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ORIGINAL ARTICLE

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Corticotrophin-releasing hormone and desmopressin tests in the differential diagnosis between Cushing's disease and pseudo-Cushing state: a comparative study

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Summary

Background We recently proposed a new and effective way of interpreting human corticotrophin-releasing hormone (hCRH) and desmopressin (DDAVP) tests, for the differential diagnosis between Cushing's disease (CD) and pseudo-Cushing state (PC), based on the simultaneous analysis of ACTH and cortisol.

Objective The study had the aims of comparing the diagnostic performance of the two tests and determining whether carrying out both tests was more beneficial than carrying out only one.

Patients and measurements We studied 30 CD, 18 PC and 12 control (CT) subjects: in these patients, hCRH test, DDAVP test, 24-h urinary free cortisol, serum cortisol after overnight 1-mg dexamethasone suppression test and serum cortisol circadian rhythm were performed.

Results The hCRH test and the DDAVP test showed an identical and excellent diagnostic performance (sensitivity 96·6% and specificity 100% for both tests); moreover, the hCRH and DDAVP tests showed almost perfect diagnostic agreement ($\kappa = 0{\cdot}93$; $P < 0{\cdot}05$) with a significantly higher number of concordant diagnoses (58 cases of 60) than those resulting from all other possible combinations among the studied tests. Interestingly, there were no subjects in whom both hCRH and DDAVP tests gave a simultaneous misdiagnosis.

Conclusions Our study indicates that the hCRH and DDAVP tests have similar diagnostic performance and present excellent agreement, without giving simultaneous misdiagnosis in any subject. Because of these characteristics, the use of both tests offers the physician a valuable tool for those cases of hypercortisolism which are difficult to interpret.

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Introduction

The differentiation between Cushing's disease (CD) and a pseudo-Cushing state (PC) poses one of the greatest challenges in the field of endocrinology.[1] Even though many studies have been published on this subject, no consensus has been reached about which test is the most reliable in differentiating between the two.[1,2]

Regarding this issue, our group recently identified new criteria for interpreting the human corticotrophin-releasing hormone (hCRH) and desmopressin (DDAVP) tests that gave an excellent diagnostic performance.[3,4] The new interpretative methodology for these two tests requires, for CD diagnosis, the presence of two parameters with the exclusion of CD in the absence of one or both.[3,4] For the hCRH test, the pair of interpretative criteria is constituted by basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l (hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l)) or, alternatively, by peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l (hCRH test (peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l)).[3] The pair of interpretative criteria that we identified for the DDAVP test is constituted by basal serum cortisol >331 nmol/l and a rise in plasma ACTH (Δ-ACTH) >4 pmol/l.[4]

Based on our previous data,[3,4] these two tests gave a better diagnostic performance than the second-level tests considered valid up to that time, i.e. midnight serum cortisol and dexamethasone-suppressed CRH stimulation test,[5] thus gaining relevance in the diagnostic management of Cushing's syndrome (CS).[2] However, although they have been assessed in diverse samples, neither of these two tests gave a clearly better diagnostic performance compared to the other and even though their sensitivity and specificity were >90%, they were not able to absolutely distinguish CD subjects from PC ones.[3,4] These results raise two problems: (i) the clinician could be in doubt as to which of the two tests he should choose to confirm or exclude CD and (ii) it is not known whether performing both tests on the same subject could be beneficial.

To clarify these two issues, we studied a sample of CD, PC and control (CT) subjects on whom both the hCRH and the DDAVP tests had been carried out.

Materials and methods

Subjects

We studied 60 subjects, consecutively admitted to our centre between 1999 and 2010: 30 with a first diagnosis of active CD, 18 with PC and 12 with CT subjects. CD and PC subjects were admitted for suspected CD, which was then confirmed or excluded, respectively; they received clinical, radiological and biochemical evaluation as part of the diagnostic work-up, and their data were evaluated retrospectively. CD and PC diagnoses were made according to previously described methods.[3,4] Of the 18 subjects with PC, 12 were affected by major depression [6] and six by both polycystic ovary syndrome and panic disorder.[6–8] The CT subjects attended our centre for diet counselling; they were recruited prospectively and underwent examination purely for research purposes; they were selected from individuals with simple obesity (body mass index > 30 kg/m^2) according to clinical and biochemical criteria previously described.[4]

Subjects taking medications known to affect any parameter addressed in the study underwent washout before hospitalization. The study was performed according to the Declaration of Helsinki and approved by the institutional ethics committee. All subjects undergoing testing at our centre were asked to sign an informed consent form at admission. Some of the data were acquired in the framework of a research protocol that entailed an additional consent form. Twenty-five CD, 14 PC and seven CT subjects had already been evaluated at an earlier date.[3,4]

Study protocol

All subjects underwent comprehensive physical examination and the following tests, according to previously described methods:[3,4] (i) 24-h urinary free cortisol (UFC); (ii) serum cortisol after overnight 1-mg dexamethasone suppression test (OST); (iii) serum cortisol circadian rhythm; (iv) hCRH test; and (v) DDAVP test. hCRH and DDAVP tests were carried out with a 48-h interval between them. Bone mineral density was assessed in all subjects by dual X-ray absorptiometry (software version 3·61; DPX Lunar Radiation, Madison, WI, USA).

The parameters necessary for interpreting the hCRH and DDAVP tests, i.e. basal serum cortisol, peak plasma ACTH and peak serum cortisol for hCRH test and basal serum cortisol and Δ-ACTH for DDAVP test, were calculated according to previously described methods.[3,4]

Assays

Chemiluminescent immunometric assays were used to measure plasma ACTH (Immulite, DPC, Los Angeles, CA, USA) and serum cortisol and UFC (Advia Centaur; Bayer Diagnostics, Newbury, UK), the latter after urine extraction with dichloromethane. Method sensitivity was 0·99 pmol/l for plasma ACTH and 11 nmol/l for both urinary and serum cortisol; intra-assay and interassay variation coefficients were 3·4% and 4·8% for plasma ACTH and 4·4% and 6·0% for both urinary and serum cortisol, respectively. Normal ranges in our laboratory are 0–10 pmol/l for plasma ACTH, 41–413 nmol/24 h for UFC and 138–634 nmol/l for morning serum cortisol (0830 h).

Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM) if normally distributed and as median (interquartile range) if not normally distributed. The prevalence of clinical signs was analysed by the χ^2 test or, where appropriate, Fisher's exact test. Shapiro–Wilk's test was applied to verify the normal distribution of quantitative variables. Comparisons among groups were made with ANOVA followed by Fisher's least significant difference *post hoc* test for normally distributed values and with Kruskal–Wallis test followed by Mann–Whitney's U test (if significant differences were detected) for not normally distributed variables; *P*-values were corrected using the Bonferroni–Holm method.[9]

Sensitivity (SE), specificity (SP), positive likelihood ratio (LR+) and negative likelihood ratio (LR−) were calculated according to standard statistical methods;[10] diagnostic accuracy (DA) was calculated as the proportion of correctly rated patients out of the total number of patients tested. The diagnostic performance of the hCRH and DDAVP tests was analysed by comparing their SE values with the McNemar test; the same procedure was applied to compare the SP values.

When we considered the various combinations among the tests, we defined as 'concordant cases' those subjects in whom the two tests gave the same diagnosis; the McNemar test was used to statistically compare the number of 'concordant cases' among the various test combinations. Among the 'concordant cases', those subjects in whom the two tests simultaneously gave a mistaken diagnosis were defined as 'simultaneous misdiagnosis'; the χ^2 test was used to compare the frequency of 'simultaneous misdiagnosis' (no. of simultaneous misdiagnosis/no. of concordant cases) among the various test combinations.

The κ statistic was used as a measure of agreement between the tests;[11] κ statistic is a measure of agreement above or below what is expected by chance alone. The coefficient can range from −1·0 to 1·0, with negative values indicating agreement worse than chance, a value of zero indicating agreement no better than chance agreement and a value of 1·0 indicating perfect agreement. Values were interpreted as previously suggested:[11] <0 indicates 'poor agreement', 0–0·2 indicates 'slight agreement', 0·2–0·4 indicates 'fair agreement', 0·4–0·6 indicates 'moderate agreement', 0·6–0·8 indicates 'substantial agreement', and 0·8–1·0 indicates 'almost perfect agreement'.

Exact 95% binomial confidence intervals (CI) were computed for SE and SP.[12] Significance was set at $P < 0{\cdot}05$. Statistical analyses were performed using SPSS 16 package (SPSS Inc., Chicago, IL, USA).

Results

Demographic, biochemical and clinical characteristics of the studied subjects

Table 1 shows the demographic and biochemical characteristics of the studied subjects; from a clinical point of view, CD and PC did

Table 1. Demographic and biochemical data of the studied subjects

	Sex (males/females)	Age (years)	Body mass index (kg/m^2)	UFC (nmol/24 h)	OST serum cortisol (nmol/l)	Midnight serum cortisol (nmol/l)
CD (n = 30)	3/27	39·3 ± 1·16	33·8 ± 0·83	859·4 (562–1692·6)*,‡	488·5 (126·9–571·3)†,‡	621 (229–770)†,‡
PC (n = 18)	0/18	34·7 ± 2·32	31·5 ± 0·95	533·8 (455·7–606·9)	96·6 (66·2–118·6)	138 (80–248·4)
CT (n = 12)	1/11	34·9 ± 2·00	32·9 ± 0·83	188·9 (166–260·7)†	22 (19·3–30·3)†	135·2 (88·3–157·3)

UFC, urinary free cortisol; CD, Cushing's disease; CT, control; PC, pseudo-Cushing state.
Values are expressed as mean ± SEM if normally distributed, and as median (interquartile range) if not normally distributed.
*$P < 0{\cdot}01$ *vs* PC; †$P < 0{\cdot}001$ *vs* PC; ‡$P < 0{\cdot}001$ *vs* CT; not significant unless specified.

not differ significantly ($P > 0{\cdot}05$) in hypertension, hirsutism, impaired fasting glycaemia/diabetes, dyslipidaemia, oligomenorrhoea, acne, muscle weakness, bruising and osteoporosis (data not shown). Purple striae were more prevalent ($P < 0{\cdot}05$) in CD subjects, while obesity and psychiatric problems were more prevalent ($P < 0{\cdot}05$) in PC subjects (data not shown).

Comparison between the diagnostic performance of hCRH and DDAVP tests

Table 2 compares the diagnostic performance of the hCRH and DDAVP tests; both the hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and the DDAVP test gave an identical and excellent diagnostic performance (SE equal to 96·6% and SP equal to 100% for both tests). On the contrary, the interpretative criteria of hCRH based on the simultaneous presence of 'peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l' showed a significantly lower SP compared to that of the DDAVP test without a statistically significant difference in SE (Table 2).

The diagnostic performance of the other tests studied appears in Table 3.

hCRH and DDAVP tests: concordance and simultaneous misdiagnosis

Table 4 shows diagnostic concordance between the hCRH and DDAVP tests according to the two possible combinations between the two tests: hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP test had an 'almost perfect agreement'[11] ($\kappa = 0{\cdot}93$; $P < 0{\cdot}05$); the number of concordances from this combination (58 cases of 60) was significantly higher than that deriving from the combination between hCRH (peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l) and DDAVP tests (Table 4). Moreover, there were no subjects in whom both the hCRH and the DDAVP tests gave simultaneous misdiagnosis (Table 4).

Other possible combinations among all the tests studied: concordance and simultaneous misdiagnosis

Table 5 presents diagnostic concordance among all the other possible combinations of the tests studied. None of these combinations showed a κ index, indicating an 'almost perfect agreement';[11] moreover, in all the combinations among the tests reported in Table 5, the number of concordant diagnoses was significantly lower than that deriving from the combination of the hCRH (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP tests shown in Table 4.

It is noteworthy that in six of the combinations, there is a frequency of simultaneous misdiagnosis which is significantly ($P < 0{\cdot}05$) or nearly significantly ($P = 0{\cdot}07$) higher than that deriving from the combination of the hCRH (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP tests shown in Table 4.

Table 2. Comparison between the diagnostic performance of human corticotrophin-releasing hormone (hCRH) and desmopressin (DDAVP) test in confirming or excluding CD

Diagnostic tests	Cut-off	SE (CI) (%)	SP (CI) (%)	LR+	LR−	DA (%)
hCRH test*,†	Basal serum cortisol > 331 nmol/l and peak plasma ACTH > 12 pmol/l	96·6 (82·7–99·9)	100 (90·5–100)	–	0·03	98·3
	Peak serum cortisol > 580 nmol/l and peak plasma ACTH > 10 pmol/l	90 (73·4–97·8)	83·3 (65·2–94·3)	5·38	0·12	86·6
DDAVP test*,†	Basal serum cortisol > 331 nmol/l and Δ-ACTH > 4 pmol/l	96·6 (82·7–99·9)	100 (90·5–100)‡	–	0·03	98·3

CD, Cushing's disease; DA, diagnostic accuracy; –, impossible to calculate; SP, specificity; *CD diagnosis based on the presence of both parameters; absence of either or both excludes CD; †cut-offs previously given.[3,4]
hCRH test *vs* DDAVP test [hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) *vs* DDAVP test; hCRH test (peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l) *vs* DDAVP test]; comparison between the SE and comparison between the SP: ‡$P < 0{\cdot}05$ *vs* hCRH test (peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l); not significant unless specified.

Table 3. Diagnostic performance of the other tests studied in confirming or excluding CD

Diagnostic tests	Cut-off	SE (CI) (%)	SP (CI) (%)	LR+	LR−	DA (%)
UFC	Urinary free cortisol > 413 nmol/24 h*	83·3 (65·2–94·3)	40 (22·6–59·4)	1·38	0·41	61·6
OST serum cortisol	Serum cortisol > 50 nmol/l†	96·6 (82·7–99·9)	50 (31·3–68·7)	1·93	0·06	73·3
OST serum cortisol	Serum cortisol > 138 nmol/l†	73·3 (54·1–87·7)	90 (73·4–97·8)	7·33	0·29	81·6
Midnight serum cortisol	Serum cortisol > 207 nmol/l‡	90 (73·4–97·8)	73·3 (54·1–87·7)	3·37	0·13	81·6

DA, diagnostic accuracy; SP, specificity; CD, Cushing's disease; UFC, urinary free cortisol.
*upper limit of the normal UFC range in our laboratory; †cut-off commonly used for Cushing's syndrome diagnosis;[5] ‡cut-off according to Papanicolaou *et al.*[29]

Discussion

This study aimed at comparing the diagnostic performance of the hCRH and DDAVP tests in the differential diagnosis between CD and PC, according to criteria recently proposed by our group,[3,4] to find out whether one of the two tests was preferable to the other in clinical practice. To do this, we examined a sample of CD, PC and CT subjects who had undergone both the hCRH test and the DDAVP test. The hCRH (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP tests showed comparable diagnostic performance; on the contrary, the interpretative criteria of hCRH, previously identified, which require the simultaneous presence of peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l,[3] are shown in this study to be less effective than the DDAVP test. On the basis of these results, the clinician who is called upon to differentiate CD from PC can use either the hCRH test or the DDAVP test without distinction, as their diagnostic performance is comparable. However, the physician must bear in mind the particular characteristics of the two tests: the weakness of the hCRH test lies in the fact that its interpretation is based entirely on absolute cut-off values, rather than on their increment, making the analysis heavily dependent on assay methodology; moreover, the DDAVP test was found to be very useful even in cases of mild hypercortisolism given its independence from UFC, OST serum cortisol and midnight serum cortisol[4] while this evidence does not exist for the hCRH test; furthermore, the DDAVP test is less expensive than the hCRH test.[13] The hCRH test, in contrast to the DDAVP test, has the advantage, according to the method previously proposed by us, of being able to distinguish not only CD from PC but also CD from ectopic CS using a single test.[3]

We then decided to find out whether carrying out both tests could be more beneficial than performing just one. Regarding this point, the practical importance of a concordant diagnosis of the two tests must be stressed as it is this result that influences the clinician in making a decision; this decision can be correct if both tests give a correct diagnosis or mistaken if both tests have got the diagnosis wrong. Discordance between the tests creates a problem as it leaves the physician uncertain in his diagnosis. In our sample, the hCRH (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP tests gave a very high number of concordant diagnoses, higher than those deriving from the other possible combination between hCRH and DDAVP (Table 4). Obviously, this is the result of the excellent diagnostic performance which the same interpretative methods of the hCRH and DDAVP tests have in our sample. But, above all, we observed that in both the combinations between the hCRH and DDAVP tests, no simultaneous misdiagnosis was ever made by either test; this aspect is very important because it ensures that no cases are overlooked by both tests. From a practical point of view, this is because of the fact that as both the hCRH and DDAVP tests have high diagnostic performance, it is therefore highly unlikely that both of them will misdiagnose in the same subject. From a molecular point of view, the explanation of this phenomenon could lie in the fact that hCRH and DDAVP use two different receptor systems, respectively, corticotrophin-releasing hormone receptor and vasopressin (V3) receptor,[14] which are overexpressed in the corticotroph pituitary adenoma in an extremely variable way.[15] It must be stated that our data on this point are in agreement, although indirectly, with those presented in a previous

Table 4. Diagnostic concordance between the human corticotrophin-releasing hormone (hCRH) and desmopressin (DDAVP) test and frequency of simultaneous misdiagnosis

	DDAVP test*†: basal serum cortisol > 331 nmol/l and Δ-ACTH > 4 pmol/l
hCRH test*†: basal serum cortisol > 331 nmol/l and peak plasma ACTH > 12 pmol/l	**58**‡ (0/58) $\kappa = 0{\cdot}93$§
hCRH test*†: peak serum cortisol > 580 nmol/l and peak plasma ACTH > 10 pmol/l	**51** (0/51) $\kappa = 0{\cdot}70$§

CD, Cushing's disease;
The number of concordant diagnosis between the two tests appears in bold; frequency of simultaneous misdiagnosis deriving from the two tests appears in brackets. *CD diagnosis based on the presence of both parameters; absence of either or both excludes CD; †cut-offs previously given.[3,4]
Comparison of the number of concordant diagnosis deriving from the combination hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l)/DDAVP test with that from the combination hCRH test (peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l)/DDAVP test: ‡$P < 0{\cdot}05$.
Comparison of the frequency of simultaneous misdiagnosis deriving from the combination hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l)/DDAVP test with that deriving from the combination hCRH test (peak serum cortisol >580 nmol/l and peak plasma ACTH >10 pmol/l)/DDAVP test: not significant unless specified.
κ index:[11] §$P < 0{\cdot}05$.

Table 5. Diagnostic concordance among the other combinations of the tests and frequency of simultaneous misdiagnosis

	UFC >413 nmol/24 h*	OST serum cortisol >50 nmol/l†	OST serum cortisol >138 nmol/l†	Midnight serum cortisol >207 nmol/l‡
OST serum cortisol > 50 nmol/l†	**51**§ (15/51)¶ $\kappa = 0{\cdot}62^{**}$			
OST serum cortisol > 138 nmol/l†	**36**§ (5/36)¶ $\kappa = 0{\cdot}25^{**}$			
Midnight serum cortisol > 207 nmol/l‡	**42**§ (9/42)¶ $\kappa = 0{\cdot}35^{**}$	**47**§ (8/47)¶ $\kappa = 0{\cdot}53^{**}$	**40**§ (2/40) $\kappa = 0{\cdot}35^{**}$	
hCRH test††,‡‡: peak serum cortisol > 580 nmol/l and peak plasma ACTH > 10 pmol/l	**33**§ (2/33) $\kappa = 0{\cdot}07$	**42**§ (3/42)§§ $\kappa = 0{\cdot}38^{**}$	**49**§ (4/49)¶ $\kappa = 0{\cdot}63^{**}$	**41**§ (1/41) $\kappa = 0{\cdot}36^{**}$
hCRH test††,‡‡: basal serum cortisol > 331 nmol/l and peak plasma ACTH > 12 pmol/l	**38**§(1/38) $\kappa = 0{\cdot}27^{**}$	**43**§ (0/43) $\kappa = 0{\cdot}44^{**}$	**48**§ (0/48) $\kappa = 0{\cdot}59^{**}$	**48**§ (1/48) $\kappa = 0{\cdot}60^{**}$
DDAVP test††,‡‡: basal serum cortisol > 331 nmol/l and Δ-ACTH > 4 pmol/l	**36**§ (0/36) $\kappa = 0{\cdot}21$	**43**§ (0/43) $\kappa = 0{\cdot}44^{**}$	**48**§ (0/48) $\kappa = 0{\cdot}59^{**}$	**48**§ (1/48) $\kappa = 0{\cdot}60^{**}$

CD, Cushing's disease; hCRH, human corticotrophin-releasing hormone; DDAVP, desmopressin; UFC, urinary free cortisol.
The number of concordant diagnosis deriving from the various combinations of tests appears in bold; frequency of simultaneous misdiagnosis deriving from the various combinations of the tests appears in brackets. *Upper limit of the normal UFC range in our laboratory; †cut-off commonly used for Cushing's syndrome diagnosis;[5] ‡cut-off according to Papanicolaou *et al.*;[29] ††CD diagnosis based on the presence of both parameters; absence of either or both excludes CD; ‡‡cut-offs previously given.[3,4]
Comparison of the number of concordant diagnosis deriving from the various combinations with that deriving from the combination hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l)/DDAVP test shown in Table 4: §$P < 0{\cdot}05$.
Comparison of the frequency of simultaneous misdiagnosis deriving from the various combinations of the tests with that deriving from the combination hCRH test (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l)/DDAVP test shown in Table 4: ¶$P < 0{\cdot}05$;§§$P = 0{\cdot}07$; not significant unless specified.
κ index:[11] **$P < 0{\cdot}05$; not significant unless specified.

study that compared the diagnostic performance of hCRH and DDAVP tests;[16] that report, although it evaluated the two tests in the differential diagnosis between CD and ectopic CS and obviously used different interpretative criteria from ours, found that there were no cases in which both tests gave an erroneous diagnosis.[16]

It must also be noted that the number of concordances deriving from the combination of the hCRH (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP tests was greater than those deriving from all the other combinations among the various tests (Table 5); in six of these combinations, the frequency of simultaneous misdiagnosis was higher than those deriving from the combination between the hCRH (basal serum cortisol >331 nmol/l and peak plasma ACTH >12 pmol/l) and DDAVP tests (Table 5). In the light of these considerations, given that neither the hCRH test nor the DDAVP test gave SE and SP equal to 100%[3,4] and therefore was not able to separately discriminate with absolute certainty subjects affected by CD from those affected by PC, it could be useful to employ both tests in some particular cases, for example in cases where one of the two tests gives a diagnosis but with results that are borderline compared to the cut-off, or alternatively, the two tests could be carried out with a time gap between them in cases where a re-evaluation is necessary before arriving at a therapeutic decision.[17]

Clearly, the results of this study have to be carefully considered. In the first place, we must point out that our analysis is not meant to be a re-evaluation of the diagnostic performance of the hCRH and DDAVP tests compared to the single first- and second-level tests as this aspect has already been evaluated in larger samples which included a large part of the subjects considered in this article.[3,4] On the contrary, this study only aims at comparing the diagnostic performance of hCRH and DDAVP test and evaluating whether carrying out both tests could be diagnostically beneficial. Further studies based on completely new samples will be necessary to test again the validity of the hCRH and DDAVP test compared to other tests. In the second place, it is important to underline that even though various studies have used similar criteria for identifying PC,[3,4,18–25] the lack of a universal and specific definition of PC creates great difficulties when generalizing and comparing the results of various studies; it is therefore desirable that the scientific community urgently define more selective diagnostic criteria for this clinical condition. Third, the fact of not having analysed late-night salivary cortisol, either in this study or in our previous ones,[3,4] prevents comparison with a convenient test found to be effective in distinguishing PC from CS;[17,20,26] however, regarding this, one can hypothesize a complementary role of hCRH and DDAVP tests with late-night salivary cortisol, rather than an alternative one. Findling and Raff in fact proposed a flow chart for CS diagnosis that envisages late-night salivary cortisol as an initial screening test and only later, in the case of ambiguous or

contradictory results or to confirm CS diagnosis, carrying out a dexamethasone-suppressed CRH stimulation test.[27,28] We suggest that the hCRH and/or DDAVP test could be performed instead of the dexamethasone-suppressed CRH stimulation test after having excluded the presence of adrenal CS by measuring plasma ACTH levels;[1] it should be noted that it is preferable to carry out the hCRH rather than the DDAVP test when faced with a case of suspected ectopic CS.

In conclusion, our study shows that the hCRH test and DDAVP test had comparable diagnostic performance and excellent diagnostic concordance without making a simultaneous misdiagnosis in any subject. Because of these characteristics, these tests offer the clinician a useful diagnostic tool in cases of hypercortisolism which are difficult to interpret.

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