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The Application of the Combined Corticotropin-Releasing Hormone plus Desmopressin Stimulation during Petrosal Sinus Sampling Is Both Sensitive and Specific in Differentiating Patients with Cushing's Disease from Patients with the Occult Ectopic Adrenocorticotropin Syndrome

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Context: Although bilateral inferior petrosal sinus sampling (BIPSS) with CRH stimulation is the most accurate procedure for the differential diagnosis of ACTH-dependent Cushing's syndrome (CS), 4–15% of patients with Cushing's disease (CD) fail to demonstrate diagnostic gradients. Preliminary data suggest that a more potent stimulation by the combined administration of CRH plus desmopressin during BIPSS may provide some diagnostic advantage. A crucial issue, however, is whether such an amplified stimulation may affect the specificity of the procedure, and this was the main aim of the present study.

Objective: We investigated the diagnostic accuracy of BIPSS performed by CRH plus desmopressin stimulation.

Design and Setting: A retrospective analysis was conducted at a single tertiary care center.

Participants: Fifty-four patients were admitted for the investigation of ACTH-dependent CS. CD was diagnosed in 47 patients; occult ectopic ACTH syndrome (oEAS) was histologically confirmed in seven patients.

Intervention(s): All patients underwent BIPSS with CRH plus desmopressin administration. Additional noninvasive tests included CRH test, high-dose dexamethasone suppression test, desmopressin test, and pituitary magnetic resonance imaging.

Main Outcome Measures: Gradients of inferior petrosal sinus (IPS) to peripheral (IPS/P) ACTH were calculated before and after stimulation with CRH plus desmopressin.

Results: The sensitivity for a basal IPS/P gradient greater than 2 was 61.7%, with 100% specificity and a diagnostic accuracy of 66.7%. After stimulation with CRH plus desmopressin, receiver operating characteristic (ROC) curve analysis showed that a cutoff gradient of more than 2 offers the best test performance. In total, 46 of 47 patients with CD had an IPS/P gradient greater than 2, but none of the patients with oEAS, resulting in a sensitivity of 97.9%. The specificity was 100%, diagnostic accuracy was 98.2%, and the positive and negative predictive values were 100 and 87.5%, respectively. A subgroup of 18 patients (16 with CD and two with oEAS) had contradictory responses to routine tests with CRH and/or high-dose dexamethasone suppression test; sensitivity, specificity, and accuracy of BIPSS in this subgroup were 100%.

Conclusions: The application of a combined stimulation with CRH plus desmopressin during BIPSS is associated with a high sensitivity but no loss of specificity. **(*J Clin Endocrinol Metab* 92: 2080–2086, 2007)**

BILATERAL INFERIOR PETROSAL sinus sampling (BIPSS) has been validated as a highly accurate investigative tool in the differential diagnosis of ACTH-dependent Cushing's syndrome (CS) (1–4). Administration of CRH to induce stimulation of ACTH secretion during BIPSS is being routinely used to elicit diagnostic gradients in patients with Cushing's disease (CD) and to improve the sensitivity of the procedure. Nevertheless, with the exception of the study of Oldfield *et al.* (1), which quoted an accuracy approaching 100%, in subsequent series a false-negative rate of 4–15% has been reported (2, 5–9). This illustrates that in many centers a significant proportion of patients with CD will undergo further unnecessary tests and sustain considerable delay of curative transsphenoidal surgery, based on such false-negative results.

The possible causes for a false-negative result during BIPSS in patients with CD are currently unclear and likely multifactorial. Besides technical factors or anatomical variation (10), insufficient ACTH stimulation by CRH used as the sole stimulus and/or CRH unresponsiveness may well be a reason of a false-negative outcome of BIPSS. It is well established that 10–20% of patients with CD do not demonstrate positive ACTH and cortisol responses in peripheral blood samples during routine CRH testing (7, 11). Some of these patients may fail to demonstrate diagnostic inferior petrosal sinus (IPS) to peripheral (IPS/P) ACTH gradients, as is sug-

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Abbreviations: BIPSS, bilateral inferior petrosal sinus sampling; CD, Cushing's disease; CS, Cushing's syndrome; HDST, high-dose dexamethasone suppression test; IPS/P, inferior petrosal sinus to peripheral; MRI, magnetic resonance imaging; oEAS, occult ectopic ACTH syndrome; ROC, receiver operating characteristic.

gested by the worse sensitivity and diagnostic accuracy of BIPSS in patients with equivocal responses to CRH and/or high-dose dexamethasone suppression test (HDST) (8).

Simultaneous use of CRH and desmopressin has been reported to be a much more potent stimulus for ACTH in patients harboring corticotroph adenomas (12) and, as we have reported (13), this combined stimulation during BIPSS induces a higher ACTH output, which could prove to reduce substantially the rate of false-negative results. One might argue, however, that the more potent stimulus of CRH plus desmopressin could raise the number of false-positive responses in patients with the occult ectopic ACTH syndrome (oEAS), compromising the specificity of the procedure. Therefore, in this report we sought to determine whether the application of the combined stimulation with CRH plus desmopressin affects the specificity of the procedure. To this end, we present our experience using this combined stimulus during BIPSS in a large group of patients with ACTH-dependent CS, including seven consecutive patients with histologically confirmed oEAS.

Patients and Methods

Catheterization of the petrosal sinuses has been performed in Evangelismos Hospital, since 1991, in 102 patients with various forms of CS. Stimulation with a combination of CRH plus desmopressin was introduced in 1997 and was subsequently used in all our patients with CS undergoing BIPPS. All catheterization studies were performed by a single invasive radiologist (I.S.K.). In 83 of 102 BIPSS attempts, the procedure was technically successful (overall catheterization success rate, 81.3%). The reasons for the unsuccessful procedures were: in 11 procedures, only unilateral catheterization was possible; in four cases, procedural limitations resulted in a bilateral catheterization of the high internal jugular veins; in one procedure, one of the two catheters was placed in the IPS, and the other sampling was received from the corresponding high jugular vein; two procedures were not completed due to anatomical reasons; and one procedure was interrupted because of patient's noncompliance.

In this series, only patients that have undergone stimulation with a combination of CRH plus desmopressin during successful catheterization were included for analysis. Confirmation of a pituitary or ectopic source of ACTH production was available for 54 patients, 47 with CD and seven with oEAS. The diagnosis of CD was confirmed histologically in 35 patients after transsphenoidal surgery; in 12 cases, no tumor was found but sustained clinical and laboratory remission resulted after transsphenoidal surgery. Among the patients with oEAS, six had histologically confirmed bronchial carcinoids and one medullary thyroid cancer; all these tumors had positive immunostaining for ACTH. In none of these patients was the origin of ACTH hypersecretion evident at presentation, and the possibility of an ectopic source was raised after the hormonal and BIPSS investigations.

The study was approved by the ethical committee of our institution, and informed consent was obtained from all patients.

All patients had routine testing with the CRH (human CRH 100 μg iv bolus; Ferring Pharmaceuticals Ltd., Malmo, Sweden) and all but two patients with the desmopressin test (DDAVP; 10 μg iv bolus, Ferring Pharmaceuticals Ltd.). Our testing protocol was as follows. After an overnight fast, an indwelling catheter was inserted at 0800 h with the subject remaining supine during the whole study period. At 0830 h (0 min), 100 μg CRH or 10 μg desmopressin was given as an iv bolus injection. Blood samples for ACTH and cortisol measurements were obtained at −15, 0, 15, 30, 45, 60, 90, and 120 min. Blood pressure and heart rate were recorded during the study period. After testing with desmopressin, restriction of fluids was advised for the rest of the day. No side effects were reported during the study period or for the rest of the day. All but two patients with CD underwent the HDST (2 mg dexamethasone orally every 6 h for 2 d; blood samples for cortisol determination were obtained at 0800 h on the day before and at 0800 h on the morning after dexamethasone treatment).

A decrease of more than 50% of serum cortisol after the HDST or an increment of cortisol more than 20% and/or ACTH more than 50% over the baseline values after the CRH or the DDAVP test were considered as representing a positive response (11, 14). Pituitary imaging was performed with magnetic resonance imaging (MRI) both before and after the injection of gadolinium.

Catheterization protocol

Bilateral IPS catheter insertion was performed as previously described (1). In each patient the position of the catheters was checked after injecting a small amount of nonionic contrast medium. Correct catheter placement with the tip at the junction of the vertical and horizontal segment of the IPS was confirmed by venous angiography before sampling in all patients included in the present study. After optimal catheter placement, blood samples (6 ml) were collected simultaneously from both catheters and a peripheral vein, over a 2-min period before and at 3–5, 8–10, and 13–15 min after the bolus administration of 100 μg human CRH (Ferring Pharmaceuticals Ltd.) plus 10 μg DDAVP (Ferring). Apart from facial flushing, and ear discomfort reported when the catheter reached the petrosal sinus, no other side effects have been observed during or after the procedure. Samples for ACTH measurements were kept in ice until the completion of the procedure and then were spun at 4 C, and the plasma was separated and stored at −20 C until assay. The highest ratio of ACTH values in IPS samples to the level simultaneously measured from the peripheral vein sample was used as an indicator of the diagnosis of CD. Based on previous reports and our current ROC curve analysis, an IPS/P ratio of more than 2, either before or after stimulation, was taken as suggestive of CD (2, 13, 15–17).

Hormone assays

Plasma ACTH was measured by a highly specific immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Inter- and intraassay coefficients of variation were 8 and 4%, respectively. Serum cortisol was assayed using a direct RIA featuring an iodine-125 radioligand and cortisol antibody-coated tubes (Coat-A-Count, Cortisol RIA; Diagnostic Products, Los Angeles, CA) until 2000, and subsequently by an immunofluorometric assay (Chiron, East Walpole, MA).

Statistics

The results are expressed as mean ± SE. Statistical comparisons of the data between groups were performed with the *t* test. The level of significance was set at 0.05 in all statistical tests. ROC curves were constructed to examine the diagnostic test performance, that is the ability to discriminate between patients with CD and EAS. Sensitivity against 100% specificity of poststimulation data was plotted at each level, and the area under the curve was computed by the nonparametric Wilcoxon statistic. Area under the curve represents the probability of correctly identifying CD and oEAS patients. Regarding calculation of the diagnostic accuracy of MRI, findings that appeared negative or equivocal were classified as negative.

Results

The clinical and laboratory characteristics of the patients are shown in Table 1.

The mean basal ACTH levels from IPS samples were higher in the patients with CD than those with oEAS (336.6 ± 458.1 pg/ml *vs.* 170.0 ± 65.2 pg/ml, respectively), but this difference was not statistically significant. Basal IPS/P gradients greater than 2 were observed in 29 of the 47 patients with CD with a mean baseline ACTH IPS/P ratio of 4.6 and in none of the patients with oEAS (mean baseline ACTH IPS/P ratio: 1.07) (Table 2). Thus, the sensitivity of a baseline ratio greater than 2 was only 61.7%, specificity was 100%, and diagnostic accuracy was 66.7%.

After stimulation with CRH plus desmopressin, the mean peak ACTH levels from the dominant IPS samples were

TABLE 1. Clinical and laboratory characteristics of patients

	CD (n = 47)	oEAS (n = 7)
Age (yr)	42.4 ± 12.6	44.0 ± 20.4
Sex (F/M)	36/11	1/6
HDST (S/NS)	35/10[a]	2/5
CRH (R/NR)	39/8	0/7
No. of CRH responders and HDST suppressors	29/45[a]	0/7
No. of patients with basal ratio > 2	29/47	0/7
No. of patients with stimulated ratio > 2	46/47	0/7
Mean basal IPS/P ratio	4.6 ± 3.9	1.07 ± 0.1
Mean stimulated IPS/P ratio	19.6 ± 23.7	1.3 ± 0.1
MRI of pituitary gland		
Negative[b]	15	3
Equivocal[c]	9	
Micro[d]	21	3
Macro[e]	2	

F, Female; M, male; S, suppressors; NS, nonsuppressors; R, responders; NR, nonresponders (as defined in *Patients and Methods*).
[a] In two patients, HDST was not available.
[b] No visible tumor.
[c] Hypointense region < 5 mm.
[d] Hypointense lesion 5–9 mm.
[e] Hypointense lesion ≥ 10 mm.

3126.6 ± 2965.8 pg/ml for the patients with proved CD and 601.6 ± 878.3 pg/ml for the patients with oEAS ($P = 0.00$) (Table 2). To determine an optimal threshold for the stimulated IPS/P ratio, we performed ROC analysis (Fig. 1). The area under the curve for the stimulated ratio was 0.997, indicating an excellent test performance. Using a cutoff value for the stimulated IPS/P ratio of 2 or greater, both maximal sensitivity (97.9%) and specificity (100%) for the distinction between CD and oEAS were achieved.

An IPS/P gradient above 2 was obtained in 46 of the 47 patients with Cushing's disease (one false-negative result). The mean stimulated IPS/P ratio for the patients with a pituitary source of ACTH was 19.6 (range, 1.6–133.9). All seven patients with oEAS had a stimulated IPS/P ratio of less than 2 (mean stimulated ratio, 1.3); therefore, there were no false-positive results (Fig. 2). Using the stimulated ratio, the sensitivity of BIPSS was 97.9%, specificity was 100%, and diagnostic accuracy was 98.2%. The positive and the negative predictive value were 100 and 87.5%, respectively.

Regarding the one patient with histologically confirmed corticotroph adenoma who failed to reach diagnostic IPS/P gradients (basal IPS/P, 1.1; stimulated IPS/P, 1.6), she displayed a significant response to peripheral CRH testing as well as to the peripheral administration of desmopressin. In this patient, the decision for transsphenoidal surgery was based on 90% cortisol suppression during the HDST, a positive response on CRH test, and the evidence of a pituitary adenoma on MRI imaging.

All our seven patients with oEAS studied after a combined stimulation during BIPSS had a basal and stimulated IPS/P ratio of less than 2. They were all unresponsive to peripheral CRH test, but two had a greater than 50% suppression of cortisol during HDST, and three had a significant response to peripherally administered desmopressin.

Eighteen patients (16 with CD and two with oEAS) had contradictory responses to CRH testing and/or HDST. Among the patients with CD, eight did not show suppression of their cortisol levels during HDST, six were unresponsive to CRH, whereas in two patients both tests were negative. All these patients had a stimulated IPS/P gradient above 2 (mean, 12.7). Moreover two of the eight patients who did not display a positive response to CRH were also unresponsive to desmopressin when administered alone. However, diagnostic stimulated IPS/P gradients were obtained even in these patients. In the two patients with oEAS who had a significant suppression of cortisol during HDST, BIPSS established the correct diagnosis. Thus, the sensitivity, specificity, and accuracy of BIPSS in this subgroup were 100%.

In this series, MRI of the pituitary was positive in only 23 patients with CD, negative in 15, and equivocal in nine. In the patients with oEAS, a lesion suggestive of a microadenoma on MRI scan was observed in three of the six that had undergone pituitary imaging. Thus, the diagnostic accuracy of MRI was only 49%.

Discussion

In a previous study, we observed significantly higher peak ACTH levels from dominant IPS samples in those patients with Cushing's disease that received a combined stimulus with CRH plus desmopressin during BIPSS, compared with those who received stimulation with CRH alone (13). As a result, the proportion of patients with false-negative BIPSS was lower in those who received the combined stimulation during the procedure, suggesting that such a combined stimulus may provide some diagnostic advantage over the traditional CRH stimulation. A crucial issue, however, was whether such an amplified stimulation may affect the specificity of the procedure, and this was the main aim of the present study. In our initial report, only one patient with oEAS underwent BIPSS with a combined CRH-desmopressin stimulation (13). In the present study, the amplified stimulation with CRH plus desmopressin was administered to all consecutive histologically confirmed cases with oEAS that were diagnosed in our department. Indeed, none of the seven patients with oEAS tested had a false-positive outcome dur-

TABLE 2. ACTH levels and IPS/P ratios in patients during BIPSS

Patient no.	Basal petrosal ACTH	Peak stimulated petrosal ACTH	Basal IPS/P ratio	Dominant stimulated IPS/P ratio	Nondominant stimulated IPS/P ratio
CD					
1	33	512	1.1	1.6	1.4
2	94	299	1.5	2.2	1.5
3	223	850	1.4	2.3	0.9
4	108	3,308	1.2	2.3	1.8
5	65	360	1	3.0	1.2
6	45	5,345	1.2	3.1	2.3
7	83	1,047	1	3.1	1.2
8	2,530	7,583	2.6	3.2	2.8
9	81	250	1.2	3.8	1.5
10	130	797	1.4	4.1	1.2
11	856	1,381	10	4.9	1.6
12	260	3,238	3.2	4.9	1.3
13	265	578	5.9	6.3	1.3
14	289	1,242	2.6	6.7	1.2
15	38	1,056	1	7.2	1.2
16	291	1,095	8.5	7.2	3.6
17	378	1,206	4.5	8.2	1.2
18	759	4,236	9.4	8.2	4.5
19	305	1,171	6	8.4	1.3
20	81	1,049	1.5	8.7	1.3
21	199	1,472	1.8	9.3	1.6
22	33	2,932	1.2	9.6	1.0
23	92	388	7.1	12.0	2.2
24	120	3,933	1.1	12.7	1.5
25	168	2,975	6.1	13.0	1.6
26	285	591	10.4	13.2	2.2
27	1,485	7,160	10.1	13.3	5.0
28	20	2,741	1	13.5	1.3
29	1,281	6,788	8.5	15.0	2.1
30	393	1,909	12.4	15.8	15.2
31	235	3,020	3.4	16.2	1.3
32	391	1,391	9.6	17.0	1.7
33	425	3,396	3.1	19.6	1.1
34	131	3,700	5	22.3	1.2
35	55	1,091	3.6	24.5	24.0
36	738	3,656	11	25.0	9.1
37	336	2,780	5	26.2	7.4
38	968	6,235	18.6	30.4	23.0
39	198	2,600	6	32.3	1.5
40	350	8,152	3.3	35.0	21.8
41	82	4,218	1.4	36.0	17.0
42	55	1,091	6.2	43.7	1.1
43	119	9,032	1.5	51.0	11.7
44	34	1,242	2.6	55.0	5.8
45	116	3,557	2.6	58.5	6.9
46	135	11,366	4.7	69.9	1.2
47	464	12,931	1.4	133.9	61.8
Mean ± SD	336.6 ± 458.1	3,126.6 ± 2,965.8	4.6 ± 3.9	19.6 ± 23.7	5.7 ± 10.3
oEAS					
48	175	299	0.9	1.1	0.9
49	224	250	1.2	1.1	1.1
50	64	135	1.1	1.3	1.1
51	108	192	1	1.3	1.1
52	186	425	1.1	1.3	1.0
53	255	328	1.1	1.3	1.1
54	178	2,582	1.1	1.4	1.3
Mean ± SD	170.0 ± 65.2	601.6 ± 878.3	1.07 ± 0.1	1.3 ± 0.1	1.1 ± 0.1

ing BIPSS after stimulation by the combination of CRH plus desmopressin. Therefore, these data demonstrate that the application of an enhanced stimulation by CRH plus desmopressin during BIPSS in a substantial number of the rare oEAS patients does not compromise the specificity of the procedure.

In general, in the era of CRH stimulation alone, false positive results have been rarely reported in patients with oEAS (18). Most of the cases are related to ectopic CRH production by the tumor (19, 20) or to intermittent secretion of ACTH leading to inadequate suppression of pituitary corticotrophs (21). Also, on the basis of the limited existing data, administration of desmopressin alone does not seem to predispose to false-positive results during BIPSS. So far, desmopressin

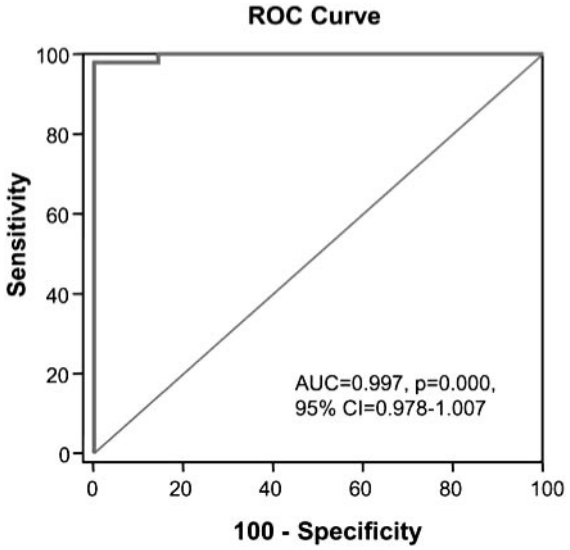

FIG. 1. ROC curve of the performance of the stimulated IPS/P ratio during BIPSS in the differential diagnosis of CD and oEAS.

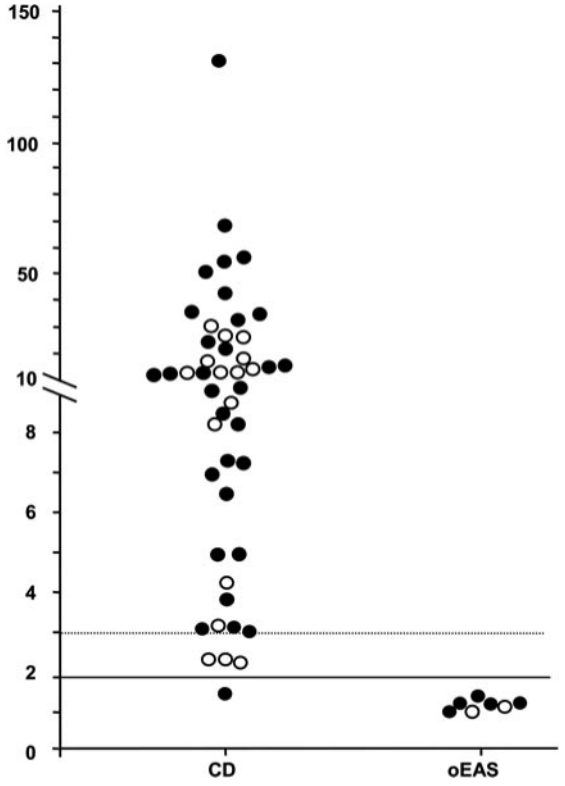

FIG. 2. Maximum IPS/P ACTH ratios after CRH plus desmopressin stimulation in patients with Cushing's disease (CD) or occult ectopic ACTH tumors (oEAS). The *continuous line* crosses the y-axis at an IPS/P ratio of 2; the *hatched line* crosses the y-axis at an IPS/P ratio of 3. *Open circles* show patients with equivocal results on routine biochemical testing with CRH and/or HDST.

alone has been applied in a few patients with CD (17, 22, 23) and in only eight patients with oEAS (17, 22, 24); all patients with oEAS had an IPS/P ratio of less than 2. The only study that raised some concern on the use of desmopressin was the description of two patients with EAS in whom the sequential administration of desmopressin after CRH stimulation led to IPS/P gradients of more than 2 in both patients (25). However, none of our seven patients with oEAS tested after a combined stimulation, by giving CRH and desmopressin concomitantly and not sequentially, had an IPS/P ratio of more than 2. Interestingly this lack of a greater than 2 IPS/P gradient occurred despite a significant peripheral ACTH rise during the procedure in five of seven patients (data not shown). As previously shown (26), this peripheral ACTH rise originates from the ectopic source and is due to stimulation of the ectopic ACTH-secreting tumor by desmopressin. This explains why in these five patients the stimulated peripheral ACTH rise could not be detected in the form of a positive IPS/P gradient. However, although this may well be the case for an adequately suppressed pituitary, a critical question is whether for an inadequately suppressed pituitary, as this occurs in those cases with periodic ectopic hypercortisolemia, a more potent stimulus may possibly lead to a higher rate of false-positive gradients. It is of note, however, that although intermittent ACTH secretion was documented in three of seven of our patients with oEAS, none of them displayed an IPS/P ratio of more than 2, when these patients were tested during a hypercortisolemic phase. Nevertheless, further study with a much greater number of patients with oEAS is still required to obtain more information with regard to the specificity of such an amplified stimulation during BIPSS and to give definitive recommendations regarding changes in the well-established ratios.

In addition to the high specificity of BIPSS in this series, we managed to obtain a remarkably high sensitivity in correctly diagnosing patients with a pituitary origin of ACTH hypersecretion. Thus, in our series, 46 of 47 patients (97.9%) with CD were correctly identified, leading to one of the lowest false-negative rate results reported so far. In fact with the exception of the very initial reports (1), additional experience with BIPSS revealed a substantial rate of false-negative results leading to varying degrees of incorrect diagnoses (18). This is particularly true in those patients with equivocal biochemical results to the less invasive tests employed in the differential diagnosis of CS. In these patients an inferior diagnostic accuracy of BIPSS has been reported (7, 27). Interestingly, Invitti *et al.* (8) analyzed a series of such patients and reported a decreased sensitivity for the CRH-stimulated gradient during BIPSS of only 76%. In contrast, in our study the use of the combined stimulation with CRH and desmopressin during BIPSS in a similar subgroup of patients with equivocal results correctly identified all the patients with CD, resulting in a sensitivity, specificity, and diagnostic accuracy of 100%. One may argue that the lower cutoff for the stimulated IPS/P gradients used in this study may of course have contributed to the higher sensitivity observed in this series. In fact by using the more stringent threshold of greater than 3, a lower sensitivity of 91.5 and 81.3% was obtained in the whole and the equivocal groups, respectively. Therefore, based on the fact that only the combination stimulus was used and there is no control with only CRH stimulation during BIPSS, we could not be sure that by using a cutoff ratio of 2 the combined procedure would outperform CRH stimulated BIPSS.

The explanation of how the combined stimulation en-

hances the sensitivity of the procedure is not straightforward because a number of reasons may account for false-negative results during BIPSS (28). For example, incorrect placement of catheters, anatomical abnormalities, the rare occurrence of a hypoplastic IPS (10), unusual location of the adenoma (29, 30), anomalous venous drainage or dilution of the petrosal venous blood (31, 32) have been reported as possible causes in some of the false-negative cases. It is most unlikely that such settings could be affected by the enhanced ACTH release produced by the combined regime. However, another reason of a false-negative outcome of BIPSS could be the lack of ACTH stimulation due to CRH unresponsiveness, which is encountered in 10–20% of patients with Cushing's disease (7, 11). Although in many of these patients a significant rise of ACTH during BIPSS with CRH stimulation is still observed, in others the expected rise in ACTH secretion may not be seen resulting in a false-negative BIPSS. Invitti *et al.* (8) reported that only two of 10 patients with CD who had failed to respond to CRH did not show a significant IPS/P gradient during BIPSS. However, it should be taken into account that in all series reported so far, the addition of CRH stimulation significantly increased the sensitivity of obtaining diagnostic IPS/P gradients. This is due to the fact that ACTH secretion from the neoplastic corticotrophs is rather intermittent and therefore inadequate to provide enough information in an unstimulated state. This suggests that any stimulation offers a diagnostic advantage. Therefore, a more intense stimulation by means of CRH and desmopressin may well be responsible for the observed diagnostic advantage, even in some CRH responder patients.

In conclusion, based on the data presented in this study, we suggest that the application of a combined stimulation with CRH plus desmopressin is associated with a high sensitivity but no loss of specificity. It is predicted that by using a more potent stimulus the number of false-negative patients will substantially decrease and this will lead to avoiding delays of curing these patients by transsphenoidal surgery. Also, the data of this study are encouraging that such an amplified stimulus will not lead to an inappropriate number of false-positive results. Nevertheless, despite the improved diagnostic characteristics of BIPSS by using a more intense stimulation, we still think that clinical judgment should remain the "gold standard" for the safety and benefit of our Cushing's patients.

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