0021-972X/00/$03.00/0
The Journal of Clinical Endocrinology & Metabolism
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Vol. 85, No. 4
Printed in U.S.A.

Bilateral Inferior Petrosal Sinus Sampling in the Differential Diagnosis of Adrenocorticotropin-Dependent Cushing's Syndrome: A Comparison with Other Diagnostic Tests

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ABSTRACT

To compare bilateral inferior petrosal sinus sampling (IPSS) with high dose dexamethasone (HDD) and CRH testing (using recently proposed stringent response criteria) in the differential diagnosis of ACTH-dependent Cushing's syndrome, we reviewed 53 consecutive cases. The main analysis was limited to 45 cases with confirmed diagnosis: 44 with pituitary dependency, proven by confirmatory histology and/or significant biochemical improvement after pituitary surgery, and 1 with ectopic ACTH syndrome. After HDD (2 mg every 6 h for 48 h), 21 of the 44 pituitary cases met the stringent more than 90% suppression criterion. Twenty-three of the 44 pituitary cases also underwent CRH testing; 16 of 23 met a stringent response criterion of a more than 50% serum cortisol rise. For HDD and CRH testing combined, 8 of 23 fulfilled both stringent criteria, 10 of 23 had discordant results, and 5 of 23 failed to fulfil either of the stringent criteria for pituitary dependency. IPSS was performed in all 44 of the proven pituitary cases; 36 had petrosal/peripheral ACTH ratios of 2.0 or more without CRH stimulation. Thus, in patients with proven pituitary disease, stringent response criteria to HDD and CRH testing were fulfilled by only 48% and 70%, respectively. IPSS, which gave direct evidence of pituitary ACTH secretion in 82% of the cases, is therefore considered necessary in a significant proportion of cases. (*J Clin Endocrinol Metab* **85:** 1525–1532, 2000)

IN THE DIFFERENTIAL diagnosis of Cushing's syndrome the most difficult distinction is between Cushing's disease and ectopic ACTH production (1–4). From a therapeutic point of view this distinction is essential so that patients with pituitary disease can be confidently referred for the treatment of choice, pituitary microsurgery, and patients with ectopic ACTH syndrome can be spared the risks of this procedure (5–8).

The most direct method of distinguishing between pituitary and ectopic ACTH secretion is bilateral inferior petrosal sinus sampling (IPSS) (9). The high diagnostic accuracy of this technique has led ourselves and others to consider it a routine investigation in the differential diagnosis of ACTH-dependent Cushing's syndrome (10–12). However, the procedure is not available at all centers (9), and although safe in experienced hands, significant complications have been reported (13).

For this reason, other indirect, noninvasive tests continue to have an important role (9, 14). Unfortunately, these tests have been associated with limited diagnostic accuracy, especially with regard to occult ectopic ACTH syndrome (15–17). For example, in one series, a quarter of patients with ectopic ACTH secretion responded to high dose dexamethasone with greater than 50% suppression of urinary 17-hydroxysteroids (15), whereas in another report, 2 of 14 patients with an ectopic source of ACTH produced a more than 20% rise in serum cortisol after CRH stimulation (17). Reliance on such tests has led to the inappropriate referral of a significant proportion of patients with ectopic ACTH secretion for pituitary surgery (15, 16). Given these difficulties, a number of indirect tests have been reevaluated, both alone and in combination, to address the overall accuracy of the noninvasive diagnostic approach (17–25).

In particular, attention has focused on new stringent diagnostic criteria designed to maximize specificity and thus avoid the misclassification and mismanagement of patients with ectopic ACTH syndrome (17, 22, 23). Flack and colleagues, for example, have proposed that during the high dose dexamethasone (HDD) test (*i.e.* 2 mg every 6 h for 48 h), more than 90% suppression of 24-h urinary free cortisol should be required for the diagnosis of pituitary disease to achieve 100% specificity (22). In their study use of the traditional greater than 50% suppression criterion would have misdiagnosed 4 of the 10 patients with ectopic ACTH secretion. In clinical practice 0800 h serum cortisol is often used instead of urinary free cortisol on the basis of limited evidence that suppression criteria established for urinary free cortisol can be extended to serum cortisol (18, 20, 21). Although suppression criteria to obtain 100% specificity with

Received May 14, 1999. Revision received December 29, 1999. Accepted January 11, 2000.

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the HDD test using serum cortisol as an end point have not been established, it seems appropriate to adopt the greater than 90% criterion proposed for urinary free cortisol by Flack *et al.* (22).

There are differing views on criteria for the diagnosis of pituitary-dependent disease after the CRH stimulation test (17–19, 25–28). A useful meta-analysis of all relevant data published before 1989 indicated that a serum cortisol response more than 20% above basal for the diagnosis of pituitary dependency would achieve a sensitivity of 91% and a specificity of 95% (29). However, it was suggested that a greater than 50% serum cortisol response would be required to exclude confidently ectopic ACTH syndrome (29).

Previous studies have shown that when the results of the CRH test are combined with those of a separately performed HDD test, the overall diagnostic performance exceeds that obtained with either test alone (18, 19, 25). No study has assessed the usefulness of this combined approach after the stringent diagnostic criteria outlined above have been applied to both tests.

We report our experience with 53 consecutive cases of ACTH-dependent Cushing's syndrome whose investigation included HDD testing (using 0800 h serum cortisol as the end point) and bilateral inferior petrosal sinus sampling. A number of patients also underwent CRH testing. The usefulness of the various diagnostic tests is examined along with the implications of applying the new stringent diagnostic criteria.

Subjects and Methods

Subjects

We reviewed the case records of 53 consecutive patients with ACTH-dependent Cushing's syndrome. Our unit provides a regional endocrine service for the whole of Northern Ireland, and the present series is therefore representative of all cases in a defined geographical area. The diagnosis of hypercortisolism was suggested by increased 24-h urinary free cortisol excretion and lack of suppression of serum cortisol during a low dose dexamethasone suppression test (0.5 mg every 6 h for 48 h) (30). All patients had detectable ACTH levels and thus underwent further investigation to determine the source of ACTH secretion. The final diagnoses were as follows. Forty-four patients had pituitary-dependent Cushing's disease established on the basis of histological confirmation or apparent cure or significant biochemical improvement after pituitary microsurgery. One patient had ectopic ACTH secretion from an oat cell carcinoma. In 8 patients the diagnosis remains unconfirmed. Four of these, for various reasons, had bilateral adrenalectomy performed in preference to pituitary surgery, 1 had medical therapy only, and 3 unconfirmed cases underwent pituitary microsurgery but had normal pituitary histology and showed no postoperative biochemical improvement. Our main analysis is based on the 44 confirmed cases of Cushing's disease and the 1 confirmed case of ectopic ACTH syndrome.

Bilateral inferior petrosal sinus sampling

This was attempted in all 53 cases in the present series as previously described (10), without the routine use of CRH stimulation. In 1 patient (case 44), CRH at a dose of 1 μg/kg was administered after obtaining basal samples, and further petrosal and peripheral samples were drawn after 1, 3, 5, 10, and 20 min. In another patient (case 39), bilateral inferior petrosal sinus sampling was initially performed without CRH stimulation and was then repeated using CRH as described.

Plasma ACTH (picograms per mL) was estimated by RIA as previously described (31). The coefficient of variation was 7.1% at 98 pg/mL (1 pg/mL = 0.23 pmol/L). Based on our early results from petrosal sinus sampling (10), we considered a ratio of the higher of the two petrosal sinus plasma ACTH levels to that of the peripheral venous plasma ACTH of 1.5 or greater as being consistent with pituitary-dependent hypercortisolism, but not diagnostic. In view of evidence from other series that patients with ectopic ACTH syndrome may occasionally have ratios between 1.5–2.0 (11), we used a ratio of 2.0 or more as our diagnostic cut-off. In each case the ratio obtained was interpreted alongside the actual data, with the variability of the ACTH assay in mind, to ensure that the ratio represented a meaningful difference in ACTH levels. Unless otherwise stated, all petrosal/peripheral ACTH ratios given are based on the higher of the two petrosal ACTH values.

HDD testing

Formal dexamethasone testing was carried out in 40 of the 45 patients with confirmed diagnosis and in the 8 patients in whom the diagnosis remains unconfirmed. Low dose dexamethasone was administered for 48 h (0.5 mg every 6 h), followed by high dose dexamethasone for 48 h (2 mg every 6 h). Serum cortisol was measured at 0800 h on day 1, just before the first 0.5-mg dose, and then at 0800 h daily until completion of the test (days 2–5). The percent suppression of serum cortisol was calculated as follows: % suppression = (basal value − final value)/basal value × 100. Our normal practice is to take the day 1 value as basal. In 3 of the 45 patients with confirmed diagnosis (2 with pituitary dependency and 1 with ectopic ACTH secretion) the HDD test was not directly preceded by a low dose test. An additional 2 patients (pituitary dependent) suppressed fully (serum cortisol undetectable) during preliminary low dose dexamethasone testing; therefore, the HDD test was deemed unnecessary (2). (Both of these patients had evidence of cyclical hypercortisolism.) In this analysis these 2 patients have been considered fully responsive to HDD. Serum cortisol was estimated by direct RIA as previously described (30).

CRH test

The response to ovine CRH was assessed in 23 of the 45 cases with confirmed diagnosis (including the case due to ectopic ACTH secretion) and in 4 of the cases with unconfirmed diagnosis. Ovine CRH was administered by iv bolus (1 μg/kg) at 1400 h, and serum cortisol was measured at the following times: −15, −5, 0, 5, 10, 15, 30, 60, 90, and 120 min. A maximal rise more than 50% above the basal average was taken as indicative of pituitary dependency (29).

Pituitary imaging

Forty-three of the 45 patients with confirmed diagnosis and all of the patients with unconfirmed diagnosis had fourth generation, thin cut pituitary computerized tomography (CT) performed. One patient (case 42) had magnetic resonance (MR) imaging (T1 weighted images after iv gadolinium administration) of the pituitary fossa performed instead of CT. The patient with ectopic ACTH syndrome did not have pituitary imaging.

Results

Results of pituitary histology, bilateral IPSS, and the other investigations in the 45 patients with confirmed diagnosis are summarized in Table 1. Twenty-five of the confirmed pituitary cases had corticotroph adenomas confirmed by histology, and 1 case had pituitary hyperplasia. In the remaining 19 pituitary cases, the diagnosis was based on apparent cure or significant biochemical improvement after pituitary surgery. Petrosal sinus sampling was performed in all 45 cases with confirmed diagnosis, and bilateral catheterization was successfully achieved in 38 of 45 (84%). In 6 cases only unilateral catheterization was possible, and in 1 case, a 10-yr-old boy, only the right jugular bulb could be catheterized (case 33). (Despite the suboptimal sampling site, a step-up in ACTH was observed, and his results have been included in the analysis). One patient developed a left groin hematoma after the procedure, but otherwise there were no complica-

TABLE 1. Pituitary histology, inferior petrosal sinus to peripheral serum ACTH ratios (without CRH stimulation), and serum cortisol responses to high dose dexamethasone and CRH testing in 45 patients with confirmed diagnosis

Case no.	ACTH source	Pituitary histology	Peripheral ACTH (pg/mL)	IPS/peripheral ACTH ratio		Suppression after high-dose dexamethasone		Cortisol rise after CRH test >50%	Adenoma on pituitary CT imaging
				Higher	Lower	>50%	>90%		
1	Pituitary	CA	42	2.9		Yes	No	No	Yes
2	Pituitary	CA	15	10.6		Yes	No	No	No
3	Pituitary	Normal	11	7.1	5.3	Yes	No	Yes	Yes
4	Pituitary	CA	55	2.2	1.4	Yes	No		No
5	Pituitary	Normal	45	1.5	1.2	Yes	Yes		Yes
6	Pituitary	Hyperplasia	72	1.3	1.1	Yes	Yes		No
7	Pituitary	CA	25	0.9	0.8	Yes	No	Yes	No
8	Pituitary	Normal	12	3.9	1.4	Yes	Yes		No
9	Pituitary	Normal	56	7.0	6.0	Yes	Yes		No
10	Pituitary	Normal	35	11.2	0.8	Yes	Yes		Yes
11	Pituitary	Normal	76	6.2	2.0	Yes	No	Yes	No
12	Pituitary	CA	69	2.6	1.2	Yes	No	No	Yes
13	Pituitary	CA	83	12.7	3.9	Yes	Yes	No	Yes[a]
14	Pituitary	Normal	46	1.5	1.3	Yes	Yes		Yes
15	Pituitary	CA	30	1.5	1.0	Yes	Yes		Yes
16	Pituitary	Normal	20	20.0	19.3	Yes[b]	Yes[b]	Yes	Yes
17	Pituitary	CA	56	2.8	1.0	Yes	No		Yes
18	Pituitary	Normal	85	3.1	0.9	Yes	Yes		No
19	Pituitary	Normal	132	8.6	6.0	Yes	No	Yes	No
20	Pituitary	CA	102	4.8	1.1	Yes	No		Yes
21	Pituitary	CA	77	3.7	1.2	Yes	Yes	Yes	Yes
22	Pituitary	CA	36	17.8	1.2	No	No		No
23	Pituitary	CA	35	16.4	15.5	Yes	No		Yes
24	Pituitary	Normal	60	12.0	1.3	Yes[c]	Yes[c]	Yes	No
25	Pituitary	Normal	46	11.1		Yes	No	Yes	No
26	Pituitary	CA	74	1.8	1.6	No	No	No	Yes[a]
27	Pituitary	Normal	54	1.8	1.2	Yes	No	Yes	No
28	Pituitary	CA	50	11.5	0.6	Yes	Yes	Yes	Yes
29	Pituitary	CA	165	25.5		Yes	No	Yes	No
30	Pituitary	Normal	15	5.1		Yes[b]	Yes[b]	Yes	No
31	Pituitary	CA	54	4.9	1.2	Yes	No		Yes
32	Pituitary	CA	35	7.9	0.9	Yes	Yes		No
33	Pituitary	CA	31	2.0[d]		No	No		Yes
34	Pituitary	CA	75	6.7	1.2	Yes	Yes		Yes
35	Pituitary	CA	128	4.3	3.8	Yes	Yes	Yes	Yes[a]
36	Pituitary	Normal	44	32.3	4.2	Yes	Yes		No
37	Pituitary	Normal	42	10.7	3.8	Yes[c]	No[c]		Yes
38	Pituitary	CA	102	5.8	1.1	Yes	No		No
39	Pituitary	CA	53	1.1[e]	1.0[e]	Yes	Yes	Yes	No
40	Pituitary	Normal	35	6.4	1.6	Yes	No	Yes	No
41	Pituitary	CA	54	10.0	1.1	Yes	No		No
42	Pituitary	CA	49	23.4	1.0	Yes	Yes	No	N/A[f]
43	Pituitary	CA	80	8.9	2.0	Yes	No	No	No
44	Pituitary	Normal	10	7.4[g]	1.3[g]	Yes	Yes	Yes	No
45	Ectopic	N/A	2565	0.7		No	No	No	N/A

IPS, Inferior petrosal sinus; CA, corticotroph adenoma.
[a] Macroadenoma.
[b] Suppressed to 30 nmol/L or less after low dose dexamethasone; full suppression to high dose dexamethasone (HDD) inferred.
[c] Separate HDD test (in other cases HDD test was part of formal 5-day test).
[d] Right jugular bulb.
[e] Repeated with CRH stimulation; on repeat, basal IPS/peripheral ratio was 11.6, rising to 39.0 after CRH.
[f] Microadenoma seen on MR scan.
[g] Basal values; after CRH, the maximum IPS/peripheral ratio was 10.0.

tions, and overall patient tolerance was good. Several patients described an awareness of noise after insertion of the catheter into the petrosal sinus. Of the 44 proven pituitary cases, 36 had petrosal/peripheral ACTH ratios of 2.0 or more (without CRH stimulation), giving a sensitivity of 82%. One of these 36 had CRH stimulation, after which the petrosal/peripheral ACTH ratio increased from 7.4 to 10.0 (case 44). Forty-one of the 44 pituitary-dependent cases (93%) had a petrosal/peripheral ACTH ratio of 1.5 or more.

Three patients with proven pituitary disease had a petrosal/peripheral ratio of 1.5 or less. The first of these had IPSS repeated with CRH stimulation (case 39); on repeat testing the ratio was 11.6 under basal conditions and rose to 39.0 after CRH administration. The second (case 6) had evidence of intermittent cortisol excess before sampling (for original description, see Ref. 10). In this patient serum cortisol was suppressed by 97% after HDD; at surgery no definite tumor was identified, and 70% of the pituitary was removed. His-

topathological examination demonstrated nodular pituitary hyperplasia with predominance of ACTH-secreting cells on immunochemistry. Serum cortisol levels remained variable postoperatively. The third case with a ratio less than 1.5 (case 7) achieved 90% suppression of serum cortisol in response to HDD, and CRH stimulation produced a 144% cortisol rise. Pituitary surgery resulted in apparent cure, and histopathological examination confirmed a basophil adenoma. The 1 case of ectopic ACTH secretion (case 45) had a petrosal/peripheral ACTH ratio of 0.7.

The results of HDD testing (Table 1) are illustrated in Fig. 1. Of the 44 proven pituitary cases, 41 met the traditional greater than 50% suppression criterion, but only 21 met the more stringent greater than 90% criterion. The patient with ectopic ACTH syndrome failed to suppress by either criterion.

Results from the CRH stimulation test (Table 1) are illustrated in Fig. 2. Sixteen of the 23 confirmed pituitary cases who had the test performed produced a serum cortisol rise exceeding 50%. The patient with ectopic ACTH secretion showed a flat cortisol response. Pituitary CT was performed in 43 of the 44 confirmed cases of Cushing's disease. A pituitary microadenoma was identified in 17 patients, and a macroadenoma was found in 3 patients, 1 of whom had evidence of suprasellar invasion (Table 1). In the remaining pituitary-dependent case a pituitary microadenoma was identified on MR imaging after gadolinium enhancement (case 42).

The efficacy of a combined diagnostic approach was evaluated in the 23 patients with confirmed pituitary-dependent Cushing's syndrome who underwent both HDD and CRH testing (Table 2). If a diagnosis of pituitary dependency was made on the basis of either a more than 90% suppression of serum cortisol after HDD, a greater than 50% cortisol rise after CRH, or both, then 78% sensitivity (18 of 23) was achieved. If, however, a positive response to both tests was required to make a confident diagnosis of pituitary disease, then only 35% (8 of 23) of pituitary-dependent patients were correctly diagnosed. Four of the 5 pituitary-dependent patients who had a negative response to both tests had a petrosal/peripheral ACTH ratio greater than 2.0 (cases 1, 2, 12, and 43 with ratios of 2.9, 10.6, 2.6, and 8.9, respectively), and the fifth (case 26) had a ratio of 1.8.

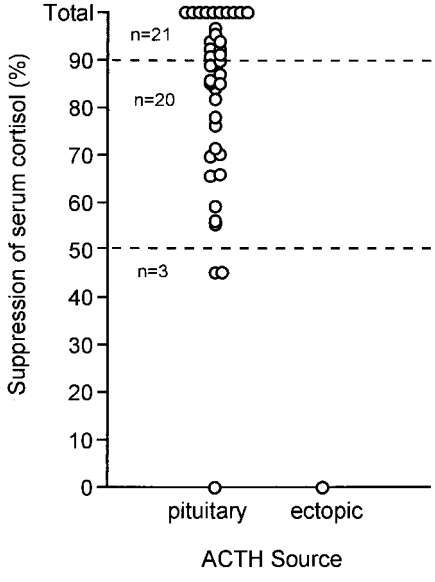

FIG. 1. Suppression of serum cortisol after HDD (2 mg every 6 h for 48 h) in 44 patients with confirmed pituitary-dependent Cushing's syndrome and 1 patient with ectopic ACTH secretion.

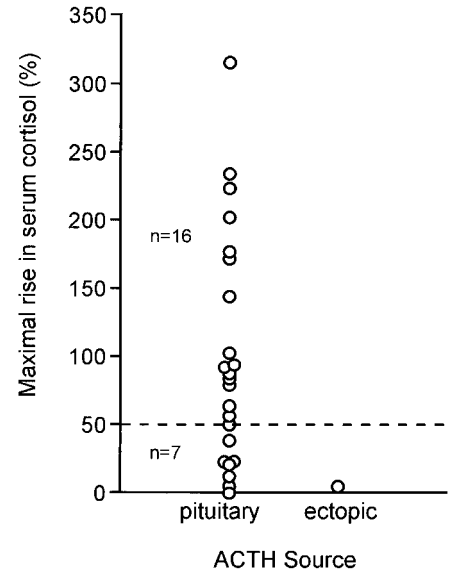

FIG. 2. Serum cortisol rise after CRH stimulation in 23 patients with pituitary-dependent Cushing's syndrome and 1 with ectopic ACTH secretion.

TABLE 2. Combined results of CRH and high dose dexamethasone tests in 23 confirmed pituitary cases who had both tests performed

Outcome	No. (%)
Positive to both tests	8/23 (35)
Positive response to one test only	10/23 (43)
Negative response to both tests	5/23 (22)

Positive response to high dose dexamethasone test defined as greater than 90% suppression of serum cortisol after dexamethasone (2 mg every 6 h for 48 h). Positive response to CRH test defined as maximal cortisol rise greater than 50% above basal after iv bolus of ovine CRH (1 μg/kg).

The results of diagnostic tests in patients with unconfirmed diagnoses are summarized in Table 3. During follow-up (as shown in Table 3), no evidence of an ectopic source of ACTH secretion emerged in any of these patients. Cases 46–48 were thought to have pituitary-dependent Cushing's syndrome, but pituitary surgery was unsuccessful and pituitary histology was negative. In case 46, hypophysectomy was complicated by excessive bleeding, and bilateral adrenalectomies were subsequently performed, the histology of which showed bilateral hyperplasia. This patient died of an acute myocardial infarction after 6 yr of follow-up. In case 47, only a small volume of pituitary tissue was present in the sella turcica. She, too, had bilateral adrenalectomies, and histological examination confirmed bilateral hyperplasia. In case 48 the first attempt at pituitary surgery was abandoned due to hypoxia and unusual anatomy. At a repeat procedure 70% of the pituitary was successfully removed,

TABLE 3. Results of diagnostic tests in patients with unconfirmed diagnosis

Case no.	Age (yr) and sex	Peripheral ACTH (pg/mL)	IPS/peripheral ACTH ratio		Suppression after high-dose dexamethasone		Cortisol rise after CRH test >50%	Adenoma on pituitary CT imaging	Follow-up (yr)
			Higher	Lower	>50%	>90%			
46	60/F	13	3.4	0.8	Yes	No	No	No	6
47	42/F	34	9.1	1.2	Yes	No		No	10
48	65/F	46	1.9	1.3	Yes	No	No	No	4 months
49	55/F	22	1.6	1.0	No	No		Yes	13
50	35/M	51	1.1	1.0	Yes	Yes		Yes	13
51	52/F	26	NA[a]	NA[a]	Yes	No	No	No	4
52	61/F	29	1.0		Yes	No		No	10
53	15/M	43	NA[b]	NA[b]	Yes	Yes	No	No	4

[a] Peripheral sample unavailable.
[b] Patient with cyclical Cushing's syndrome; peripheral and central ACTH levels undetectable at time of sampling.

but this was complicated by postoperative meningitis. She died of bronchopneumonia 3 months after surgery. At autopsy, no residual adenoma was identified in the pituitary gland, nor was an occult source of ACTH secretion found.

For various reasons cases 49–53 were not referred for pituitary surgery. In case 49, HDD testing and IPSS were not diagnostic of pituitary dependency despite the appearance on CT scanning of a 6- to 7-mm pituitary adenoma. CT scan of adrenals showed massive bilateral enlargement; this appearance was consistent with cortical adenomas. Bilateral adrenalectomies were performed, and histological examination showed bilateral cortical nodular hyperplasia. Case 50 had severe osteoporosis at presentation, and bilateral adrenalectomy was believed to be the treatment of choice. Histological examination showed bilateral adrenal hyperplasia. In case 51, HDD testing produced 62% suppression of serum cortisol, and CRH administration led to a 9% rise in serum cortisol. On CT scanning, the pituitary fossa and chest were normal, but the adrenal glands were bilaterally enlarged, with the suspicion of a small adenoma on the left side. IPSS was performed, but unfortunately only samples from the right jugular bulb could be obtained, and peripheral ACTH levels were unavailable (samples presumed to be lost in transit). Given the diagnostic uncertainty, bilateral adrenalectomies were performed, and histology confirmed bilateral cortical nodular hyperplasia. In case 52, HDD produced 68% suppression of serum cortisol, but IPSS was not diagnostic of pituitary dependency. CT scans of pituitary fossa and chest were normal, whereas CT scanning of the adrenal gland was suggestive of a small left-sided adrenal adenoma. Bilateral adrenalectomies were performed, and pathology showed bilateral pigmented nodules, the histological appearance of which was consistent with micronodular adrenocortical disease. Finally, case 53 is a young boy with learning difficulties (that predate his features of Cushing's syndrome) who has cyclical hypercortisolism. IPSS was not helpful, as both central and peripheral ACTH levels were undetectable at the time of sampling. After discussion with his parents, it was decided to control his hypercortisolism with metyrapone.

Discussion

Most patients with ACTH-dependent Cushing's syndrome have Cushing's disease (9). The object of further investigation is to identify those few patients with ectopic ACTH secretion, especially those with an occult tumor, in whom the clinical features may be indistinguishable from those of Cushing's disease (32). Tests used for the diagnosis of pituitary dependency, therefore, must be highly specific if they are to be of discriminatory value. To this end, more stringent diagnostic criteria have been suggested for several of the standard tests used in this situation (17, 22, 23, 29). As the ectopic ACTH syndrome is rare, these criteria are necessarily derived from data from very large series or from meta-analyses. In the present analysis of 45 patients with ACTH-dependent Cushing's syndrome with confirmed diagnosis, there was only 1 case of ectopic ACTH secretion. We are not therefore in a position to comment upon the specificity of the diagnostic tests used, but, rather, our analysis focuses upon the sensitivities of the various tests, with possible implications for their relative roles, when the more stringent criteria are applied to both the HDD suppression test and the CRH stimulation test.

In the present series of 53 cases, the diagnosis remains unconfirmed in 8 patients (15%). This is in keeping with a previous series in which 32 of 281 cases were undiagnosed (11%) even after biochemical testing and IPSS (11). Although such patients represent an important subgroup in clinical practice, we have not included them in our main analysis as we believe that the sensitivity of diagnostic tests is most reliably calculated in those patients with confirmed diagnosis. This is similar to the approach taken in previous series (11, 17, 18). It must be remembered that in patients with occult ectopic ACTH syndrome, there is often an interval of several years from the clinical diagnosis of hypercortisolism until the appearance of the underlying ACTH-secreting tumor (16).

In 44 patients with confirmed Cushing's disease, bilateral IPSS without CRH stimulation yielded a petrosal/peripheral ACTH ratio of 2.0 or more in 36 (82%). After HDD, serum cortisol was suppressed by more than 90% in only 21 of 44 (48%). Of 23 proven pituitary cases who had a CRH test, 16 (70%) produced a cortisol rise of greater than 50%. Pituitary CT scanning showed evidence of a pituitary lesion in 20 of 43 (47%). This is in accordance with previous series that reported detection of pituitary adenomas on CT scan in 30–53% of patients with Cushing's disease (33, 34). With MR imaging higher detection rates have been reported, partic-

ularly when iv contrast enhancement is used (34–37). However, as pituitary microadenomas are relatively common in the general population (36, 38), it is generally agreed that pituitary imaging is of only limited diagnostic value in this situation (9, 14); it is the most useful if there is clear evidence of a pituitary macroadenoma, a situation seen in only 20% of cases of Cushing's disease (8). Although pituitary imaging has only a small role to play in deciding whether to proceed to pituitary surgery (9), there is little doubt that MR imaging is the most accurate method of tumor localization before surgery (9, 37).

There is now substantial evidence that bilateral IPSS is the most reliable method of distinguishing between pituitary and nonpituitary ACTH excess (11, 14). Indeed, when combined with CRH stimulation, a diagnostic accuracy of 100% has been reported in a series of 220 patients, including 17 with ectopic ACTH syndrome (11). Although in the past we have not routinely used CRH stimulation, we now advocate this approach, in keeping with most other centers (11, 14). Obviously, the present analysis reflects our previous experience using IPSS without CRH stimulation and to some extent underestimates the current usefulness of the procedure when CRH is routinely used.

Even with CRH stimulation, the results of IPSS can be misleading in certain unusual situations. Two cases of proven ectopic ACTH syndrome have been reported in whom false positive results occurred after CRH stimulation (39). Periodic hypercortisolism was documented in each, and it was suggested that the patients may not have been hypercortisolemic at the time of sampling, emphasizing that petrosal sinus sampling is only a useful discriminatory test in the presence of hypercortisolism (40).

The need for simultaneous bilateral sampling has previously been emphasized (10) and is supported by the data from the present analysis. Of the 38 patients with proven pituitary disease who had bilateral sampling successfully performed, 27 had a lower petrosal/peripheral ACTH ratio of less than 2.0. It is recognized, however, that even in the most experienced hands, bilateral catheterization is not always possible (11). Results from unilateral sampling are only of diagnostic value if an elevated petrosal/peripheral ratio is found. Similarly, the need for sampling higher than that in the jugular bulb has long been recognized, as blood from the jugular bulb is admixed with blood from different areas of the brain (41). In a young boy from our series (case 33) sampling was only possible from the right jugular bulb due to the patient's age and sinus development. In that case the ratio of jugular bulb to peripheral ACTH was 2.0. If anything, one might have expected a higher ratio had the inferior petrosal sinuses been sampled, and his results were therefore interpreted as indicative of pituitary disease.

For many years the HDD suppression test has retained a central role in the differential diagnosis of Cushing's syndrome (9, 14, 42), and traditionally greater than 50% suppression of urinary 17-hydroxysteroid after HDD for 48 h has been taken as evidence of pituitary disease (2). This suppression criterion has subsequently been extended to urinary free cortisol (43, 44) and serum cortisol (18, 20, 21). Although this provides a sensitive test for the diagnosis of pituitary dependency (93% sensitivity in the present series), it is clear from previous reports that patients with ectopic ACTH syndrome may achieve greater than 50% suppression of serum cortisol after HDD (20, 21). In the absence of specific criteria for serum cortisol suppression after HDD to achieve 100% specificity, we have used the greater than 90% suppression criterion developed for urinary free cortisol (22). Given limited evidence that the suppressibility of serum cortisol in response to HDD may be slightly less than that of urinary free cortisol (21), it is likely that the new 90% criterion established for urinary free cortisol can be extended to serum cortisol without loss of test specificity.

Although various centers, including our own, routinely use IPSS in the differential diagnosis of ACTH-dependent Cushing's syndrome (10–12), others advocate a more selective use of IPSS, based on the results of biochemical and radiological investigations (37). For example if a patient with ACTH-dependent Cushing's syndrome has a clearly defined adenoma on CT or MR scanning, suppresses on HDD testing by 50% or more, and has no evidence of a neoplasm on chest x-ray, many physicians may consider referral for pituitary surgery without IPSS. Had we used such an approach, based on pituitary imaging and HDD testing, 19 of the 44 proven pituitary cases (43%) would have been diagnosed without the need for IPSS. However, any reduction in the number of cases requiring IPPS must be balanced against the small, but significant, risk of failing to diagnose ectopic ACTH syndrome and inappropriate referral of such patients for pituitary surgery. Given the diagnostic limitations of both pituitary imaging and traditional HDD testing already discussed, we do not recommend that patients be referred for pituitary surgery on the basis of these tests alone.

If IPSS is to be avoided, then whatever diagnostic strategy is used in its place should be highly specific so that the small number of patients with ectopic ACTH secretion are accurately diagnosed. One approach might be to employ a combination of biochemical tests using stringent diagnostic criteria to achieve 100% specificity. In the present analysis 23 patients with proven pituitary disease underwent both HDD and CRH testing. Using stringent response criteria for the diagnosis of pituitary dependency, the sensitivity of the 2 tests combined was 78% (18 of 23) if a positive response to 1 or other of the tests (or both) is taken as evidence of pituitary disease. If these criteria were 100% specific, then a positive response to either test would completely exclude the possibility of ectopic ACTH secretion and obviate the need for IPSS. Unfortunately, the evidence to date does not permit this degree of confidence. Although the data used to develop the stringent response criteria were based on large numbers of patients, the actual numbers with ectopic ACTH secretion were still relatively small (22, 29). Since publication of the greater than 90% suppression criterion for urinary free cortisol (22), at least 1 patient with an ACTH-secreting bronchial carcinoid tumor has been reported in whom urinary free cortisol was suppressed by more than 90% after HDD (14). With regard to the CRH test, a separate patient with ectopic ACTH secretion has been noted to produce a greater than 50% cortisol rise in response to CRH (17). It seems only a matter of time before more such cases are reported. Based on

the evidence available, we believe that reliance on 1 indirect test (even with the stringent response criteria described) does not afford the degree of confidence required to refer a patient with ACTH-dependent Cushing's syndrome for pituitary surgery.

A more cautious approach might be to insist that stringent criteria for both the HDD test and the CRH test are fulfilled before a confident diagnosis of pituitary dependency is made. In the present series this would have identified only 35% (8 of 23) of proven pituitary cases. The chance of a patient with ectopic ACTH secretion surpassing both of the stringent response criteria would appear small, and it could be argued that further testing in such cases would be unnecessary. However, bilateral IPSS would be required to establish the diagnosis in the remaining 65%. It has been suggested previously that when the HDD and CRH tests are combined, only cases with discordant results require further evaluation to exclude ectopic ACTH secretion (19). Clearly, when stringent response criteria are applied, such recommendations are no longer appropriate, as in the present study a significant number of cases with proven pituitary disease had concordant negative results (5 of 23).

In conclusion, bilateral inferior petrosal sinus sampling without CRH stimulation was diagnostic of pituitary dependency in 82% of confirmed cases of Cushing's disease. The procedure was safe and well tolerated. If the procedure is not to be performed in all cases, then it is essential that stringent response criteria are applied to whatever alternative tests are used, and preferably a positive response to more than one indirect test should be sought before referring a patient for surgery. This means that a significant proportion of patients will still require petrosal sinus sampling.

Acknowledgments

We thank Sister R. Humphries and the staff of the Metabolic Unit for their assistance.

References

1. **Aron DC, Tyrell JB, Fitzgerald PA, Findling JW, Forsham PH.** 1981 Cushing's syndrome: problems in diagnosis. Medicine. 60:25–35.
2. **Crapo L.** 1979 Cushing's syndrome: a review of diagnostic tests. Metabolism. 28:955–977.
3. **Findling JW.** 1990 Eutopic or ectopic adrenocorticotropic hormone-dependent Cushing's syndrome? A diagnostic dilemma. Mayo Clin Proc. 65:1377–1380.
4. **Trainer PJ, Grossman A.** 1991 The diagnosis and differential diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf). 34:317–330.
5. **Atkinson AB.** 1989 1989 Cushing's syndrome: a review of current diagnostic procedures and management. Saudi Med J. 10:89–93.
6. **Atkinson AB.** 1991 Management of Cushing's syndrome. Clin Endocrinol (Oxf). 34:507–514.
7. **Melby JC.** 1989 Therapy of Cushing's disease: a consensus for pituitary microsurgery. Ann Intern Med. 109:445–446.
8. **Bochicchio D, Losa M, Buchfelder M, European Cushing's Disease Survey Study Group.** 1995 Factors influencing the immediate and late outcome of Cushing's disease treated by transsphenoidal surgery: a retrospective study by the European Cushing's Disease Survey Group. J Clin Endocrinol Metab. 80:3114–3120.
9. **Orth DN.** 1995 Cushing's syndrome. N Engl J Med. 332:791–803.
10. **McCance DR, McIlrath E, McNeill A, et al.** 1989 Bilateral inferior petrosal sinus sampling as a routine procedure in ACTH-dependent Cushing's syndrome. Clin Endocrinol (Oxf). 30:157–166.
11. **Oldfield EH, Doppman JL, Nieman LK, et al.** 1991 Petrosal sinus sampling with and without corticotropin-releasing hormone for the differential diagnosis of Cushing's syndrome. N Engl J Med. 325:897–905.
12. **Findling JW, Kehoe ME, Shaker JL, Raff H.** 1991 Routine inferior petrosal sinus sampling in the differential diagnosis of adrenocorticotropin (ACTH)-dependent Cushing's syndrome: early recognition of the occult ectopic ACTH syndrome. J Clin Endocrinol Metab. 73:408–413.
13. **Miller DL, Doppman JL, Peterman SB, Nieman LK, Oldfield EH, Chang R.** 1992 Neurologic complications of petrosal sinus sampling. Radiology. 185:143–147.
14. **Findling JW, Doppman JL.** 1994 Biochemical and radiological diagnosis of Cushing's syndrome. Endocrinol Metab Clin North Am. 3:511–537.
15. **Jex RK, van Heerden JA, Carpenter PC, Grant CS.** 1985 Ectopic ACTH syndrome. Diagnostic and therapeutic aspects. Am J Surg. 149:276–282.
16. **Findling JW, Tyrell JB.** 1986 Occult ectopic secretion of corticotrophin. Arch Intern Med. 146:929–933.
17. **Nieman LK, Oldfield EH, Wesley R, Chrousos GP, Loriaux DL, Cutler Jr GB.** 1993 A simplified morning ovine corticotropin-releasing hormone stimulation test for the differential diagnosis of adrenocorticotropin-dependent Cushing's syndrome. J Clin Endocrinol Metab. 77:1308–1312.
18. **Grossman AB, Howlett TA, Perry L, et al.** 1988 CRF in the differential diagnosis of Cushing's syndrome: a comparison with the dexamethasone suppression test. Clin Endocrinol (Oxf). 29:167–178.
19. **Nieman LK, Chrousos GP, Oldfield EH, Avgerinos PC, Cutler Jr GB, Loriaux L.** 1986 The ovine corticotropin-releasing hormone stimulation test and the dexamethasone suppression test in the differential diagnosis of Cushing's syndrome. Ann Intern Med. 105:862–867.
20. **Howlett TA, Drury PL, Perry L, Doniach I, Rees LH.** 1986 Diagnosis and management of ACTH-dependent Cushing's syndrome: comparison of the features in ectopic and pituitary ACTH production. Clin Endocrinol (Oxf). 24:699–713.
21. **Blunt SB, Sandler LM, Burrin JM, Joplin GF.** 1990 An evaluation of the distinction of ectopic and pituitary ACTH dependent Cushing's syndrome by clinical features, biochemical tests and radiological findings. Q J Med. 77:1113–1133.
22. **Flack MR, Oldfield EH, Cutler Jr GB, et al.** 1992 Urine free cortisol in the high-dose dexamethasone suppression test for the differential diagnosis of the Cushing's syndrome. Ann Intern Med. 116:211–217.
23. **Averingos PC, Tanovski JA, Oldfield EH, Nieman LK, Cutler Jr GB.** 1994 The metyrapone, and dexamethasone suppression test for the differential diagnosis of the adrenocorticotropin-dependent Cushing's syndrome: a comparison. Ann Intern Med. 121:318–327.
24. **Dichek HL, Nieman LK, Oldfield EH, Pass HI, Malley JD, Cutler Jr GB.** 1994 A comparison of the standard high dose dexamethasone suppression test, and the overnight 8-mg dexamethasone suppression test for the differential diagnosis of adrenocorticotropin-dependent Cushing's syndrome. J Clin Endocrinol Metab. 78:418–422.
25. **Hermus AR, Pieters GF, Pesman GJ, Smals AG, Benraad TJ, Kloppenborg PW.** 1986 The corticotropin-releasing-hormone test *vs.* the high-dose dexamethasone test in the differential diagnosis of Cushing's syndrome. Lancet. 2:540–544.
26. **Muller OA, Stalla GK, von Werder K.** 1983 Corticotropin-releasing factor: a new tool for the differential diagnosis of Cushing's syndrome. J Clin Endocrinol Metab. 57:227–229.
27. **Chrousos GP, Heinrich MS, Oldfield EH, Gold PW, Cutler Jr GB, Loriaux DL.** 1984 The corticotropin-releasing factor stimulation test. An aid in the evaluation of patients with Cushing's syndrome. N Engl J Med. 310:622–626.
28. **Nieman LK, Cutler Jr GB, Oldfield EH, Loriaux DL, Chrousos GP.** 1989 The ovine corticotropin-releasing hormone (CRH) stimulation test is superior to the human CRH stimulation test for the diagnosis of Cushing's disease. J Clin Endocrinol Metab. 69:165–169.
29. **Kaye TB, Crapo L.** 1990 The Cushing syndrome: an update on diagnostic tests. Ann Intern Med. 112:434–444.
30. **Kennedy L, Atkinson AB, Johnston H, Sheridan B, Hadden DR.** 1984 Serum cortisol concentrations after low dose dexamethasone suppression to screen for Cushing's syndrome. Br Med J. 289:1188–1191.
31. **Atkinson AB, Chestnutt A, Crothers E, Woods R. Weaver JA, Kennedy L. Sheridan B.** 1985 Cyclical Cushing's disease: two distinct rhythms in a patient with a basophil adenoma. J Clin Endocrinol Metab. 60:328–332.
32. **Wajchenberg BL, Mendonca BB, Liberman B, et al.** 1994 Ectopic adrenocorticotropic hormone syndrome. Endocr Rev. 15:752–787.
33. **Saris SC, Patronas NJ, Doppman JL, et al.** 1987 Cushing's syndrome: pituitary CT scanning. Radiology. 162:775–777.
34. **Buchfelder M, Nistor R, Fahlbusch R, Huk WJ.** 1993 The accuracy of CT and MR evaluation of the sella turcica for detection of adrenocorticotrophic hormone-secreting adenoma in Cushing's disease. Am J Neuroradiol. 14:1183–1190.
35. **Colombo N, Lolo P, Vignati F, Scialfa G.** 1994 MR of corticotrophin-secreting pituitary microadenomas. Am J Neuroradiol. 15:1591–1595.
36. **Hall WA, Luciano MG, Doppman JL, Patronas NJ, Oldfield EH.** 1994 Pituitary magnetic resonance imaging in normal human volunteers: occult adenomas in the general population. Ann Intern Med. 120:817–820.
37. **de Herder WW, Uitterlinden P, Pieterman H, et al.** 1994 Pituitary tumour localization in patients with Cushing's disease by magnetic resonance imaging. Is there a place for petrosal sinus sampling? Clin Endocrinol (Oxf). 40:87–92.

38. **Kontogeros G, Kovacs K, Horvath E, Scheithauer BW.** 1991 Multiple adenomas of the human pituitary. A retrospective autopsy study with clinical implications. J Neurosurg. 74:243–247.
39. **Yamamoto Y, Davis DH, Nippoldt TB, Young WF, Houston III J, Parasi JE.** 1995 False-positive inferior petrosal sinus sampling in the diagnosis of Cushing's disease. Report of two cases. J Neurosurg. 83:1087–1091.
40. **Yanovski JA, Cutler Jr GB, Doppman JL, et al.** 1993 The limited ability of inferior petrosal sinus sampling with corticotropin-releasing hormone to distinguish Cushing's disease from pseudo-Cushing states and normal physiology. J Clin Endocrinol Metab. 77:503–509.
41. **Findling JKW, Aron DC, Tyrell JB, et al.** 1981 Selective venous sampling for ACTH in Cushing's syndrome: differentiation between Cushing's disease and the ectopic ACTH syndrome. Ann Intern Med. 96:647–652.
42. **Liddle GW.** 1960 Tests of pituitary adrenal suppressibility in the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab. 20:1539–1560.
43. **Eddy R, Jones A, Lloyd G, et al.** 1973 Cushing's syndrome: a prospective study of diagnostic methods. Am J Med. 55:621–629.
44. **Burke CW, Beardwell CG.** 1973 Cushing's syndrome. A evaluation of the clinical usefulness of urinary free cortisol and other urinary steroid measurements in diagnosis. Q J Med. 42:175–204.