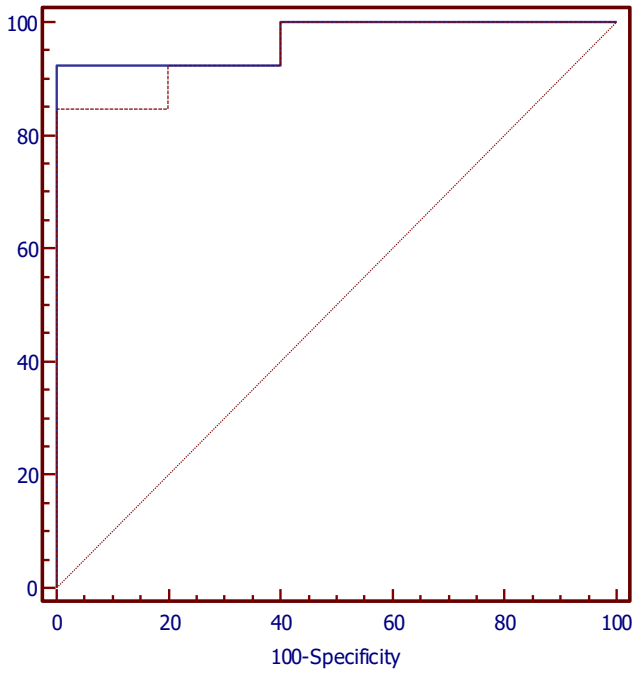
Figure 2: ROC curves using MSMC and MSVC as criteria for the diagnosis of CS. Blue solid line shows results for MSC and red dashed line shows results for MSVC. Diagonal dashed line represents AUC = 0.5.