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The role of non-invasive dynamic tests in the diagnosis of Cushing's syndrome

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ABSTRACT. ***Objective:*** **To evaluate the role of non-invasive dynamic tests in the diagnosis and differential diagnosis of Cushing's syndrome (CS).** ***Methods:*** **We studied laboratory features of 74 patients with endogenous CS, subdivided as follows: 46 (62.1%) with Cushing's disease (CD), 21 (28.3%) with an adrenal tumor, and 7 (9.5%) with ectopic ACTH syndrome (EAS).** ***Results:*** **In 100% of cases of CS we found serum cortisol levels greater than 1.8 µg/dl after low-dose dexamethasone suppression tests (LDDST), as well as elevation of midnight serum or salivary cortisol. However, urinary free cortisol was normal in 11.5% of patients. ACTH levels were suppressed in patients with adrenal tumors, normal or high in CD and invariably increased in EAS. After the 8-mg overnight dexamethasone suppression test (HDDST), serum cortisol suppression >50% was observed in 79.5% of cases of CD and in 28.6% of subjects with EAS, whereas cortisol suppression >80% was only found in CD. After stimulation with CRH or desmopressin an ACTH rise ≥35% occurred in 86.5% of individuals with CD and 14.3% of those with EAS, whereas an ACTH rise ≥50 achieved 100% specificity. Moreover, the combination of serum cortisol suppression >50% after HDDST and an ACTH increase ≥35% after the administration of CRH or desmopressin only occurred in CD.** ***Conclusion:*** **Our findings demonstrate that LDDST had 100% sensitivity for the diagnosis of CS and that HDDST and stimulation tests with CRH or desmopressin may be very useful for confirmation of CS etiology when analyzed together or when more stringent cut-offs are used.**

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INTRODUCTION

Among endocrine disorders, Cushing's syndrome (CS) is certainly one of the most challenging to endocrinologists due to the difficulties that often appear during its investigation. The diagnosis of CS involves two steps: confirmation of hypercortisolism and determination of its etiology. Biochemical confirmation of the hypercortisolemic state must be established before any attempt at differential diagnosis. Failure to do so will result in misdiagnosis, inappropriate treatment, and poor management (1, 2). It should also be kept in mind that hypercortisolism may occur in some patients with depression, alcoholism, anorexia nervosa, generalised resistance to glucocorticoids, and in late pregnancy. However, in contrast with true endogenous CS, the biochemical findings improve when the underlying disorder has been resolved (3). Moreover, exogenous or iatrogenic hypercortisolism should always be excluded (4). Endogenous CS is classified as either ACTH-dependent or ACTH-independent (adrenal autonomy). The former category is the most frequent (80%) and encompasses ACTH-secreting pituitary adenomas [Cushing's disease (CD)] or ACTH-producing ectopic tumors, most commonly bronchial carcinoids. CD accounts for 80% to 90% of ACTH-dependent diseases, whereas 10% to 20% is caused by ectopic sources. Adrenal disorders autonomously secreting glucocorticoids are found in 20-30% of patients with CS, and this group comprises discrete and multiple adrenal lesions, such as adenomas, carcinomas, or micronodular and macronodular hyperplasia (1-4). The 3 most useful tests for the confirmation of hypercortisolism are the measurement of 24-h urinary free cortisol (UFC) levels, low-dose dexamethasone-suppression tests (LDDST), and determination of midnight serum cortisol or late-night salivary cortisol. However, none of these tests is perfect, each has different sensitivities and specificities, and several are usually needed to provide better diagnostic accuracy (3-5).

The greatest challenge in the investigation of CS involves the differentiation between CD and ectopic ACTH syndrome (EAS). This task requires the measurement of plasma ACTH levels, non-invasive dynamic tests [high-dose dexamethasone suppression test (HDDST) and CRH stimulation test or desmopressin stimulation test], invasive dynamic tests (inferior petrosal sinus sampling), and imaging studies (4-6).

The main objective of this study is to evaluate the role of non-invasive dynamic tests in the diagnosis and differential diagnosis of CS.

MATERIALS AND METHODS

Patients

A retrospective analysis of medical records was performed on 74 patients with endogenous CS routinely followed in the Division of Endocrinology of Hospital das Clínicas, Federal University of Pernambuco, and in Pernambuco Center for Diabetes & Endocrinology, in Recife, northeast of Brazil, from 2000 to 2007.

Key-words: Cushing's syndrome, diagnosis.

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Study design

For the confirmation of the biochemical hypercortisolism at least two of the following tests were performed in every patient: LDDST and measurement of the 24-h UFC and midnight serum or salivary cortisol. Serum cortisol at 08:00 h was also measured in all patients. The LDDST comprises the overnight and the 48-h dexamethasone-suppression tests (DST). In the overnight test, 1 mg of dexamethasone was given at 23:00 h and the concentration of cortisol in serum measured the next day at 08:00-09:00 h. In the 48-h test, dexamethasone was given at a dose of 0.5 mg every 6 h for 2 days at 09:00 h, 15:00 h, 21:00 h, and 03:00 h with measurements of serum cortisol at 09:00 h at the start and end of the test.

In the interpretation of both LDDST, we used the cut-off value of 1.8 μg/dl, as previously suggested (7). Midnight salivary cortisol samples were collected at home, stored in a standard refrigerator and delivered to the laboratory the next morning. Midnight serum cortisol samples were obtained after 2 days of hospitalization, in order to reduce the stress induced by hospitalization itself. Patients were woken up at midnight and blood samples drawn within 5 min. Midnight serum cortisol values >1.8 μg/dl were considered to be elevated (8).

Once a diagnosis of CS was established, the next step was to define its cause. The first test done was the measurement of morning plasma ACTH levels (between 08:00 h and 09:00 h) in two subsequent days. Patients with reduced ACTH levels were submitted to abdominal computerized tomography (CT) in search of adrenal tumors. Patients with normal or elevated ACTH levels underwent two non-invasive dynamic tests: HDDST and stimulation test with CRH or desmopressin. The HDDST consisted in the administration of a single 8-mg dose of dexamethasone orally at 23:00 h with measurement of serum cortisol at 08:00 h before and after administration. A cortisol reduction greater than 50% was considered indicative of CD (9, 10). In the CRH or desmopressin stimulation tests, plasma ACTH and cortisol levels were measured at –15 and 0 min before the iv administration of ovine CRH (100 μg) or desmopressin (10 μg), and 15, 30, 45, 60, 90, and 120 min afterwards. After CRH or desmopressin administration, a rise above basal levels ≥35% for ACTH and ≥20% for cortisol was considered suggestive of CD (11, 12).

All patients with ACTH-dependent CS were submitted to a magnetic resonance imaging (MRI) of the sellar region (Sigma LX GE, Milwaukee, WI), 1.5T, and gradient of 23 mT/m. The slices were axial, coronal, and sagittal in T1, pre- and post-gadolinium, and in T2. Patients suspected of having EAS also underwent chest and abdominal CT scans.

Bilateral inferior petrosal sinus sampling (BIPPS) was reserved for ACTH-dependent CS patients with discordant results between non-invasive dynamic tests and normal pituitary MRI findings, as well as for subjects with signs and symptoms highly suggestive of an ectopic source of ACTH. Serial samples for central and peripheral plasma ACTH concentrations were drawn –1 and 0 min before and 3, 5, and 10 min after the iv administration of CRH (100 μg) or desmopressin (10 μg). A central-to-peripheral ACTH gradient ≥2 (basal) or ≥3 (after CRH or desmopressin) was considered to be consistent with CD while lower gradient values were assumed to be indicative of EAS, as previously suggested (13-15).

Assays

Immunochemiluminometric assays (Diagnostic Products Corporation, Immulite 2000, Los Angeles, CA) were used for measurement of serum cortisol and UFC. The UFC normal range was 10-90 μg/24 h. Salivary cortisol levels were determined by a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, CA). Values >8.5 nmol/l (3.0 ng/ml) were considered to be elevated (4). Plasma ACTH levels were obtained using an immunoradiometric assay (Nichols Institute Diagnostics, San Clemente, CA) or a immunochemiluminometric assay (Diagnostic Products Corporation, Immulite 2000, Los Angeles, CA). Their reference values were 10-60 pg/ml and up to 46 pg/ml, respectively.

Statistical analysis

In the analysis of qualitative variables, the χ^2 test or Fisher's exact test (when necessary) were used. Both analysis of variance and the Student's t-test were performed for the comparative analysis of quantitative variables. Results are presented as percentages or as mean values ±SD. *p*-values <0.05 were considered to be statistically significant.

RESULTS

Etiology

Among the 74 evaluated patients, 46 (62.1%) had CD, 21 (28.4%) adrenal tumors (15 adenomas and 6 carcinomas), and 7 (9.5%) EAS (5 bronchial carcinoid tumors and 2 thymic carcinoid tumors).

ACTH immunoreactivity of the neoplastic tissue was documented in all cases of EAS. Among patients with CD, the diagnosis was confirmed by detection of ACTH immunostaining in the pituitary adenoma and/or reversal of hypercortisolism after its surgical resection.

Diagnosis

The performance of diagnostic tests is summarized in Table 1. Serum cortisol levels at 08:00 h were high in all but 7 patients (9.5%), 5 with CD, and 2 with adrenal adenomas. Loss of the diurnal (circadian) rhythmicity, expressed by elevation of midnight serum or salivary cortisol concentrations, was found in all patients that underwent these tests. The midnight serum cortisol concentrations ranged from 8.3 to 28.2 μg/dl (mean, 21.9±8.6) whereas midnight salivary cortisol levels varied from 12.6 to 36.7 nmol/l (mean, 18.2±6.1). UFC levels were found to be elevated in 47 of 53 patients (88.7%) but normal in 6 cases of CD. Mean UFC levels were significantly higher ($p<0.001$) in patients with EAS (696.3 μg/24 h) than in those with CD (364.7 μg/24 h) or adrenal tumors (424.6 μg/24 h).

LDDST were performed in virtually all patients. As shown in Table 1 and Figure 1, afterwards there was no suppression of serum cortisol levels to less than 1.8 μg/dl in any case. Following the overnight 1-mg DST, serum cortisol levels between 2 and 5 μg/dl, between 5.1 and 10 μg/dl, and >10 μg/dl were found in 5%, 21%, and 74% of patients, respectively. After the 48h-LDDST, the corresponding figures were 14%, 29%, and 57%, respectively (Fig. 1).

Differential diagnosis

Plasma ACTH levels (in pg/ml) ranged from 2.5-9.8 (mean, 7.4±2.1), 18-380 (88.1±53.1) and 175-1820 (mean, 516.5±654.1) in patients with adrenal tumors, CD and EAS, respectively. ACTH levels were reduced (<10 pg/ml) in 100% of patients with adrenal tumors, normal (in 37%) or elevated (in 63%) in patients with CD, and invariably high in EAS. There was a great overlap when pa-

Table 1 - *Performance of tests used to the diagnosis of Cushing'syndrome.*

Test	Reference value	No. of patients	Range (mean±SD)	Rate of patients with elevated values (%)
Serum cortisol at 08:00 h (µg/dl)	5-25	74	20.4-49.2 (30.3±5.5)	90.5
Midnight serum cortisol (µg/dl)	≤1.8	35	8.3-28.2 (21.9.3±8.3)	100
Midnight salivary cortisol (nmol/l)	≤8.5	26	12.6-36.7 (18.2±6.1)	100
Urinary free cortisol (µg/24 h)	10-90	53	62-1760 (390.7±336.4)	88.7
Serum cortisol (µg/dl) after 1 mg-overnight DST	≤1.8	74	3.6-28.5 (12.9±4.4)	100
Serum cortisol (µg/dl) after 2 mg/48 h LDDST	≤1.8	73	2.0-18.2 (6.9±3.8)	100

DST: dexamethasone-suppression test; LDDST: low-dose dexamethasone-suppression test.

tients with CD or EAS were compared but mean ACTH values were significantly higher in EAS cases ($p<0.001$). Stimulation tests with CRH (no.=19) or desmopressin (no.=25) were performed in 44 patients with ACTH-dependent CS. Among 37 patients with CD, 32 (86.5%) have shown an ACTH-positive response (rise ≥35% above basal), which was also found in 1 (14.3%) patient with EAS. An ACTH rise ≥50% above basal levels was only seen in CD (sensitivity of 75.7% and specificity of 100%) (Table 2). Cortisol rise ≥20% following the stimulation with CRH or desmopressin was detected in 29 of 37 (78.4%) patients with CD and 2 of 7 (28.6%) with EAS. A cortisol rise ≥50% was found in 54% of cases of CD and in 2/7 (28.6%) patients with EAS (Table 2). In patients with CD, the rate of positive response of ACTH (rise >35%) and cortisol (rise >20%) did not differ significantly (86.5% *vs* 78.4%; $p=0.620$). However, the specificity of ACTH response was greater than that of cortisol (83.7% *vs* 71.4%; $p=0.047$).

As shown in Table 3, the diagnostic sensitivity and specificity of CRH stimulation test and desmopressin stimulation test were similar and did not differ significantly when different criteria for ACTH and cortisol responses were compared.

After HDDST, cortisol suppression >50% was found in 31 of 39 (79.5%) patients with CD and in 2 of 7 (28.6%) of those with EAS. Nevertheless, cortisol suppression >80% only occurred in CD, being found in 22 of 39 cases (56.4%) (Table 2). Moreover, the combination of ACTH response to CRH or desmopressin (rise ≥35% above basal) and cortisol suppression >50% in HDDST was found in 19 of 30 (63.3%) patients with CD but in none of the 7 patients with EAS. Among patients with Cushing's disease, cortisol suppression >50% was more frequent in cases of microadenomas than macroadenomas (84.8% *vs* 50%; $p<0.001$).

Imaging studies

Pituitary MRI was performed in the 53 patients with ACTH-dependent CS. Among the 46 patients with CD, it revealed a microadenoma in 24 (52.1%), a macroadenoma in 9 (19.5%) whereas in 10 MRI was found to be normal. MRI also disclosed a 6 mm pituitary microadenoma in a female patient harboring a 5.6 cm ACTH-secreting thymic carcinoid tumor (Fig. 2). This patient did not respond to desmopressin stimulation and did not show suppression of serum cortisol after HDDST; moreover, there was no central-to-peripheral ACTH gradient on BIPPS (basal and after desmopressin administration).

The diameters of thymic carcinoid tumors ranged from 2.7-5.6 cm (mean, 4.1±2) and those of bronchial carcinoid tumors varied from 1.2-4 cm (mean, 2.7±1.7). They were all identified by CT scans. However, in 2 cases of

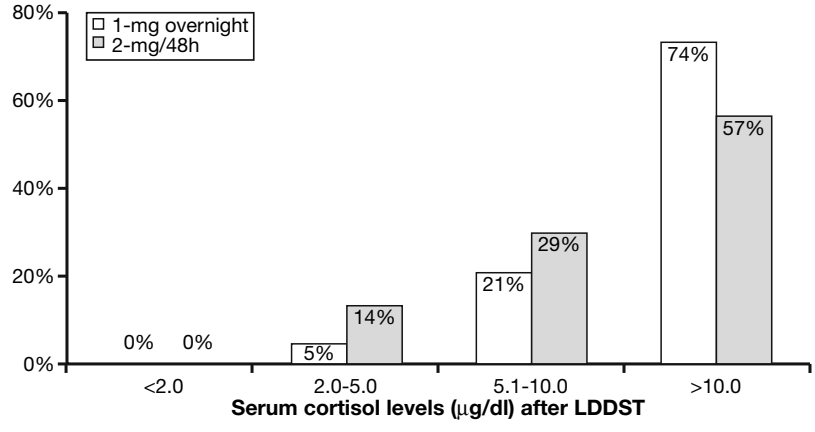

Fig. 1 - *Distribution of the patients according to serum cortisol levels after low-dose dexamethasone suppression tests (LDDST).*

Table 2 - *Performance of tests used to the differential diagnosis of the 53 patients with ACTH-dependent Cushing's syndrome, 46 with Cushing's disease (CD) and 7 with ectopic ACTH syndrome (EAS).*

Test (adopted criteria)	Sensitivity	Specificity	Diagnostic accuracy	Predictive value	
				Positive	Negative
Stimulation test with ovine CRH or desmopressin (performed in 37 patients with CD and 7 with EAS)					
ACTH rise ≥35%	86.5%	85.7%	86.4%	97%	54.5%
ACTH rise ≥50%	75.7%	100%	79.5%	100%	43.8%
Cortisol rise ≥20%	78.4%	71.4%	77.3%	93.5%	38.5%
Cortisol rise ≥50%	54.1%	71.4%	54.5%	90.9%	22.7%
HDDST (performed in 39 patients with CD and 7 with EAS)					
Serum cortisol suppression >50%	79.5%	71.4%	78.3%	93.9%	38.5%
Serum cortisol suppression >80%	56.4%	100%	63.0%	100%	29.2%
HDDST+stimulation test with ovine CRH or desmopressin (performed in 30 patients with CD and 7 with EAS)					
Cortisol suppression ≥50% in HDDST+ ACTH rise ≥35% after CRH or desmopressin	63.3%	100%	70.3%	100%	38.9%
BIPSS (performed in 10 patients with CD and 3 with EAS)					
Basal central-to-peripheral ACTH gradient ≥2	90%	100%	92.3%	100%	75%
Central-to-peripheral ACTH gradient ≥3 after CRH or desmopressin administration	90%	100%	92.3%	100%	75%

HDDST: high-dose dexamethasone suppression test; BIPSS: bilateral inferior petrosal sinus sampling.

bronchial carcinoid, the lesion could only be detected by the CT scan 7 to 25 months after the diagnosis of CS. Adrenal carcinomas measured 6-13.5 cm (mean, 9.1±3.0 cm) whereas the adenomas diameters ranged from 1.5 to 4 cm (mean, 2.8±0.7 cm).

Bilateral inferior petrosal sinus sampling

BIPSS was performed in 13 patients with ACTH-dependent CS of whom 10 subsequently were confirmed to have CD and 3 EAS. A central-to-peripheral ACTH gradient ≥2 (basal) and ≥3 following the administration of CRH (4 patients) or desmopressin (9 patients) was found in 9 patients with CD but in none of those with EAS (Table 2).

DISCUSSION

This study evaluated the laboratory features of a cohort of 74 patients with endogenous CS and confirms the inherent difficulties in the diagnosis of this condition. With reference to screening tests to confirm hypercortisolism, we observed that using a cut-off value of 1.8 µg/dl, none of the patients had cortisol suppression after the LDDST. Comparable results were found in studies from the United Kingdom that adopted the same cut-off value for the LDDST (sensitivity of 98-100%) (7, 8). In a previous study, we have shown that the 48-h LDDST allowed a clear distinction between obese subjects and patients with CS (16). In the current study, all tests used to confirm hypercortisolism, except UFC, provided a sensitivity of 100%. Indeed, 6 out of 53 patients (11.3%) had normal UFC values. This percentage varied from 5-15% in most studies (2, 17-21) . In the present study, ACTH levels were low (<10 pg/ml) in all patients with adrenal tumors, normal or elevated in cases of CD, and invariably elevated in EAS. There was, however, a great overlap in the hormone values in patients with CD or EAS. Similar results were reported by other authors (2, 4, 22). Nevertheless, in a recent study (23), plasma ACTH levels were found to be normal in 32% of 79 patients with EAS.

Serum cortisol suppression >50% after the HDDST was observed in 78.2% of our patients with CD but the same also happened in 28.6% of subjects with EAS. In three recent studies (22-24), cortisol suppression after HDDST was found in 9% to 25% of patients with EAS. These percentages are in agreement with those of previous studies that have shown that the overnight 8 mg-HDDST had sensitivity and specificity of 77-92% and 57-100%, respectively (9, 10, 25).

The main issue for the classical criteria of HDDST (cortisol suppression >50%) is that its performance statistically falls below the pre-test likelihood of CD, at least in women (i.e. around 90%) (2, 3, 26). Therefore, some authors have advocated abandoning the HDDST (3,26). Nevertheless, among our patients, cortisol suppression >80% only occurred in CD (found in 56.4% of the cases). Furthermore, response to both HDDST and CRH (or desmopressin) stimulation test was only found in patients with CD (sensitivity of 63.3% and specificity of 100%). Only very rarely is this observed in patients with EAS (22, 23). Conversely, lack of response to both CRH (or desmopressin) test and HDDST is highly suggestive of EAS (4).

Table 3 - *Performance of CRH and desmopressin stimulation tests in the differential diagnosis of the ACTH-dependent Cushing's syndrome.*

Adopted criteria	After CRH (no.=19)[a]	After desmopressin (no.=25)[b]	*p*
ACTH rise ≥35% above basal			
Sensitivity	93.5%	85.7%	0.62
Specificity	100%	75%	0.82
ACTH rise ≥50% above basal			
Sensitivity	81.2%	76.2%	1.00
Specificity	100%	100%	1.00
Cortisol rise ≥20% above basal			
Sensitivity	81.2%	76.2%	1.00
Specificity	66.7%	75%	0.88
Cortisol rise ≥50% above basal			
Sensitivity	62.5%	47.6%	0.57
Specificity	66.7	75%	0.88

[a]The CRH test was performed in 16 patients with Cushing's disease and 3 with ectopic ACTH syndrome (EAS). [b]The desmopressin test was performed in 21 patients with Cushing's disease and 4 with EAS.

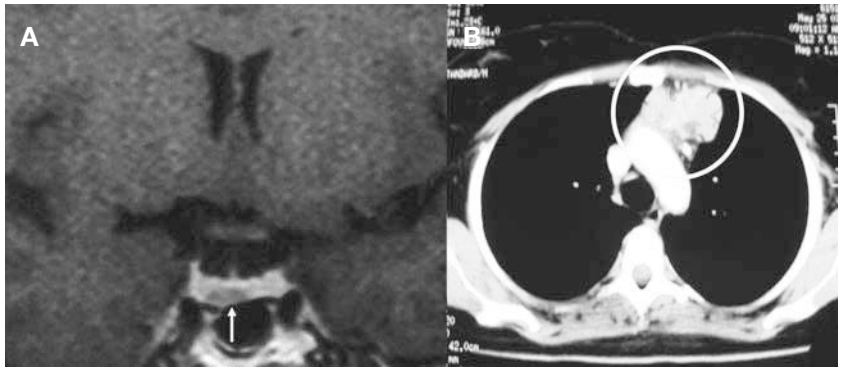

Fig. 2 - *Presence of a 6-mm pituitary adenoma (A) in a patient with a 5.6-cm ACTH secreting thymic carcinoid tumor (B).*

It was found in 71.4% of our EAS patients and in 79% of those reported by Ilias et al. (22).

In the Italian multicenter study suppression >80% of serum cortisol or UFC also had 100% specificity for CD (27). However, it should be noted that there are 4 reported cases of EAS with cortisol suppression >90% after the HDDST (26, 28).

In a combined analysis of the stimulation tests with CRH or desmopressin we found that, among our patients with CD, the rate of positive response of ACTH (rise >35%) and cortisol (rise >20%) was similar (86.5% *vs* 78.4%; p=0.620). Conversely, the specificity of ACTH response was greater than that of cortisol (83.7% *vs* 71.4%; p=0.045). In the largest individual series to date, where the same cut-off values were used, the sensitivity and specificity of the ACTH response to CRH were 93% and 100%, respectively, whereas the corresponding figures for the cortisol response were 91% and 88%, respectively (12). On the other hand, as shown in Table 2, an ACTH rise >50% after CRH or desmopressin was found in 70% of patients with CD but in none of the patients with EAS. In the Italian multicenter study, an ACTH increase >50% following CRH administration was also exclusively found in cases of CD (27). However, the specificity of this criteria was 90-95% in two further studies (18, 29).

As shown in Table 3, the sensitivity and specificity of both ACTH criteria and cortisol criteria did not differ significantly after the administration of CRH or desmopressin. However, we did not perform a head-to-head comparison of them and the number of patients with EAS was relatively small. In some studies, the desmopressin test was found to be less accurate than CRH test when they were assessed in the same patients (2-4), whereas in others both tests had similar diagnostic sensitivity (11). In the patients with CD from the series reported by Terzolo et al. (11), the frequency of ACTH response was of 90% after both CRH or desmopressin tests while the figures for cortisol were 73% after CRH and 77% after desmopressin, respectively. Moreover, in the 15 patients who underwent both tests the magnitude of ACTH and cortisol responses induced by the 2 stimuli was fully comparable. Nevertheless, among patients with EAS, a positive ACTH response was found in 2/9 cases (22%) after the CRH test and in 2/5 patients (40%) after the desmopressin test. In the study by Malerbi et al. (30), desmopressin administration produced a significant rise of cortisol secretion in 15 of 16 (93.7%) patients with CD whereas 1 patient with proven EAS and 2 patients with suspected EAS were unresponsive. Colombo et al. (31), using similar criteria, demonstrated a positive cortisol and ACTH response in 14 of 17 (82.3%) patients with CD; no response was observed in the single patient with non-histologically confirmed EAS studied. Newell-Price et al. (2) have also reported positive cortisol and ACTH responses to desmopressin in 1/5 and 3/5 patients with EAS, respectively. A possible explanation for the relatively poorer specificity of the desmopressin test is the more common expression of the V_{1b} (or V_3) receptor in ACTH-secreting non-pituitary tumors (32, 33). However, it should be noted that some patients with CD respond only to one peptide or the other (2, 4, 32).

A recent paper also highlighted the importance of non-invasive dynamic tests in the definition of the etiology of CS (34). In that study, among patients with positive concordant endocrine tests (CRH or desmopressin and HDDST) who did not undergo BIPSS, the rate of remission and recurrence after pituitary surgery did not significantly differ in cases with positive or negative MRI (34).

In summary, our findings demonstrate that the non-invasive dynamic tests may still be very useful in the investigation of CS. Both the 1-mg-DST and the 48-h-LDDST had 100% sensitivity for its diagnosis using the cut-off value of 1.8 µg/dl. We also found that a suppression of serum cortisol to less than 80% of the baseline concentration after HDDST and an ACTH rise >50% above basal levels after CRH or desmopressin stimulation were only seen in patients with CD. Moreover, a positive response to both CRH or desmopressin test and HDDST was exclusively observed in CD. Finally, we were also able to show that the sensitivity and specificity of CRH and desmopressin tests did not differ significantly.

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