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CLINICAL STUDY

The usefulness of combined biochemical tests in the diagnosis of Cushing's disease with negative pituitary magnetic resonance imaging

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Abstract

Objective: The etiological diagnosis of ACTH-dependent Cushing's syndrome is often a problem. In fact, no endocrine or radiological examination can conclusively distinguish the ectopic from the pituitary source of disease. The aim of our study was to evaluate the role of stimulation and suppression endocrine tests in the diagnostic and therapeutic approach of patients with Cushing's disease (CD) and negative pituitary magnetic resonance imaging (MRI), considering their post-surgical outcome in comparison with patients with CD and positive MRI.

Patients and methods: We retrospectively analyzed 31 patients (25 women and 6 men, median age 40 ± 15 years) with a confirmed diagnosis of CD who underwent transsphenoidal pituitary surgery by the same neurosurgeon between 2001 and 2005. Preoperative endocrine assessment included corticotropin-releasing hormone (CRH), desmopressin (dDAVP), and overnight 8 mg dexamethasone suppression tests (8-DST) in all patients. Fifteen patients had a normal pituitary MRI and sixteen had a clearly evident pituitary microadenoma. Bilateral inferior petrosal sinus sampling (BIPSS) was performed in patients with discordant biochemical results or with signs and symptoms highly suggestive of an ectopic source of ACTH. Post-surgical median follow-up was 38.4 ± 22.0 months.

Results: Among patients with negative MRI, 60% had concordant positive endocrine tests and underwent neurosurgery without other examinations. BIPSS was performed in three other patients prompted by discordant endocrine tests (negative dDAVP) and in two patients with clinical suspicion of ectopic disease. Among patients with positive MRI, 87% underwent neurosurgery without BIPSS that was performed in two patients because of negative concomitant response to dDAVP and CRH tests. A pituitary adenoma, confirmed by pathological examination, was found in 40 and 81% of patients with negative and positive MRI respectively ($P<0.05$), corticotroph hyperplasia resulted more frequent in the group with negative MRI. Remission rate was not different between patients with negative and positive MRI (73 and 75% respectively; $P=0.61$) and between patients with negative MRI who did not undergo BIPSS and patients with positive MRI ($P=0.56$). The recurrence rate was also similar between groups ($P=0.64$), but higher, although not statistically different ($P=0.07$) in patients with corticotroph hyperplasia at histology.

Conclusions: An accurate evaluation of presurgical endocrine tests results enabled us to reduce the number of BIPSS in patients with a negative MRI without any fallout on their post-surgical outcome. In the hands of an expert pituitary surgeon, the outcome after surgery and the subsequent recurrence rate are much the same in patients with negative or positive MRI.

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Introduction

The diagnosis of Cushing's syndrome (CS) is often a challenge because it is still difficult to differentiate between an adrenocorticotrophic hormone (ACTH)-secreting pituitary adenoma (Cushing's disease; CD) and the rare form of ectopic ACTH secretion by tumors.

Pituitary magnetic resonance imaging (MRI) should be performed in all patients with suspected ACTH-dependent CS, but this may reveal pituitary adenoma in no more than 36–78% of cases in adult series (1–5). It is also important to bear in mind that the pituitary gland may harbor incidental tumors in the general population too, since autopsy studies have shown that 10–20% of normal individuals have pituitary microadenomas (6). In the diagnostic approach to CS, endocrinologists have to deal with two different problems, i.e., the possibility of CD despite normal pituitary MRI findings, and the possibility of an incidental pituitary adenoma in an ectopic form of CS.

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Several tests have been used in the differential diagnosis of CS, such as the high-dose dexamethasone suppression (HDDST) and CRH stimulation tests (1, 7), none of which completely discriminates between CD and an ectopic source of ACTH, however. There is some controversy regarding the combined use of these tests, but no studies have compared these data in relation to normal/dubious MRI findings and a clinical suspicion of CD.

The desmopressin (dDAVP) test has been introduced in recent years as a tool for the differential diagnosis of CS. Acting through pituitary V3 receptors, desmopressin increases ACTH secretion in 80–90% of patients with CD, but only rarely in normal individuals or patients with pseudo-Cushing's syndrome. From 20 to 50% of ectopic ACTH-secreting tumors may respond to dDAVP, however, thus limiting its usefulness in distinguishing the source of ACTH (8).

Bilateral inferior petrosal sinus sampling (BIPSS) is considered the gold standard for establishing the origin of ACTH secretion. It is recommended in patients with CS whose clinical, biochemical, or radiological studies are discordant or equivocal in identifying the pituitary or ectopic origin of their ACTH production (9). Its availability is limited, however, by its cost, technical difficulty and important related risks. A recent report (10) analyzed the results of BIPSS in several series, finding a 73–95% sensitivity and a 67–100% specificity, and these percentages improve slightly after CRH stimulation. In some patients with a negative BIPSS, the origin of their ACTH excess is never found: these cases need to be followed up with caution because at least some of them may have false negative results (10–13).

These observations prompt interest in combining biochemical tests and imaging methods for a better selection of patients who might benefit from undergoing a BIPSS.

The aim of our study was to evaluate the role of stimulation and suppression endocrine tests in the diagnostic and therapeutic approach of patients with CD and negative pituitary MRI, considering their post-surgical outcome in comparison with patients with CD and positive MRI.

Subjects and methods

Patients

Thirty-one consecutive patients were retrospectively analyzed (25 women, mean age 41.0±13.4 years (range 13–67); and 6 men, mean age 36.5±19.3 years (range 15–64)) from those managed at the Endocrinology Unit of Padova University who underwent transsphenoidal adenomectomy as primary treatment for CD between 2001 and 2005.

We only considered patients with a confirmed diagnosis of CD (pituitary source of ACTH hypersecretion confirmed by histology or BIPSS), evaluated by means of stimulation and suppression tests with CRH, dDAVP, overnight 8 mg dexamethasone suppression test (8-DST), pituitary MRI, and BIPSS, as appropriate.

Preoperative biochemical and radiological evaluation

The diagnosis of CS was suspected on clinical grounds and confirmed by increased 24 h urinary free cortisol (UFC) levels (mean of three samples), loss of circadian rhythm in plasma cortisol (at 0800 and 2300 h), and failure of 1 mg dexamethasone overnight to suppress cortisol secretion (normal values <3 μg/dl considered from 2001 to 2003 and <1.8 μg/dl since 2003) (9, 14).

The diagnosis of ACTH-dependent CS was based on the presence of detectable plasma ACTH concentrations and the source of ACTH secretion was investigated by endocrine assessment and standard pituitary MRI before and after gadolinium injection.

Endocrine assessment included overnight 8-DST, the CRH stimulation test (100 μg i.v. of ovine CRH), and the dDAVP test (10 μg i.v.). For analyzing the percentage of ACTH and cortisol increment, after the collection of a baseline blood sample, serial measurements of their plasma level were performed at 15-min intervals for 2 h after hormone injection. The diagnosis of pituitary ACTH secretion was suspected when morning plasma cortisol levels dropped by more than 50% of the baseline values after 8-DST and ACTH levels rose by more than 50% with $\delta > 20$ pg/ml and/or cortisol more than 20% above their baseline values after consecutive CRH and dDAVP tests.

MRI was performed with a superconducting magnet scanner (TESLA 1.0). In all patients, sagittal and coronal T1-weighted images and additional coronal T2-weighted spin echo MRI images were obtained in thin sections (3 mm) before gadolinium, followed by a dynamic coronal T1 sequence beginning simultaneously with the contrast injection (15). Pituitary MRI studies were reviewed independently by the neuroradiologist, the pituitary neurosurgeon, and at least by one endocrinologist. Full agreement was reached in discovering the microadenoma from the normal or ambiguous MRI pictures.

We evaluated 15 patients with negative MRI (no visible adenoma) and a group of 16 patients with positive MRI (definite evidence of pituitary adenoma). In the group with positive MRI, 15 patients presented a pituitary lesion <10 mm (diameter range 3–8 mm) and only one patient presented a macroadenoma (diameter 11 mm).

A BIPSS was performed in patients with discordant biochemical results or with signs and symptoms highly suggestive of an ectopic source of ACTH. The threshold criteria for a pituitary source were defined as a ratio of inferior petrosal sinus to peripheral basal ACTH levels $\geq 2{:}1$ at the baseline or $\geq 3{:}1$ after CRH stimulation (11).

Surgery

All patients were operated by the same surgeon with extensive experience of pituitary tumor surgery. The same transsphenoidal technique was used in all patients. After opening the sellar floor and sellar dura, the hypophysis was exposed. After identification, selective adenomectomy was performed. When no adenoma was seen, the surgeon cut the gland at the middle and explored lobes. If no tumor was identified with this technique, the lower third or two-third of hypophysis were excised.

Histological examination

Pituitary specimens were analyzed by the same anatomopathologist and the presence of ACTH-secreting cells was evaluated by immunocytochemistry using specific anti-ACTH antibodies.

Thirty-one formalin-fixed, paraffin-embedded pituitary biopsy specimens were investigated. Standard hematoxylin and eosin stain, periodic acid-Schiff (PAS) reaction, and reticulin stain were used for histological examination.

Immunohistochemistry was used to characterize the cell composition of the pituitary adenoma and immunostaining was done using the avidin–biotin-peroxidase complex method. Antisera were directed against pituitary hormones, growth hormone, prolactin, ACTH, follicle-stimulating hormone, luteinizing hormone, and thyrotropin; both MIB1 and p53 labeling indices were evaluated.

Classic light microscopic features of ACTH-secreting adenoma were sheets of cells with amphophilic to basophilic and periodic acid-Schiff positive cytoplasm, variable but generally strong ACTH immunoreactivity.

Diagnosing pituitary hyperplasia could prove challenging because clusters and nodules of ACTH immunoreactive cells are a normal feature of pituitary tissue.

Pituitary hyperplasia could be focal, nodular, or diffuse, and the latter often escapes detection because adenohypophyseal cells are unevenly distributed in the normal gland too.

Nodular hyperplasia is more easily diagnosed and is characterized by changes in cell architecture, with an expansion rather than an effacement of the acinar pattern, best seen with reticulin stain.

Postoperative biochemical evaluation

The biochemical evaluation was repeated within 2–4 weeks after surgery. Corticotropic deficiency was considered as 0800 h plasma cortisol levels below 5 μg/dl with clinical evidence of adrenal insufficiency necessitating glucocorticoid replacement (1, 9). These patients were given replacement therapy with oral cortisone acetate (12.5–25 mg/day) until the pituitary–adrenal axis returned to normal, as assessed 3, 6, and 12 months after surgery and each year thereafter. We considered in remission only those patients with normal mean value of UFC, normal cortisol rhythm (0800 and 1800 h) and restored suppression of plasma cortisol levels by overnight 1 mg dexamethasone after surgery (<1.8 mcg/dl).

Statistical analysis

Data were analyzed with the Statistic program (Stat Soft rel. 6.0).

Comparisons between means were drawn using Student's *t*-test as data were normally distributed. Distributions of gender, post-surgical evolution, and histological findings were analyzed using Fisher's exact test. Data are given as mean $\pm$ S.D. Statistical significance was considered as a P value of <0.05.

Results

Preoperative evaluation

Patients with negative and positive MRI were similar in terms of sex ratio, age, basal UFC, ACTH levels, and follow-up (Table 1).

In the group with a negative MRI, 9 out of 15 patients (60%) underwent transsphenoidal surgery without performing BIPSS, because of positive concordant response to the three tests, while one patient with a negative response to 8-DST refusing further diagnostic procedures, underwent pituitary surgery achieving clinical and biochemical remission. Other two male patients had BIPSS because of strong clinical suspicion of ectopic CS (hypokalemia, severe muscle hypotrophy, and very high ACTH levels) although they had presented positive concordant response to the reported three tests. Other three patients with negative response to dDAVP performed BIPSS (Fig. 1).

In the group of patients with a positive MRI, 12 out of 16 (75%) had concordant biochemical response to the three tests and underwent transsphenoidal pituitary surgery; nine of them achieving a clinical and biochemical remission. BIPSS was performed in two other patients with discordant biochemical response to

Table 1 Clinical and biochemical characteristics of Cushing's disease patients with negative and positive magnetic resonance imaging (MRI).

	Negative MRI	Positive MRI	*P*
No. of patients	15	16	NS
Mean age (S.D.)	38.6 $\pm$ 16.6	42.1 $\pm$ 12.9	0.53
Sex M/F	4/11	2/14	0.29
UFC (mcg/24 h)	565.4 $\pm$ 374.1	538.8 $\pm$ 358.8	0.84
ACTH (pg/ml)	62.6 $\pm$ 30.6	52.0 $\pm$ 39.5	0.44
Median follow-up	38.0 $\pm$ 23.5	38.7 $\pm$ 21.4	0.93

M, male; F, female; UFC, urinary free cortisol (normal 13.7–75.4 mcg/24 h); plasma ACTH (normal 5–78 pg/ml); plasma cortisol at 0800 h (normal 9.4–26.7 mcg/dl).

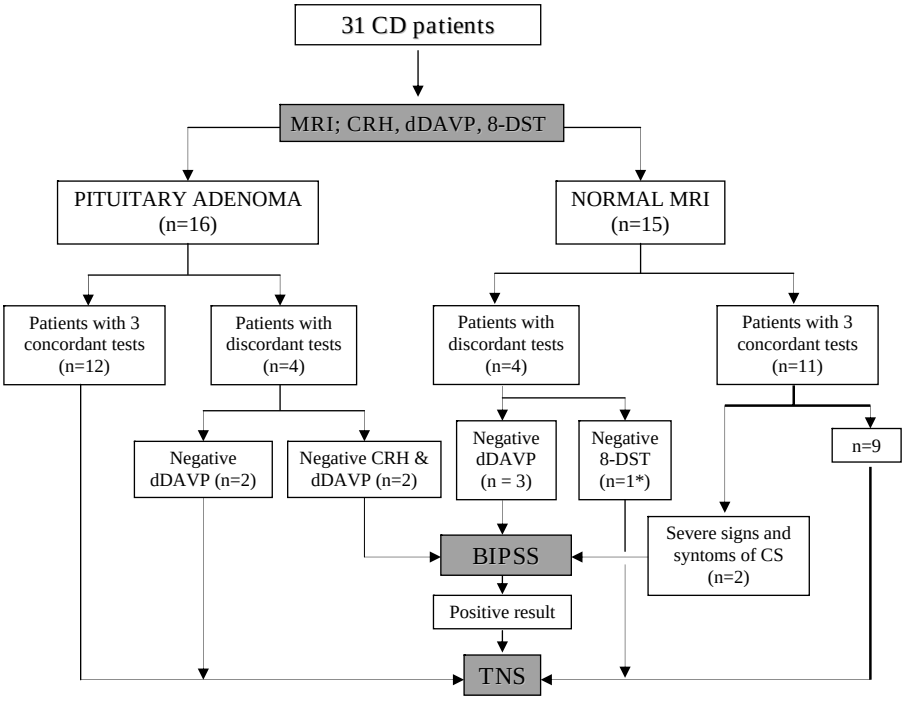

Figure 1 Flow diagram displaying diagnostic approach we used in 31 patients with Cushing's disease (CD). BIPSS, bilateral inferior petrosal sinus sampling; TNS, transsphenoidal surgery; *this patient refused other examinations.

the suppression and stimulation tests (negative for the CRH and dDAVP tests). Other two patients had a negative response to the dDAVP test, but underwent transsphenoidal pituitary surgery without BIPSS (Fig. 1). These patients also achieved a clinical and biochemical remission after surgery.

The clinical and biochemical characteristics of patients who underwent BIPSS are reported in Table 2.

Histological findings

Group with negative MRI Among these patients, the pathologist confirmed a pituitary adenoma in 6 out of 15 patients (40%), corticotroph hyperplasia in five (nodular in 2), and no morphological alteration in four patients. The diagnosis of pituitary hyperplasia was based on reticulin stain which revealed alterations in both tissue architecture and cell morphology. In two cases with nodular hyperplasia, the diagnosis was easier, because in the surgical tissue we could appreciate clusters of irregular nodules without replacement of the acini; the reticulin network was focally disrupted with occasionally confluence of *acini*, which chiefly consisted of PAS and ACTH-positive corticotrophs cells. In the other three cases, there was a higher number of basophilic cells with a cellular monomorphism suggestive of adenoma, but the reticulin stain showed expansion rather than absence of the reticulin fiber network of pituitary *acini*. In these cases, to avoid the presence of an ACTH-secreting microadenoma, we did serial sectioning of the available tissue.

Table 2 Clinical and biochemical characteristics of patients who underwent bilateral petrosal sinus sampling.

	ACTH/cortisol Δ% CRH	ACTH/cortisol Δ% dDAVP	Cortisol Δ% ODDST	ACTH (pg/ml)	MRI	Hypo-k	Clinical CS signs	Outcome after surgery
1	59/63 (+)	50/28 (+)	61 (+)	60.9	−	+++	+++	Remission
2	810/68 (+)	389/51 (+)	79 (+)	54	−	+++	+++	Remission
3	117/183 (+)	40/18 (−)	79 (+)	183	−	+++	+++	Remission
4	28.3/29.4 (+f)	12.8/29.8 (−)	82 (+)	19.7	−	−	+	Remission
5	67/59 (+)	4/2 (−)	84 (+)	45.4	−	++	++	Persistence
6	43/7 (−)	4/17 (−)	90 (+)	32.5	+	−	+	Remission
7	16/17 (−)	33/47 (+f)	61 (+)	178	+	+	+++	Persistence

CRH, corticotropin-releasing hormone test; dDAVP, desmopressin stimulation test; ODDST, overnight 8 mg dose dexamethasone suppression test; hypo-k, hypokalemia; CS, Cushing's syndrome; (+), positive result; (−), negative result.

Pathological findings in patients with persistent disease were as follows: adenoma in two cases, hyperplasia in one, and no evidence of pituitary tumor in one case in whom presurgical BIPSS had suggested a pituitary origin of the ACTH secretion. Recurrence was recorded in three patients with hyperplasia (Fig. 2a).

Group with positive MRI Adenoma was confirmed in 13 out of 16 cases (81%), corticotroph hyperplasia in one patient (with persistent disease after surgery), and negative histological report in the remaining two cases. Recurrence was recorded in one patient without histopathological findings and in two with adenoma (Fig. 2b).

Histologically confirmed adenoma was significantly more frequent among patients with a positive MRI ($P<0.01$).

Postoperative evaluation

Outcome after surgery The postoperative remission rate did not differ statistically between the two groups, being 11 out of 15 negative-MRI patients (73%) and 12 out of 16 positive-MRI patients (75%; $P=0.61$; Fig. 3). Among patients with a negative MRI, the remission rate did not differ significantly between ten patients who had no BIPSS and the group with positive MRI (70 vs 75% respectively; $P=0.56$).

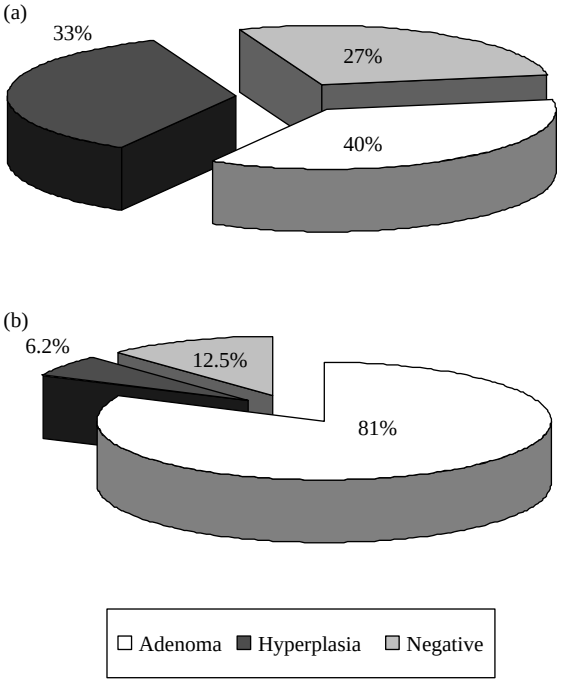

Figure 2 Histological findings in patients with negative MRI (a) and in patients with positive MRI (b).

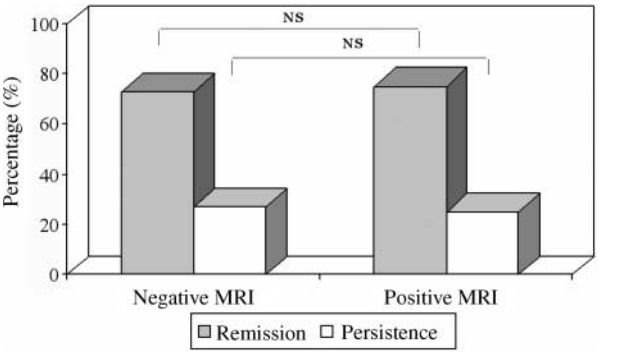

Figure 3 Remission rate of the disease in patients with positive and negative MRI.

Hypercortisolism persisted in 4 out of 15 patients with a negative MRI and 4 out of 16 patients with a positive MRI.

During the follow-up (mean ± S.D.: 38.4 ± 22.0 months), 17 out of 23 patients achieving initial remission, maintained mean normal levels of UFC, plasma cortisol, and the suppression after 1 mg dexamethasone. Recurrence was observed in 3 out of 15 patients with negative and in 3 out of 16 patients with positive MRI (20 vs 18.7%; $P=0.64$), at a mean follow-up of 38.0 ± 23.5 and 38.7 ± 21.4 months respectively. In the group of patients with recurrent disease, four had normal cortisol levels immediately after surgery (three had a negative and one had a positive MRI); the other two (both with positive MRI) had hypocortisolism immediately after surgery. No patients presented post-surgical hypopituitarism or visual defects.

Comparing data of patients with histological findings of corticotroph hyperplasia and ACTH secreting adenoma, the postoperative remission rate did not differ in the two groups (67 vs 72% respectively; P: 0.58) but a higher recurrence rate was evident in patients with hyperplasia at mean follow-up of 34 months (50 vs 11%; P: 0.07).

Discussion

This study suggests that an accurate evaluation of endocrine data from suppression and stimulation tests may help containing the amount of BIPSS performed in patients with Cushing's disease with no evidence of pituitary adenoma at MRI without any fall out on their post-surgical outcome.

With some differences in the criteria used between different studies (16–19), an increase in ACTH and cortisol after CRH stimulation is considered the most reliable non-invasive diagnostic procedure for the differential diagnosis of ACTH-dependent CS (16). There are reports of no ACTH response to CRH in patients with CD, however, and a CRH receptor mRNA

expression was observed in one case of a bronchial carcinoid producing Cushing's syndrome (8).

Desmopressin is a long-acting vasopressin analog with a high affinity for the V2 vasopressin receptor, but a relatively low affinity for the V3 receptor that predominates in the normal pituitary gland.

Desmopressin has been shown to increase ACTH levels in patients with CD but not in normal subjects (20, 21), though some degree of ACTH and cortisol response in healthy subjects was demonstrated in another study (22). V2 receptor expression has also been demonstrated in tumors of patients with ectopic ACTH secretion (8, 23). These observations reduce the value of the dDAVP test as a tool for the differential diagnosis of ACTH-dependent CS.

Using the criterion of a cortisol suppression $>50\%$ of the baseline level after high-dose dexamethasone, there were initial reports of a 92% sensitivity and 100% specificity (24, 25), but later studies demonstrated a sensitivity and specificity of 88 and 57% respectively (26).

These data suggest that none of the existing tests has sufficient diagnostic accuracy alone to differentiate the source of ACTH production, so these non-invasive tests are frequently used consecutively in clinical practice. Some authors found that when CRH test results are combined with information obtained from low- or high-dose dexamethasone suppression tests, the accuracy of the diagnosis is better than when each test is used alone (27, 28); other authors disagree, however (16). The combined use of CRH and desmopressin stimulation in the differential diagnosis of ACTH-dependent CS was first proposed by Newell Price *et al.* (29), and subsequently evaluated by other authors too (25). These studies found no overlap in cortisol responses between patients with CD and those with ectopic forms, thus discriminating clearly between the two entities. But Tsagarakis *et al.* (8) showed that after CRH–dDAVP stimulation, only ACTH, not cortisol responses were useful in the differential diagnosis, but still far from capable of fully discriminating between patients with pituitary and ectopic forms.

We retrospectively evaluated patients with CD and negative MRI to assess the usefulness of endocrine tests in the diagnostic approach based on test's sensitivity and specificity previously reported.

In our experience, 93% of patients had a positive ACTH and cortisol response to the CRH test. Despite a positive MRI, two patients with a negative response to the CRH test underwent BIPSS, showing that CRH was the test that more influenced our decision of performing BIPSS because of its low incidence of false negative results.

Only one patient in our group had a negative response to the 8 mg dexamethasone suppression test; perhaps this high percentage of positive tests was due to the plasma cortisol suppression cut-off used (Δ 50%): this choice could confer to the test a higher sensitivity but a lower specificity.

In this study, dDAVP test confirms to have the lowest concordance with the final diagnosis. However, a positive dDAVP test supporting the results of CRH and HDDST was important for us in the decision of not performing BIPSS, particularly in patients with negative MRI. A negative dDAVP result should prompt BIPSS in patients with normal/dubious MRI, whereas in those with radiological evidence of a pituitary adenoma, the test could be avoided. We based this statement on previous evidences that confer to the dDAVP test a lower sensitivity than the other tests and a lower specificity than MRI in the diagnosis of CD (2, 17, 24).

No single patient had a negative ACTH and/or cortisol response to all the three tests suggesting that consecutively applying more than one test may help in the diagnosis of ACTH overproduction.

Patients with CD and a normal MRI can pose numerous problems in terms of diagnostic and therapeutic approach. The European Cushing's Disease Survey Group (30) found a lower pituitary neurosurgery success rate in patients with CD and a normal MRI. A recent study found a similar outcome of pituitary surgery regardless of any microadenoma observable on pituitary MRI (31), and the same goes for our study.

BIPSS has proven to be highly accurate in localizing the source of ACTH, but false negatives have been described (10). It is also an undoubtedly invasive procedure, raising the risk of thromboembolism (32, 33), therefore it should be reserved for particular dubious cases, which are strongly suspected of being ectopic forms of ACTH secretion.

There is no agreement in the literature about the kind of patients who are candidates to this diagnostic procedure. Some authors advise to perform BIPSS in all patients with negative MRI (30). Others support the utility of BIPPS in all patients with negative MRI and in those with positive MRI but discordant biochemical tests (34). Therefore, what is the utility of endocrine tests in patients with negative MRI if they undergo BIPSS in any case?

The systematic use of stimulation and suppression tests in the diagnosis of CS enables us to avoid the use of invasive examinations (BIPSS) in a high percentage of patients (77%), and particularly those with a negative MRI (60%), for which this procedure is usually more indicated. A strong clinical suspicion of ectopic CS made us consider BIPSS in three patients despite their concordant non-invasive test results, but if we consider test result concordance alone, then the percentage of patients not requiring BIPSS would be higher (73%).

Considering the low predictive value of BIPSS in the adenoma lateralization (5), the surgical approach in patients with negative MRI who did not undergo this test did not differ from patients who perform BIPSS with a significant ACTH ratio for the pituitary origin of the disease.

In our group of patients, there were no differences in clinical and biochemical outcome after surgery between patients with negative and positive MRI findings. These

results are similar to those of a recent report (30), in which the diagnosis of CD was confirmed by BIPSS in all patients with a negative MRI. This means that we reduced the morbidity associated with the invasive procedure (BIPSS) in 67% of patients with negative MRI, while obtaining the same remission rate.

We observed an overall recurrence rate of 19.3%, after mean follow-up of 38 months, and most of the patients involved had normal plasma cortisol levels in the early postoperative period. As demonstrated previously (30, 35), the probability of recurrence is higher in patients with normal cortisol levels after surgery than in cases with corticotropic deficiency. We found histological evidence of an ACTH-secreting pituitary adenoma less frequently in the group with negative MRI, as it was described in a recent report in which histology confirmed the adenoma in only 53% of these patients (30). Failure to identify a pituitary lesion in some patients could be due to many causes, the main on being that most Cushing's adenomas are microadenomas and it can be difficult to find them at surgery or in the minute specimens obtained by transsphenoidal surgery. In our group, the remission rate was much the same regardless of positive or negative histology.

We found a high incidence of pituitary hyperplasia, particularly among patients with a negative MRI. Pathological studies had revealed varying amounts of hyperplastic pituitary tissue in patients with CD (36, 37). The diagnosis of corticotroph hyperplasia is still discussed but different reports have demonstrated that it cannot be so rare (38) and some authors propose that the hyperplastic lesions could be the predecessor of the adenoma (39). Moreover, corticotroph hyperplasia is reported as a possible cause of disease relapse after partial hypophysectomy (40). Our data showed the same remission rate between patients with corticotroph hyperplasia and those with histological evidence of an adenoma but, as expected, a higher frequency of relapse was observed in patients with hyperplasia.

In conclusion, the accurate evaluation of results of stimulation and suppression tests, enabled us to perform BIPSS only in selected cases in which the diagnosis of pituitary ACTH secretion was still dubious. Our opinion is that, in patients with CS and negative pituitary MRI, the finding of concomitant positive results of the three tests could avoid performing BIPSS.

The lack of any significant difference in remission rate between patients with negative MRI who did not undergo BIPSS and patients with positive MRI (our group of patients as a whole) confirms the validity of this diagnostic approach.

MRI remains a useful tool in the diagnosis of Cushing's disease, but – in the hands of an expert pituitary surgeon – the outcome after surgery and the subsequent recurrence rate are much the same, regardless of whether or not microadenoma is evident at MRI.

Histopathological findings may be unable to predict outcome after surgery in some patients with CD. A larger study is necessary to establish whether finding no adenoma at MRI correlates with other morpho-histopathological features of the pituitary gland in these patients.

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