Original article

Performance of the 4-mg intravenous dexamethasone suppression test in differentiating Cushing disease from pseudo-Cushing syndrome

Évaluation de la performance du test de freination à la dexaméthasone 4 mg IV pour différencier une maladie de Cushing d’un pseudo-Cushing

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Abstract

Context. – Discriminating Cushing disease (CD) from pseudo-Cushing syndrome (PCS) is a challenging task that may be overcome with the 4-mg intravenous (IV) dexamethasone suppression test (DST). Objective. – Assess the performance of the 4-mg IV DST in the differential diagnosis between CD and PCS in well-characterized patients. Design. – Retrospective comparative study of subjects seen in a tertiary care unit (November 2008 to July 2011). Methods. – Thirty-six patients with PCS and 32 patients with CD underwent 4-mg IV dexamethasone infusions from 11 am to 3 pm. Areas Under ROC Curves (AUCs) were estimated and compared for ACTH and cortisol measured at 4 pm the same day (day 1) and 8 am the next day (day 2). The ROC curve of the marker with the highest AUC was used to determine the threshold with the highest specificity for 100% sensitivity. Results. – The AUC of ACTH at 8 am on day 2 was estimated at 98.4% (95% CI: [92.1–100]), which is significantly greater than that of ACTH at 4 pm on day 1 (P = 0.04) and that of cortisol at 8 am on day 2 (P = 0.05). For ACTH at 8 am on day 2, the threshold with the highest specificity for 100% sensitivity was estimated at 14.8 ng/L. At this threshold, the sensitivity was estimated at 100% [89–100] and the specificity at 83.3% [67–94]. Conclusion. – The 4-mg IV DST is an easy and accurate tool in distinguishing CD from PCS. It deserves thus a better place in establishing the diagnosis of CD.

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Keywords: Cushing disease; Pseudo-Cushing syndrome; Dexamethasone suppression test; ACTH; Pituitary tumor

Résumé

Contexte. – Le diagnostic différentiel entre une maladie de Cushing (MC) et un pseudo-Cushing (PCS) est difficile mais peut être facilité par l’utilisation du test de freination à la dexaméthasone 4 mg intraveineuse (DXM-IV 4 mg). Objectif. – Évaluer la performance du test DXM-IV 4 mg dans le diagnostic différentiel entre le CD et PCS chez les patients présentant au moins un test de première ligne positif.

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Design. – Étude comparative rétrospective des sujets vus dans une unité de soins tertiaires (novembre 2008 à juillet 2011). Méthodes. – Trente-six patients avec PCS et 32 patients avec MC ont été perfusés de 11–15 heures avec 4 mg de dexaméthasone. Les aires sous les courbes ROC (AUC) de l’ACTH et de cortisol mesurées à 16 heures le même jour (j1) et 8 heures le lendemain (j2) ont été analysées. Résultats. – L’AUC de l’ACTH à 8 h à j2 a été estimée à 98,4 % (IC 95 % : [92,1–100]), ce qui est nettement supérieure à celle de l’ACTH à 16 h à j1 (p = 0,04) et du cortisol à 8 h à j2 (p = 0,05). Pour l’ACTH à 8 h à j2, le seuil avec la plus grande spécificité pour 100 % de sensibilité a été estimé à 14,8 ng/L. À ce seuil, la sensibilité est de 100 % [89–100] et la spécificité à 83,3 % [67–94]. Conclusion. – Le test de freination par DXM-IV 4 mg est de réalisation simple et performant pour distinguer une MC d’un PCS. Ce test mérite d’être utilisé pour le diagnostic de CD.
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Mots clés : Maladie de Cushing ; Pseudo-Cushing ; Test de freination ; Dexaméthasone ; ACTH ; Tumeur hypophysaire

1. Introduction

The diagnosis of Cushing disease (CD) remains a great challenge, especially regarding its differentiation from pseudo-Cushing syndrome (PCS). Actually, PCS is still not clearly understood; it would result from a physiological hyperactivation of the hypothalamic axis that produces symptoms of glucocorticoid overproduction. Besides, PCS is considered typically associated with alcoholism, depression, anorexia, and obesity [1].

The classical phenotype of Cushing syndrome (with cardiovascular, metabolic, dermatological, musculoskeletal, and psychiatric manifestations) is rather easy to diagnose but remains uncommon. In fact, the clinical manifestations of Cushing syndrome (CS) are variable and differ widely in severity depending on the degree and duration of the hypercortisolism. This leads to a clinical overlap with PCS.

Despite the fact that some symptoms (ecchymosis, purple striae, and proximal myopathy) are more specific of CD than PCS [2], the diagnosis of CD is still dependent on laboratory results.

For the diagnosis of CD, the Endocrine Society Clinical Practice guidelines recommended one of the following tests: at least two 24-hour urinary free cortisol tests, 1-mg overnight dexamethasone suppression test, or two measurements of late salivary cortisol [3,4]. The Endocrine Society consensus considers the 2 mg/d for 48 h dexamethasone suppression test as a first step test. Once CD is suspected, the second step consists in performing a Dexamethasone/Corticotropin-Releasing Hormone (DEX/CRH) test, or a midnight serum cortisol test.

Nearly 30 years ago, our group demonstrated the interest of a 4-mg intravenous (IV) Dexamethasone suppression test (DST) in differentiating obese subjects from CD patients [5]. The test was based on beta-lipotropin and cortisol measurements. In 2010, Jung et al. [6] evaluated the performance of a 4-mg IV DST based on plasma cortisol and ACTH measurements in differentiating CS from control subjects (normal and overweight) and from patients with low probability of CS. Here, we evaluate the diagnostic accuracy of the 4-mg IV DST based on ACTH and cortisol measurements in a large and carefully characterized cohort of patients already classified as CD or PCS patients on the basis of stringent clinical and biological criteria.

2. Materials and methods

2.1. The patients

The present retrospective observational study was conducted in a tertiary care unit of Hospices Civils de Lyon (France). It included all 32 patients with Cushing disease diagnosed between July 2004 and July 2011. In this CD group, the pituitary adenoma was confirmed by histopathology after trans-sphenoidal surgery.

This CD group was compared with a PCS group that included 36 patients seen between November 2008 and July 2011. In this PCS group, the diagnosis was based on biochemical features compatible with CS; i.e., 24-hour urinary free cortisol higher than the reference values of the laboratory and/or lack of plasma cortisol suppression after administration of 1 mg DST (plasma cortisol post-test > 50 nmol/L), and at least one year of clinical and biochemical follow-up (Median of follow-up was 2.49 years [1–4.2]). MRI was performed in 11 patients and was normal in 10 and revealed a Rathke Cleft Cyst in one case.

The study excluded initially patients with ACTH-independent Cushing Syndrome or recurrent CD. Four patients with ectopic ACTH-producing tumors were excluded from the analysis; they had either neuroendocrine tumors or unidentified tumors that caused Cushing paraneoplastic syndrome.

The study was approved by the local ethics committee though, in accordance with the current French legislation, an observational study that does not change the routine management of patients does not need to be declared or submitted to the opinion of a research ethics board.

2.2. The 4-mg intravenous DEX infusion

Two IV catheters were placed at 08:30 am: one for blood sampling and another for DEX infusion. DEX phosphate (4 mg; Soludexadron®) was dissolved in 40 mL 0.9% saline and infused at 1 mg/h for 4 h using an IV infusion pump, starting at 11 am. Blood samples for cortisol and ACTH measurements were withdrawn at 9 am, 11 am, 4 pm, 8 pm, and midnight on day 1 then at 8 am on day 2 [5].

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2.3. The laboratory assays

All the immunoassays of all samples were performed in a single biochemistry laboratory (Lyon Est Hospital).

Plasma cortisol was quantified in duplicates by an in-house radioimmunoassay. According to the in-house controls, the inter-assay coefficients of variation were 11.9, 7.6, and 6.3% at 53, 370, and 810 nmol/L, respectively. The functional sensitivity was 40 nmol/L.

Plasma ACTH was measured by a radioimmunoassay according to the manufacturer’s instructions (ACTH RIA, BRAHMS, Germany). With these instructions, the inter-assay coefficients of variation were 4% and 4.2% at 35 ng/L and 330 ng/L, respectively, and the limit of quantification was 2.3 ng/L.

Urinary free cortisol was measured by an in-house RIA based on radioimmunoassay after dichloromethane extraction. The inter-assay coefficients of variation were 14.4%, 11%, and 7.7% at 61 nmol/L, 324 nmol/L, and 731 nmol/L, respectively. The normal values were < 145 nmol/24 h.

2.4. The statistical analyses

Quantitative variables were expressed as means ± standard deviations and compared using the non-parametric Mann-Whitney test. Qualitative variables were expressed as absolute and relative frequencies and compared using the Chi² test or Fisher exact test.

The kinetics of ACTH and cortisol over the duration of the 4-mg IV DST in the two groups of patients were studied by plotting the mean ± SD values of the two markers versus time from 9 am on day 1 to 8 am on day 2.

Empirical ROC curves were built to assess the discriminant ability of the 4-mg IV DST for cortisol and ACTH as measured at 4 pm on day 1 [6] and at 8 am on day 2. The areas under the ROC curves (AUCs) were estimated with their 95% confidence intervals (CIs) and compared using the method of DeLong [7]. The sensitivity and specificity of the markers were estimated at the threshold used by Jung et al. [6]; i.e., 10 ng/L for ACTH and 130 nmol/L for cortisol. The ROC curve of the marker with the highest AUC was used to determine the threshold with the highest specificity for 100% sensitivity. The exact binomial method was used to build the 95% CIs.

The discriminant ability of the 4-mg IV DST was also assessed using the percentage of decrease of cortisol and ACTH at 4 pm on day 1 and at 8 am on day 2 with regard to the values measured at 9 am before DEX injection.

The optimal thresholds of the markers were estimated for a 43% prevalence of CD, which corresponds to the prevalence estimated in the population of patients referred to our centre for suspicion of CD. The same weight was applied to false positives and false negatives. This corresponds to the threshold that maximizes the number of well-classified patients. The Bayesian method developed by Subtil and Rabilloud [8] was used to estimate the optimal threshold and the corresponding sensitivity and specificity.

All the analyses were carried out with Stata software, version 11.2, except the estimations of the optimal thresholds; these were carried out with R software. A P-value of 0.05 was considered for statistical significance.

3. Results

3.1. Patient clinical and biological characteristics

The PCS group included 21 obese patients (of whom 11 with hypertension and 5 with depression) and 15 non-obese patients (of whom 6 with hypertension and 5 with depression) (Table 1).

The BMI was significantly higher in the PCS group than in the CD group (30.3 ± 5.6 kg/m² vs. 26.2 ± 4.1 kg/m², P < 0.01). Obesity was more frequent in the PCS group than in the CD group (the difference just reached statistical significance: P = 0.05). Hirsutism, proximal amyotrophy, and bruises were significantly more frequent in the CD group than in the PCS group (P < 0.01). Urinary free cortisol was much higher in the CD group than in the PCS group (1058 ± 1433 nmol/24 h vs. 132 ± 119 nmol/24 h, P < 0.001).

3.2. ACTH and cortisol kinetics during the 4-mg IV DST

In CD as in PCS patients, ACTH and cortisol levels decreased during DEX infusion. ACTH decreased from 65.9 ± 34.4 ng/L at baseline to 31.3 ± 26.5 ng/L at 4 pm in CD patients and from 19.7 ± 7.7 ng/L at baseline to less than 5 ng/L at 4 pm in PCS patients. Cortisol levels followed the same trend; they decreased from 746.4 ± 225 to 364.6 ± 216 nmol/L at 4 pm in the CD group and from 580.5 ± 147 to 151.8 ± 62 nmol/L at 4 pm in the PCS group (Fig. 1).

In both groups, a rebound of ACTH and cortisol levels appeared during the test. This rebound occurred earlier and was higher in CD than in PCS patients. Indeed, in the CD group, ACTH increased from 31.3 ± 26.5 ng/L at 4 pm on day 1 up to 64.7 ± 35.1 ng/L at 8 am on day 2 whereas, in the PCS group,
ACTH remained suppressed and undetectable until nearly the end of the test when it spiked up to 8.3 ± 7.1 ng/L. In CD patients, cortisol level increased up to 609.6 ± 256.5 nmol/L at 8 am on day 2 whereas, in PCS patients, it increased only at 8 am on day 2 up to 123.8 ± 115 nmol/L. At 8 am on day 2, the mean ACTH and cortisol levels were significantly higher in the CD group than in the PCS group (P < 0.001).

3.3. Discriminant ability of cortisol during the 4-mg IV DST

The overlap between the distributions of cortisol values in PCS and CD was more important at 4 pm on day 1 than at 8 am on day 2 (Fig. 2A and B). The AUC for plasma cortisol at 8 am on day 2 was estimated at 90.5% (95% CI: [81.8–96.7]) vs. 80.9% (95% CI: [69.5–89.9]) at 4 pm but the difference did not reach statistical significance (P = 0.07).

For a cortisol threshold of 130 nmol/L at 8 am on day 2 (Fig. 2B), the sensitivity was estimated at 93.8% and the specificity at 83.3% (Table 2).

The AUC for the percentage of cortisol decrease between 9 am and 4 pm (Fig. 2C) was estimated at 78.6% (95% CI: [67.3–88.5]).
Table 2
Sensitivity and specificity of the 4 mg iv dexamethasone suppression test at different cortisol and ACTH thresholds.

<table>
<thead>
<tr>
<th>Threshold and time</th>
<th>Sensitivity [95% CI]</th>
<th>Specificity [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol &gt; 130 nmol/L, 8 am of day 2</td>
<td>93.8% [79; 99]</td>
<td>83.3% [67; 93.6]</td>
</tr>
<tr>
<td>ACTH &gt; 10 ng/L, 8 am of day 2</td>
<td>100% [89; 100]</td>
<td>80.6% [64; 92]</td>
</tr>
<tr>
<td>ACTH &gt; 14.8 ng/L, 8 am of day 2</td>
<td>100% [89; 100]</td>
<td>83.3% [67; 94]</td>
</tr>
<tr>
<td>ACTH &gt; 19.6 ng/L, 8 am of day 2</td>
<td>93.7% [80; 98]</td>
<td>88.9% [74.7; 95.6]</td>
</tr>
</tbody>
</table>

3.4. Discriminant ability of ACTH during the 4-mg IV DST

As for cortisol, the overlap between the distributions of ACTH values in PCS and CD was more important at 4 pm on day 1 than at 8 am on day 2 (Fig. 3A and B). The AUCs for plasma ACTH at 4 pm on day 1 and 8 am on day 2 were respectively estimated at 91.0% (95% CI: [81–96.5]) and 98.4% (95% CI: [92.1–100]) and the difference between the two areas was statistically significant (p = 0.04). However, ACTH value at 4 pm on day 2 was always > 10 ng/L in CD.

The percentage of ACTH decrease between 9 am and 4 pm (Fig. 3C) was unable to discriminate PCS from CD (AUC estimated at 52.7%; 95% CI: [39.4–65.1]).

At 8 am on day 2, the diagnostic value of ACTH, as measured by the AUC, was greater than that of cortisol (Fig. 4); the difference between the two AUCs just reached statistical significance (P = 0.05).

3.5. ACTH threshold and the differential diagnosis of Cushing disease

In this study, the optimal threshold was estimated at 8 am on day 2 because it was this moment that the marker discriminated the better the patients with CD from the patients with PCS. This threshold was estimated at 19.6 ng/L and corresponded to 93.7% sensitivity and 88.9% specificity (Table 2). It was the threshold that maximized the number of well-classified patients for a prevalence of CD of 43%.

At the threshold of 10 ng/L at 8 am on day 2 reported by Jung et al. [6], the sensitivity of ACTH was estimated at 100% and the specificity at 80.6% (Table 2). This threshold identified six “false positive” CD patients in the study group (Table 3), including three patients under benzodiazepine treatment.

Here, the threshold with the highest specificity for 100% sensitivity was estimated 14.8 ng/L. At this threshold, the specificity was estimated at 83.3% (Table 2).

4. Discussion

Since 1960 and the first publication of the 48-h 2 mg/day low-dose dexamethasone suppression test (LDDST) by Liddle, this test has been an important part of the diagnostic protocol in case of suspicion of Cushing syndrome. The administration of DEX, results in suppression of the hypothalamic-pituitary-adrenal axis in normal individuals and a fall in plasma and urinary cortisol.

For CD patient classification, DEX administration exists in a variety of regimen and diagnostic thresholds. In all oral DEX tests, various DEX absorption and metabolism rates influence the test result. Intravenous administration of DEX avoids the pitfalls linked to poor compliance or unpredictable gastrointestinal absorption. In 1985, Abou Samra et al. [5] reported that infusion
Cortisol
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of
syndrome.
associated
distinguishing
patients
who failed to the first-line biological screening test (plasma cortisol > 50 nmol/L after 1 mg DST and/or UFC/24 hours > 145 nmol/24 h). The specific objective here was to assess the diagnostic value of the 4-mg IV DST in more challenging conditions and not only in obese patients frequently excluded by the first-line screening test.

The estimations of the AUCs for cortisol and ACTH showed that ACTH would be the best test in this differential diagnosis (98.4% AUC at 8 am on day 2). The comparisons made at different time points showed that ACTH at 8 am on day 2 was associated with the best diagnostic value. The threshold that maximized the number of well-classified patients at a CD prevalence of 43% was estimated at 19.6 ng/L and found associated with 93.7% sensitivity and 88.9% specificity. However, the main goal in CD diagnostic tests is to optimize sensitivity and avoid missing CD cases although at the price of a reduced specificity. The ACTH threshold of 14.8 ng/L at 8 am on day 2 was the threshold with highest specificity for a 100% sensitivity. At this threshold, the specificity was estimated at 83.3%. Hence, we suggest limiting the 4-mg IV DST to a single measurement of ACTH at 8 am on day 2. According to the 14.8 ng/L threshold, we identified six “false positive” patients (Table 3) of whom three were under benzodiazepine treatment, which is a cause of false positive results [10]. Indeed, benzodiazepine may induce cytochrome P450 activity and change DEX catabolism [11–13]. Among the three others, two were obese – DEX plasma concentration changes according to the BMI in obese women [14]. The measurement of plasma DEX concentration in these patients may be useful for better test interpretation but this needs the determination of normal values in normal and obese subjects and it could not be routinely performed.

The diagnosis performance of different tests in differentiating CD from PCS has been recently evaluated in a prospective study including 73 patients [15]. The authors concluded that a cortisol level > 87 nmol/L after DEX/CRH test together with a single measurement of serum cortisol > 243 nmol/L by midnight has a high diagnostic accuracy in differentiating CD from PCS with a 100% positive predictive value (PPV) and a 90% negative predictive value (NPV). These results are in line with a study included only 8 patients with proven CD and 10 patients with PCS.

Table 3
Cortisol and ACTH values during the 4-mg IV dexamethasone suppression test in the 6 CD “false positive” patients according to the ACTH measurement.

<table>
<thead>
<tr>
<th>False positive CD patients</th>
<th>24-hour UFC</th>
<th>4-mg IV dexamethasone suppression test</th>
<th>Plasma cortisol at 8 am of day 2</th>
<th>Plasma ACTH at 8 am of day 2</th>
<th>Plasma ACTH at 4 pm of day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>124</td>
<td>336</td>
<td>20</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>549.3</td>
<td>587</td>
<td>19</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>287.62</td>
<td>370.66</td>
<td>31.43</td>
<td>5.11</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>256</td>
<td>43</td>
<td>17.53</td>
<td>17.53</td>
<td></td>
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<tr>
<td>5</td>
<td>169</td>
<td>117.02</td>
<td>28</td>
<td>&lt;5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>21.95</td>
<td>&lt;5</td>
<td></td>
</tr>
</tbody>
</table>

UFC: urinary free cortisol.

Fig. 4. ROC curves of ACTH and cortisol at 8 am on day 2. ———: ACTH; ————: cortisol.

of DEX at 1 mg/h between 11 am and 3 pm caused a sustained suppression of plasma cortisol to < 83 nmol/L until at least 9 am of the next day in normal and obese subjects whereas this level remained > 276 nmol/L at 9 am in patients with Cushing syndrome. In 2010, in a larger cohort (66 CS and 32 control subject), Jung and al. [6] confirmed, that the 4-mg IV DST was very efficient in distinguishing CS patients from controls. They reported that, at day 2, a cut-off of 130 nmol/L cortisol was associated with 100% sensitivity and 100% specificity, and that a cut-off of 10 ng/L ACTH was associated with 99% sensitivity and 86% specificity. These results are close to the ones shown here and the residual difference may reflect difference in the control populations: i.e., “normal” subjects in Jung et al. [6] but PCS patients here. Actually, Jung et al. have already underlined the difficulty of distinguishing patients with low probability of CS from patients with true CS. The former group was composed of 15 patients with suspicion of CS – on the basis of clinical features – whose features did not progress over ≥ 2 years follow-up. In a limited cohort, Tran et al. [9] suggested that the 4-mg IV DST could differentiate CD form PCS; however, their
previous study that underlined the good diagnostic performance of the DEX/CRH test though this diagnostic performance varied between studies (PPV 80 to 100% and NPV 90 to 100%) as well as the threshold of the 15-min post-CRH cortisol (44 to 110 nmol/L) [2,15–18]. Moreover, the protocols used in these different studies differed (the start time of the test and the type of CRH (ovin or human sequence) used as well as the cortisol assays used). Though it is difficult to compare diagnostic test performance between laboratories, and despite its lower specificity compared to the DEX/CRH test (83.3% vs. 90%), the 4-mg IV DST has a good diagnosis performance. The absence of a normal population could be a limitation of this study; however, we thought that such a control group would not be useful. Indeed, in general practice, biochemical testing is performed only in patients with clinical signs of Cushing syndrome. Thus, we deemed it more relevant to evaluate the diagnostic value of the 4-mg IV DST in patients with PCS but admit that the definition of the PCS is not always clear and that a one-year follow-up or even more may not exclude the presence of some cases of mild Cushing disease.

5. Conclusion

The 4-mg IV DST is an easy and reliable diagnostic test that may be used as a second-line test to distinguish CD from PCS. A plasma ACTH level > 14.8 ng/L at 8 am on day 2 was generally sufficient to establish the diagnosis of CD. In the rare false positive CD patients, the level of ACTH at 4 pm on day 1 may correct the diagnosis.

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Disclosure of interest

The authors declare that they have no competing interest.

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