Diagnostic Errors after Inferior Petrosal Sinus Sampling

BROOKE SWEARINGEN, LAURENCE KATZNELSON, KAREN MILLER, STEVEN GRINSPOON, ARTHUR WALTMAN, DAVID J. DORER, ANNE KLIBANSKI, AND BEVERLY M. K. BILLER

Neuroendocrine Clinical Center (B.S., L.K., K.M., S.G., A.K., B.M.K.B.), Division of Neurosurgery (B.S.), Biostatistics Center (D.J.D.), and Department of Radiology (A.W.), Massachusetts General Hospital, Boston, Massachusetts 02114

Although inferior petrosal sinus sampling (IPSS) is useful in the evaluation of Cushing's syndrome, false negative cases have been described, and many patients presumed to have ectopic tumors based upon negative IPSS remain without a final diagnosis. These patients are often managed as if they have as yet undiscovered ectopic tumors. To test this assumption, we conducted a retrospective review of our results to determine the ultimate diagnoses after IPSS. Between 1986 and 2002, 179 patients underwent 185 IPSS procedures as part of their evaluation for Cushing's syndrome. Confirmed diagnoses were available for 149 patients (83%): 139 patients had pituitary adenomas (94%), eight had bronchial carcinoids (5%), and two had adrenal tumors (1%). Threshold criteria for a pituitary source were defined as an inferior petrosal sinus to peripheral (IPS:P) basal ratio of 2:1 or greater without CRH or an IPS:P ratio of 3:1 or greater after CRH stimulation. There were nine patients in whom the IPS:P ratio failed to meet threshold criteria after successful sampling, but were nonetheless found to have pituitary tumors after transsphenoidal exploration (false negatives). Eight of these had received CRH and had a significant rise (>35%) in peripheral ACTH levels after CRH treatment, even though the IPS:P ratio did not reach the threshold. There were two patients in whom the IPS:P ratio reached threshold criteria, and ectopic tumors were demonstrated as the source of ACTH overproduction (false positives). The sensitivity after CRH stimulation was 90% (95% confidence interval, 80.8–95.5%) with a specificity of 67% (95% confidence interval, 11.4–94.5%). The positive and negative predictive values were 99 and 20%, respectively. Our data show that patients with an IPS:P ratio suggestive of a nonpituitary source of ACTH overproduction may still have Cushing's disease. Analyzing the CRH-stimulated peripheral ACTH levels in addition to the standard IPS:P ratios may provide improved diagnostic accuracy. Transsphenoidal exploration should be considered in all cases of unsuccessful sampling and in those cases for which no ectopic source can be identified after further body imaging, even if the IPSS is negative, and especially if peripheral ACTH levels rise significantly with CRH stimulation. (J Clin Endocrinol Metab 89: 3752–3763, 2004)

Since its introduction more than 20 yr ago, inferior petrosal sinus sampling (IPSS) has become widely used as part of the diagnostic evaluation of patients with Cushing's syndrome (1, 2). Early major series reported the procedure to be 100% sensitive and specific for the diagnosis of pituitary-dependent Cushing's disease when using threshold central: peripheral ACTH ratios of 3:1 after the administration of ovine CRH (3, 4). With additional experience, false negative results were described, initially on the basis of variants in the venous anatomy (5). Later studies described additional patients who were found to have pituitary Cushing's disease despite IPSS results indicating an ectopic source and attempted to determine the optimum inferior petrosal sinus to peripheral (IPS:P) ratio to maximize sensitivity and specificity (6–8). Although most patients with noncentralizing IPS:P ratios were found to harbor ectopic sources of ACTH production, in a number of cases the site of the presumed ectopic source was never found. These patients are usually excluded from further analysis and categorized as having as yet undiscovered ectopic tumors. To test this assumption, we reviewed our results to determine the ultimate diagnostic outcome in those patients whose IPS sampling indicated a peripheral source. In addition, false positive results are rarely reported, and we also reviewed the final diagnostic outcomes to determine the incidence of incorrect positive test results in our series.

Subjects and Methods

Patient population

Hospital records were reviewed for all patients undergoing IPSS at a single tertiary care institution between 1986 and 2002. Record review was approved by the hospital institutional review board. Mortality data and surgical results for a subset of these patients have been previously published (9).

Endocrine evaluation

All patients had undergone extensive clinical evaluation for Cushing's syndrome, with the initial testing often performed by the referring physician. Before proceeding to IPSS, patients were confirmed to have Cushing's syndrome by demonstration of an elevated 24-h urinary free cortisol (UFC) level and at least one other confirmatory test (a second clearly elevated UFC, nonsuppression after 2 d of low dose dexamethasone, or a combined low dose dexamethasone-CRH test.) Serum ACTH levels were nonsuppressed. Hypercortisolism was proven on the day of IPS sampling in the majority of subjects by collection of a 24-h urine sample for UFC, concluding on the morning of the sampling procedure. One patient presented with recurrent hypercortisolism after previous bilateral adrenalectomy. No patient was taking any medication to block cortisol production at the time of the procedure.

IPSS technique

The sampling procedure was performed as described by Doppman et al. (2) by a single group of vascular radiologists. Correct catheter
placement within the petrosal sinus was confirmed by venous angiography before sampling, and intermittent fluoroscopy was performed during sampling to confirm that the catheters had not dislodged. Cannulation of the IPS was performed after systemic heparinization. Satisfactory catheter placement was defined by the cannulation of both inferior petrosal sinuses, as demonstrated by retrograde flow of contrast into the cavernous sinuses after dye injection. Placement of the catheters at the os of the IPS, without direct cannulation, was considered technically unsatisfactory. In 64 procedures CRH was not available, and five timed samples for ACTH determination were simultaneously drawn from the inferior petrosal sinuses and periphery at 5-min intervals. In 121 procedures ovine CRH was administered at a dose of 1 μg/kg to a maximum dose of 100 μg after bilateral cannulation and baseline sampling for ACTH had been performed. Samples were drawn simultaneously from both petrosal sinuses and the periphery at 2–3, 5, 10, and 15 min after the injection of CRH. The samples were drawn into prechilled tubes in an ice bath and hand-delivered to the endocrine laboratory for assay immediately at the conclusion of the procedure.

**IPSS analysis**

The procedures were considered either successful or unsuccessful, and results from unsuccessful procedures were analyzed separately. Successful IPSS procedures were defined as those in which 1) technically satisfactory cannulation of both inferior petrosal sinuses was achieved, and simultaneous bilateral samples were obtained; 2) there was normal venous anatomy; and 3) sample handling and analysis were performed according to protocol. Maximal IPS:P ratios were calculated for three groups: 1) using ACTH levels from those procedures in which CRH stimulation was not administered at any time, and only unstimulated ACTH values were available; 2) using prestimulation ACTH levels from those procedures in which baseline ACTH levels were obtained before CRH stimulation; and 3) using poststimulation ACTH levels from procedures in which CRH was administered. A central pituitary source for ACTH overproduction was presumed if the IPS:P ratio was 2:1 or greater and the test was considered negative for pituitary disease. For those cases in which a peripheral source was predicted, but a pituitary tumor was subsequently found (false negatives), the venous angiograms taken at the time of the original procedure were reviewed by an independent radiologist to confirm correct catheter placement. Sensitivity and specificity were calculated for each group, and statistical calculations were made using the R statistical package (10). The receiver operating characteristic (ROC) package for Splus from Mayo Clinic Biostatistics was used for ROC plotting and calculations (11).

**ACTH assay**

ACTH determinations were made by extraction RIA for the 12 procedures between 1986 and 1988, and by immunoradiometric assay for the remainder performed between 1989 and 2002. The interassay variability ranged from 8–10%, with an intraassay variability of 3–5%.

**Imaging**

Radiographic interpretations of the pituitary magnetic resonance imaging (MRI) from both the radiologist’s report (without knowledge of IPSS outcome) and the surgeon’s records (usually after IPSS results were available) were analyzed. These reports were based primarily upon coronal and sagittal T1-weighted images before and after the administration of gadolinium. Based upon these reports, the studies were categorized as: negative, no tumor seen; positive, the presence of an adenoma was thought to be highly likely; or suspicious, no tumor was clearly seen, but circumstantial evidence (a relative hypodensity within the gland, stalk deviation, or gland asymmetry) suggested that an adenoma was present. No patients with macroadenomas underwent IPSS, because these patients were referred directly for transphenoidal surgery, but all patients with microadenomas (<1 cm) seen on MRI were routinely referred for IPSS testing in an attempt to obtain biochemical confirmation of a pituitary source.

**Percent increase in peripheral ACTH levels after CRH stimulation**

In those 121 procedures in which CRH stimulation was used, the percent increase between pre- and post-CRH-stimulated peripheral ACTH levels was determined to simulate a true CRH stimulation test. The percent increase in baseline ACTH was calculated using the mean of the two pre-CRH peripheral ACTH values compared with the mean of the 10- and 15-min post-CRH peripheral ACTH levels, because no later ACTH levels were available.

**Diagnostic categorization**

Patients were categorized by their diagnosis at the time of last follow-up. The diagnosis was considered consistent with a pituitary source if the patient was cured by transphenoidal surgery, if an ACTH-staining pituitary adenoma was proven pathologically although the patient remained uncured, or if the patient was cured after adjunctive pituitary radiation therapy even if no pathological confirmation was ever obtained. Surgical cure was defined as profound postoperative hypoadrenalinism. An ectopic source was listed as such only if confirmed pathologically. If the patient was treated based upon IPSS results, but no tissue diagnosis was ever obtained, and follow-up was available, the patient was considered presumed pituitary or presumed ectopic. These cases were considered as unknowns for the purposes of statistical analysis. If the patient returned to the referring institution after IPSS sampling and no follow-up was available, he/she was listed as unknown.

**Results**

Between 1986 and 2002 179 patients underwent 185 IPSS procedures as part of their evaluation for Cushing’s syndrome. Six patients underwent two procedures. The female: male ratio was 3.4:1. The mean age was 39 yr (range, 8–75 yr). The final diagnosis was available in 149 patients. Successful bilateral sampling was achieved in 145 procedures (in 143 patients; 78% of procedures); the 40 procedures (in 38 patients; 22% of procedures) in which sampling was unsuccessful were analyzed separately. (Two patients each had one successful and one unsuccessful procedure and are included in both groups.)

**IPS:P ratios in successful procedures without CRH stimulation**

Of the 145 successful procedures, there were 50 in which CRH was unavailable and was not given at any point (in 49 patients). The IPS:P ratio was 2 or greater in 45 of 50 procedures (90%) and was considered predictive of a pituitary source; the IPS:P ratio was less than 2 in five of 50 procedures (10%), considered predictive of an ectopic source.

**Positive IPSS without CRH stimulation**

A final diagnosis was available after 41 of 45 of the procedures in the 44 patients in the group whose IPS:P ratio was 2 or more. ACTH-secreting pituitary adenomas were found in 39 of 41 patients (true positive results). Two patients (two procedures) were found to have nonpituitary tumors (false positive results): one had a bronchial carcinoid, and one had an adrenal carcinoma. Four procedures (in four patients) were excluded from statistical analysis as unknowns: one patient was managed as if a pituitary tumor were present, although none was found, and three patients were lost to follow-up.
Negative IPSS without CRH stimulation

A final diagnosis was made after three of five procedures in the five patients in whom the IPS:P ratio was less than 2. Of the three negative procedures with known diagnoses, a pituitary tumor was found in one patient (false negative), and confirmed ectopic tumors were found in two (true negatives). There were two patients excluded from the statistical analysis whose diagnoses remain unknown.

IPS:P ratios in successful procedures before CRH stimulation

Of the 145 successful procedures, there were 95 (in 94 patients) in which the IPS:P ratio could be calculated from the two baseline ACTH values obtained before the administration of CRH. The IPS:P ratio was 2 or more in 71 of 95 procedures (75%) and was considered predictive of a pituitary source; it was less than 2 in 24 of 95 procedures (25%), considered predictive of an ectopic source.

Positive IPSS before CRH stimulation

A final diagnosis was available after 65 of 71 of the procedures in the 71 patients whose IPS:P ratio was 2 or greater. Two patients (two procedures) were found to have nonpituitary tumors (false positive results; one bronchial carcinoid and one adrenal adenoma), whereas ACTH-secreting pituitary adenomas were found in 63 of 71 procedures (true positive results). Six procedures (in six patients) with positive IPSS were excluded from statistical analysis: two patients were managed as if a pituitary tumor were present, although none was found; one patient was proven to be factiously hypercortisoluric; and three patients were lost to follow-up.

Negative IPSS before CRH stimulation

Of the 18 negative procedures (in 17 patients) with known diagnoses, pituitary tumors were found after 16 procedures (in 15 patients; false negative results), and confirmed ectopic tumors were found in two (true negative results). There were seven patients whose IPSS results changed from negative to positive after the administration of CRH, i.e. the post-CRH IPS:P ratio increased to 3 or more. All seven proved to have pituitary tumors and were included in the pre-CRH false negative group above. No patient with a positive IPSS at baseline before CRH treatment failed to reach threshold after CRH as well.

There were six patients (six procedures) with negative IPSS who were excluded from the statistical analysis: one patient was in spontaneous remission; four patients were managed as if they had ectopic tumors, although none has been found; and the diagnosis in one patient was unknown.

Sensitivity and specificity in IPSS procedures without or before CRH

The results from procedures in which CRH was never administered were combined with the results obtained from pre-CRH data to calculate the sensitivity and specificity of the procedure without CRH. Because the IPSS procedures for the two patients with adrenal tumors are only equivocally positive (cases 3 and 4 in Table 4), they have been excluded from these calculations. Based upon these data, the sensitivity of the IPSS without or before CRH stimulation is 85% [95% confidence interval (CI), 76.8–90.6%], with a specificity of 67% (95% CI, 22.7–94.7). The positive predictive value is 98%, whereas the negative predictive value is 18%. The diagnostic outcomes in this group are outlined in Fig. 1.

IPS:P ratios in successful procedures after CRH stimulation

Of the 145 successful procedures, there were 95 procedures in 94 patients in which CRH was administered (after obtaining the baseline ACTH levels analyzed above). In 78 of these procedures the IPS:P ratio was 3 or greater after CRH (82%) and was considered positive for a pituitary source (Fig. 2). There were 17 procedures in 16 patients in which the IPS:P ratio was less than 3 and considered predictive of an ectopic source.

Positive IPSS after CRH stimulation

A final diagnosis was made after 72 of 78 procedures in 78 patients with a successful positive IPSS after CRH administration. In 70 of 72 a pituitary tumor was demonstrated (true positives), whereas two patients had nonpituitary tumors (false positives; one bronchial carcinoid and one adrenal adenoma). Six patients were excluded from statistical analysis for lack of a final diagnosis: two patients were managed as if they had pituitary tumors, although none has been found; one patient (described above) was found to be factiously hypercortisoluric; and three were lost to follow-up.

Negative IPSS after CRH stimulation

The final diagnosis was determined for 10 of 16 patients (after 17 procedures) in this group. Eight patients (nine procedures) had pituitary tumors (false negatives), and two had proven ectopic tumors (true negatives). Six patients were excluded from the statistical analysis: one patient was in spontaneous remission; four were managed as if they had ectopic tumors, although none has been found; and one patient was lost to follow-up.

Sensitivity and specificity in IPSS procedures after CRH

Excluding one patient with an adrenal adenoma, the sensitivity of the IPSS after CRH stimulation was 90% (95% CI, 80.8–95.5), and the specificity was 67% (95% CI, 11.4–94.5). The positive predictive value was 99%, and the negative predictive value was 20%.

Diagnostic outcome after negative IPSS

Successful catheterizations. There were 21 successful procedures in 20 patients which did not reach a threshold ratio of 2 or more before or without CRH and were predicted to have ectopic tumors on that basis. Four of these 20 patients were ultimately found to have ectopic tumors (bronchial carcinoids) as the source of ACTH overproduction. CRH was administered in all but three of the 21 procedures, and the predicted source correctly changed from ectopic (before CRH) to pituitary in seven patients after the administration of CRH.
Based upon the clinical picture and suggestive MRI findings, the remaining nine patients (one who never received CRH and eight who did; all had subthreshold IPS:P ratios) underwent transsphenoidal exploration despite negative IPSS, eight at our institution (one after a repeat IPSS later met criteria; Table 1). Of these nine, seven were cured after a microadenoma was found and removed at operation. One of these patients underwent pituitary exploration for an enlarging sellar lesion 3 yr after two negative IPSS procedures and a bilateral adrenalectomy had been performed (Nelson’s syndrome); an ACTH-staining adenoma was found, although the patient was not cured. The patient who underwent surgery elsewhere had a pathologically proven ACTH-staining adenoma found adjacent to, but not within, the sella.

Venous angiograms obtained at the time of the catheterization were available for review after eight of the 10 procedures (in nine patients) in which surgical results conclusively demonstrated a pituitary source after negative IPSS. (One patient in this group had two IPSS procedures, and only one of her studies was available; one other study had been sent with the patient for surgery elsewhere.) Seven of the eight venograms showed correct placement of the catheters within the IPS bilaterally, whereas in one study, one of the catheters was not clearly in the IPS at the time of filming. The formal radiology report does not indicate any difficulties with the procedure and reports correct catheter placement, but it is impossible to determine where the catheter was actually located at the time of the sampling itself. It is therefore possible that one of the false negative procedures should have been considered unsuccessful and therefore possibly excluded from the false negative group.

Characteristics of false negative catheterizations. Data from the IPSS results for these patients are shown in Table 1. In those nine...
patients (ten procedures) with falsely negative IPSS procedures, the mean IPS:P ratio at baseline (without or before CRH) was 1.4 (range, 1.1–1.8), with a mean maximum IPS:P ratio in those eight who received CRH of 1.95 (range, 1.4–2.5).

**Unsuccessful negative catheterizations.** In 12 patients (13 negative IPSS procedures) there were technical difficulties with IPS cannulation, making it impossible to determine whether the IPSS was truly negative, predicting an ectopic source, or

![Flow chart showing final diagnoses based on IPSS results after CRH stimulation.](https://example.com/flowchart.png)

**TABLE 1. Patients with false negative IPSS**

| Case no. | CRH used | ACTH right IPS (time, min) | ACTH left IPS (time, min) | ACTH peripheral (time, min) | Max IPS:P | MRI results | Results after transsphenoidal surgery | % Increase in peripheral ACTH after CRH
---|---|---|---|---|---|---|---|---|---
1 | Yes | 76 (3) | 79 (3) | 32 (3) | 2.5 | Suggestive | Cured | 294 |
2 | Yes | 88 (3) | 80 (3) | 42 (3) | 2.1 | Suggestive | Cured | 397 |
3 | Yes | 135 (15) | 105 (15) | 78 (15) | 1.7 | Suggestive | Cured | 52 |
4 | Yes | 14 (0) | 25 (0) | 14 (0) | 1.8 | Positive | Cured after 2 procedures | 983 |
5 | Yes | 136 (10) | 103 (10) | 59 (10) | 2.3 | Negative | Cured | 142 |
6 | Yes | 70 (15) | 65 (15) | 49 (15) | 1.4 | Negative | Cured | 64 |
7 | No | 71 (10) | 72 (10) | 61 (10) | 1.2 | Unknown | Cured | No CRH |
8 | Yes | 85 (3) | 74 (3) | 48 (3) | 1.8 | Suggestive | Cured | 61 |
9 | Yes | 118 (10) | 150 (10) | 83 (10) | 1.8 | Suggestive | ACTH adenoma found, not cured | 166 |
| | Yes | 45 (5) | 71 (5) | 32 (5) | 2.2 | | | 113 |

*a ACTH values are in picograms per milliliter; time represents minutes after administration of CRH to the point of maximal IPS:P ratio.
*b Calculated as \[rac{\text{(mean of peripheral ACTH at 10 and 15 min)} - \text{(mean of peripheral ACTH at 0.0 min)}}{\text{(mean of peripheral ACTH at 0.0 min)}} \times 100\%.
*c Patient underwent repeat catheterization that met criteria for a pituitary source, then curative transsphenoidal surgery.
*d Review of venogram obtained at time of initial IPSS suggests that one catheter may not have been correctly positioned, although formal report at time of procedure mentions no problems.
*e Patient had two negative IPSS procedures followed by bilateral adrenalectomy; transsphenoidal exploration of an enlarging pituitary lesion that developed 3 yr later showed an ACTH adenoma.
negative because of technical problems. None of these 12 met threshold criteria at baseline, and the eight who received CRH failed after stimulation as well. In two patients there was either uni- or bilateral jugular occlusion. In one patient there was a venous complex draining the pituitary, without separate IPS drainage. In eight at least one IPS was atretic and could not be successfully cannulated, with samples drawn from the jugular bulb or petrosal os. Multiple hemolysed samples prevent accurate assay in one other patient. Based upon clinical, endocrine, and imaging data, curative transsphenoidal surgery was nonetheless performed in seven patients. A bronchial carcinoid was found in one patient after further body imaging. No source was found despite extensive evaluation in two patients (including successful repeat negative IPSS in one), who then underwent adrenalectomy. Two patients have been lost to follow-up.

**Diagnostic outcome after positive IPSS**

One hundred and fifty catheterizations (148 patients; 96 procedures with CRH administration and 54 without) met the criteria for a pituitary source of ACTH overproduction (Figs. 1 and 2). One hundred and twenty-three procedures demonstrated normal petrosal venous anatomy. Atypical anatomy with difficulty in obtaining successful bilateral IPS cannulation was found in 27 procedures, even though suprathreshold IPS:P ratios were still obtained. The diagnosis of a pituitary source was confirmed by surgical cure, positive pathology, or response to pituitary irradiation in 125 of 150 positive catheterizations, even if the IPSS procedure was unsuccessful. Four patients (four IPSS procedures) underwent pituitary surgery, but because they were not cured and no tumor was found on pathological review, no final diagnosis was obtained, and they are considered presumed pituitary. Despite reaching threshold criteria for a pituitary source, five patients (six IPSS procedures) were found to have nonpituitary tumors (Table 2). One of these patients (no. 5 in Table 2) had two sampling procedures, both of which demonstrated abnormal anatomy and were therefore considered unsuccessful, yet still produced a marginally positive ratio, although an ectopic tumor was later demonstrated. This patient was excluded from the statistical calculations. In addition, one patient was shown on further testing to be factitially hypercortisolic, although her IPSS was positive (she is listed as an unknown and was not considered a false positive). Fourteen patients returned to their referring institutions and were lost to follow-up.

**Characteristics of false positive catheterizations**

Five patients (six procedures) were shown to have nonpituitary sources of ACTH production despite having at least one IPS:P ratio above threshold criteria for a pituitary source for at least one point in time. Three procedures were performed with CRH stimulation, and three without. Data from the IPSS procedures in these patients are shown in Table 2. The mean IPS:P ratio in the three false positive IPSS procedures without CRH was 13 (baseline ratio, 31, 5, and 2.3), whereas the mean maximum IPS:P ratio in the three procedures with false positive IPSS after CRH was 8.4 (maximum ratios of 3.2, 10, and 12). In three patients (cases 3, 4, and 5)
the peripheral ACTH levels obtained during the IPSS were very low, making IPS:P ratios difficult to interpret, although suprathreshold ratios were achieved for at least one point in time in all three. Two of these three patients (cases 3 and 5) had nonsuppressed ACTH levels before the procedure. In one patient (case 4) peripheral ACTH levels before the IPSS were low, but detectable, and imaging studies performed before the IPSS suggested lesions in both adrenals and pituitary. The IPSS procedure was performed to determine which lesion was responsible for the hypercortisolemia. The very low ACTH levels were thought to be most consistent with an adrenal source even though the IPS:P ratio reached the threshold at one point, and the patient was cured after her adrenal adenoma was removed. These three cases (no. 3-5) were not included in the calculation of sensitivity and specificity reported above. In two cases (no. 1 and 2), however, IPSS results were indistinguishable from a true pituitary source, although bronchial carcinoids were found in both patients. One of these tumors stained for CRH, and one did not; both patients required cortisol replacement after tumor resection.

**Surgical results after positive IPSS**

One hundred and twenty-two of these patients underwent transsphenoidal surgery at Massachusetts General Hospital, and the remainder returned to their referring institution. One hundred and fourteen of these patients were newly diagnosed and had undergone no previous therapy, whereas eight had undergone diagnostic evaluation and previous unsuccessful surgery elsewhere. Surgical cure rates were 91% (104 of 114) for those newly diagnosed and 75% (six of eight) for those undergoing reoperation after unsuccessful treatment elsewhere. Cure rates for a subset of these patients have been previously published (9).

**Imaging**

MRI results were available for 145 of the 148 patients with pathological or surgical confirmation of the final diagnosis. Patients imaged only by CT were considered MRI unknowns (Table 3). In those 136 patients with a confirmed pituitary tumor in whom imaging results were available, the MRI was positive or suggestive in 64 (47%) and negative in 72 (53%). Imaging results were available in nine of 10 patients with nonpituitary disease; two of nine (22%) had a suggestive pituitary MRI. The MRI results in patients with falsely negative IPSS procedures are shown in Table 4. These are patients whose IPSS results did not meet criteria, but were nonetheless found to have pituitary disease. Of these nine patients, the MRI was suggestive or positive in six, negative in two, and unknown in one. In the five patients with false positive IPSS procedures (where the IPSS results met criteria, but nonpituitary tumors were found), the MRI was negative in three, suggestive in one, and not performed in one (Table 4). There were 22 successful IPSS procedures (in 21 patients) that predicted an ectopic source; the final diagnosis was known in 13 patients. The MRI results in this group are shown in Table 5. When both the IPSS and MRI were negative, three of five (60%) cases were truly ectopic. When the IPSS was negative, but the MRI was positive or suggestive, six of seven (86%) patients proved to have pituitary disease.

**ROC curve analysis before and after CRH**

CRH was administered in 95 successful procedures to 94 patients. If we exclude the patient with factitious hypercortisoluria, the patient in spontaneous remission, and 10 patients whose final diagnosis is unknown, there were 78 patients (79 procedures) whose final diagnosis was pituitary and four patients with confirmed nonpituitary tumors (three carcinoids and one adrenal adenoma). The patient with the adrenal adenoma (Table 2, case 4) was excluded from the ROC analysis because the IPS:P ratios met threshold at only one point in time, and the peripheral ACTH levels were very low. (She was, however, included in the flow chart in Fig. 2.) The scatterplot and ROC analyses before and after CRH stimulation for the remaining 81 patients are shown in Figs. 3 and 4.

**Determination of optimal threshold criteria**

The ROC analysis can be used to determine an optimal IPS:P threshold ratio, but this determination is dependent upon the decision of whether it is relatively more or less important to permit false positive or false negative results. If the goal is to maximize the total (sensitivity plus specificity), then this goal can be achieved with a threshold IPS:P ratio of 3.3 or greater, with a sensitivity of 89.7% (95% CI, 80.8–95.5) and a specificity of 100% (95% CI, 30.5–100), because this threshold would eliminate one additional false positive re-

**TABLE 4. MRI results shown by false negative and false positive IPSS results**

<table>
<thead>
<tr>
<th>False negative IPSS</th>
<th>MRI results unknown/ no MRI</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>False positive IPSS</td>
<td>6</td>
<td>2</td>
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<tr>
<td></td>
<td>2</td>
<td>1</td>
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**TABLE 5. MRI results after successful negative IPSS**

<table>
<thead>
<tr>
<th>Pituitary</th>
<th>Ectopic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
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<td>4</td>
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There were 21 patients (22 procedures) with successful negative IPSS procedures (16 with CRH and six without). The final diagnosis is available in 13 of these patients. This table presents results by diagnosis when MRI and IPSS are concordant or discordant. When the MRI was positive or suggestive, but the IPSS negative, the MRI correctly diagnosed a pituitary tumor in six of seven (86%) cases. When both the MRI and IPSS were negative, the diagnosis was pituitary in two of five (40%) and ectopic in three of five (60%).
The percent increase in the peripheral ACTH level was calculated by comparing the mean of the two baseline pre-CRH levels with the mean of the ACTH levels at 10 and 15 min post-CRH and was correlated with the IPSS results and final diagnosis. These data are shown in Table 6. Using the published criterion for a positive peripheral CRH stimulation result (Table 2, case 2). Alternatively, a threshold ratio could be chosen that would tend to minimize false negative results, i.e., one that would minimize the risk that a patient with a pituitary tumor would falsely be labeled as having an ectopic tumor. This choice may increase the false positive rate, i.e., increase the risk of recommending transsphenoidal surgery for a patient with an ectopic tumor. The greatest negative predictive value, 40.0, was achieved by lowering the threshold to 1.8 or more.

Characterization of peripheral ACTH response to CRH stimulation

The percent increase in the peripheral ACTH level was calculated by comparing the mean of the two baseline pre-CRH levels with the mean of the ACTH levels at 10 and 15 min post-CRH and was correlated with the IPSS results and final diagnosis. These data are shown in Table 6. Using the published criterion for a positive peripheral CRH stimulation test as a greater than 35% increase (12), 70 patients with a positive response to CRH had pituitary tumors, whereas no patients with nonpituitary tumors did. All of the false negative responses after IPSS responded to CRH with an elevated peripheral ACTH level, even though the IPS:P ratio never reached the threshold criterion. There were 12 patients with a negative peripheral ACTH response to CRH; four had nonpituitary tumors, and eight had pituitary tumors. These eight patients with pituitary tumors all had a positive IPSS. If the threshold for a positive response to CRH is lowered to a 5% increase, then the 75 patients with a positive test all had pituitary tumors, whereas three of the seven negative responders did. When the results of the IPSS and the CRH agreed (either both positive or both negative), the combination of the two correctly predicted the final diagnosis in all cases. When the results disagreed, either diagnosis was possible.

Discussion

These data indicate that false negative results after IPSS procedures are significantly more common than previously appreciated, and that a negative IPSS does not rule out a pituitary source. The overall sensitivities are 85% (95% CI, 76.8–90.6%) and 90% (95% CI, 80.8–95.5%) before and after CRH, respectively, with a specificity of 67% in both cases (95% CI without CRH, 22.7–94.7%; 95% CI after CRH, 11.4–94.5%). This is in contrast to initial reports that showed a very high specificity and sensitivity (100%), although later analyses suggested sensitivities ranging from 82–85% before CRH and 88–97% after CRH (6, 7, 13). The 95% CIs reported here are broad, especially for the specificity, because the total number of ectopic tumors in the series is low. This may also explain some of the discrepancy between this series and others reported in the literature. In many of these studies, however, patients with IPSS results predictive of an ectopic source in whom final diagnoses were never obtained did not undergo pituitary exploration and were considered unknowns; our data suggest that at least some of these patients will prove to be false negatives. A summary of the reported sensitivities, specificities, and false negative results as documented in the literature is shown in Table 7. All of these studies, including the data reported here, demonstrate a significant benefit to performing the IPSS after CRH stimulation.

If the IPSS is positive for a pituitary source, its predictive value is high: 99% after CRH. For negative results predictive of an ectopic tumor, however, the predictive accuracy is much lower and is therefore not definitive in excluding the diagnosis of pituitary disease. Because it has been suggested that a pituitary source will be found in 65–75% of all patients with Cush- ing’s syndrome, a positive IPSS can be viewed as confirming the most likely outcome (14, 15). Undetected pituitary disease after a falsely negative IPSS may account for a subset of those patients previously considered to have ectopic tumors of unknown origin. In a 2001 review of ectopic Cushing’s syndrome, Aniszewski et al. (16) reported that 16% of 106 cases of ectopic hormone function had no putative source. Doppman’s 1989 study (17) of the radiographic localization of ectopic tumors by selective venous sampling reported that the source of excess ACTH in

![Fig. 3. Scatterplot of IPS:P ratios after administration of CRH. With a threshold value of 3 or greater, the sensitivity is 90%, and the specificity is 67%. For the purposes of graphic clarity, IPS:P ratios above 100 have been rounded down to 100 to expand the lower y-axis.](image)

![Fig. 4. ROC curves for IPSS with and without CRH administration. The area under the pre-CRH ROC curve is 0.838 (95% CI, 0.739–0.910), and the area under the post-CRH curve is 0.932 (95% CI, 0.853–0.976). The difference between areas is 0.094 (95% CI, 0.020 to 0.107).](image)
nine of 28 cases of presumed ectopic ACTH overproduction remained undiagnosed, pituitary tumors having been excluded by negative IPSS. Even in the original NIH series, 32 patients were excluded for lack of a final diagnosis, 12 with presumed, but unconfirmed, ectopic tumors (3). The situation is made more complicated by the fact that ectopic tumors may not present concurrently with the diagnosis of hypercortisolemia, e.g. bronchial carcinoids have been diagnosed in patients many years after the onset of hypercortisolemia (16, 18). In the setting of a negative IPSS, it can therefore be extraordinarily difficult to determine whether the IPS procedure is a false negative, with the source of ACTH still residing in the pituitary, or whether the true ectopic source has not yet been found. Data from analysis of proopiomelanocortin fragments secreted by ectopic tumors vs. pituitary adenomas suggest that fragment size may offer another means of differentiating ectopic from pituitary sources; these data await extensive clinical verification (19).

There are several possible causes of a false negative IPSS (20). Anatomical abnormalities precluding satisfactory catheterization of at least one IPS were initially described in four patients, and atrophic inferior petrosal sinuses have been described in 25% of cases, although this atrophy reportedly did not influence the success of sampling (5). Samples drawn from the jugular vein may be diluted by nonpituitary venous drainage and give artificially low ACTH levels, leading to falsely low IPS:P ratios. Thirteen of our cases with nonthreshold ratios were excluded for this reason; the remaining 21, however, appeared to be successful, and unsuccessful procedures were excluded from the ROC analyses. Correct catheter position was confirmed by venous angiography before sampling and by intermittent fluoroscopy during sample withdrawal. Some centers have advocated repeat angiography at the conclusion of sampling in addition to fluoroscopy as an additional determination of correct catheter positioning, although this has not been our practice. It is possible that

### TABLE 6. Correlation of IPSS results with peripheral ACTH response to CRH, by final diagnosis

<table>
<thead>
<tr>
<th>ACTH increase after CRH</th>
<th>Positive IPSS (IPS:P, ≥3)</th>
<th>Negative IPSS (IPS:P, &lt;3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral ACTH increased by &gt;35%</td>
<td>62 Pituitary, 0 ectopic</td>
<td>8 Pituitary (Table 1), 0 ectopic</td>
</tr>
<tr>
<td>Peripheral ACTH increased by &lt;35%</td>
<td>8 Pituitary, 1 carcinoid (Table 2, case 2), 1 adrenal adenoma (Table 2, case 4)</td>
<td>0 Pituitary, 2 carcinoid</td>
</tr>
<tr>
<td>Peripheral ACTH increased by &gt;5%</td>
<td>67 Pituitary, 0 ectopic</td>
<td>8 Pituitary (Table 1), 0 ectopic</td>
</tr>
<tr>
<td>Peripheral ACTH increased by &lt;5%</td>
<td>3 Pituitary, 1 carcinoid (Table 2, case 2), 1 adrenal adenoma (Table 2, case 4)</td>
<td>0 Pituitary, 2 carcinoid</td>
</tr>
</tbody>
</table>

### TABLE 7. Sensitivity, specificity, and false negative and positive results in the literature

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients/ successful IPSS</th>
<th>CRH used?</th>
<th>IPS-P criterion, no CRH/CRH</th>
<th>Sensitivity, no CRH/CRH</th>
<th>Specificity, no CRH/CRH</th>
<th>False negatives, no CRH/CRH</th>
<th>False positives</th>
<th>Unknown/presumed ectopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mampalam (40)</td>
<td>39</td>
<td>No</td>
<td>≥2/NA</td>
<td>2/NA</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snow (41)</td>
<td>10</td>
<td>No</td>
<td>≥2/NA</td>
<td>1/NA</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McCance (29)</td>
<td>29/27</td>
<td>24</td>
<td>≥2/2</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oldfield (3)</td>
<td>281/278</td>
<td>262</td>
<td>≥2/3</td>
<td>95%/100%</td>
<td>0/0</td>
<td>32/12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McNally (21)</td>
<td>8/8</td>
<td>8</td>
<td>≥2/2</td>
<td>92%/92%</td>
<td>4/2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zarrilli (42)</td>
<td>26</td>
<td>26</td>
<td>≥2/3</td>
<td>10/3</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lopez (8)</td>
<td>32/30</td>
<td>24</td>
<td>≥2/3</td>
<td>3/1</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bonelli (7)</td>
<td>128/86</td>
<td>124</td>
<td>≥2/2</td>
<td>73%/97%</td>
<td>19/21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kalsas (13)</td>
<td>92/82</td>
<td>92</td>
<td>≥2/2</td>
<td>92%/92%</td>
<td>4/6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wiggam (36)</td>
<td>53/38</td>
<td>2</td>
<td>≥2/NA</td>
<td>82%/NA</td>
<td>3/NA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colao (6)</td>
<td>97/97</td>
<td>78</td>
<td>≥2/3</td>
<td>85%/88%</td>
<td>11/36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>This study</td>
<td>179/143</td>
<td>120</td>
<td>≥2/3</td>
<td>85%/90%</td>
<td>16/8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- This patient was taking cyproheptadine before IPSS.
- Six false negatives were reported: four patients failed to meet threshold before CRH; two patients failed after CRH and one of whom met criteria before, but not after, CRH.
- One patient developed MRI evidence of microadenoma 2 yr after negative IPSS, which was surgically confirmed.
- Four patients (0.8%) had hypoplastic IPS.
- Both patients were cured after transsphenoidal surgery.
- Of the six, two patients had malpositioned catheters, one patient had IPSS after two surgical procedures, and two patients met threshold criteria before CRH, but not after.
- One patient met criteria after CRH, one patient had periodic hormonogenesis and nodular pituitary hyperplasia, and one patient had confirmed pituitary adenoma.
- There were 11 false negatives without CRH, eight false negatives after CRH, and three false negatives in 68 patients who had both pre- and post-CRH IPS:P ratios available.
- Of the 16 patients with false negative results after CRH, seven reached a positive threshold after CRH, eight did not reach the threshold even after CRH, and one never received CRH.
petrosal venous blood could be diluted by a nonpituitary source even when the catheters are correctly positioned; this may account for some of the variability between centers (or radiologists) in relative sensitivity and specificity. To correct for this, it has been recommended that IPS samples be normalized against other pituitary hormones (21). It has also been suggested that more proximal sampling of pituitary venous drainage from the cavernous sinus, as opposed to inferior petrosal sinus, might better localize ACTH secretion, although a direct comparison has not supported this conclusion (22–26). An abnormally located corticotroph adenoma, if originating ectopically in the sphenoid sinus, might demonstrate aberrant venous drainage not detected by IPS sampling or may show a suprathereshold IPS:P ratio even though the tumor is found to be extrasellar on surgical exploration (27, 28). One of the false negatives described here reportedly had tumor in the sphenoid sinus, rather than in the sella. In addition, pituitary corticotroph adenomas may possess varying degrees of responsiveness to exogenous CRH. If an adenoma is minimally responsive to CRH, one might not see the expected rise in ACTH secretion after CRH administration, with a resultant false negative IPSS. If a pituitary adenoma were to produce ACTH cyclically, intercycle suppression of normal corticotroph production could lead to a false negative IPSS if sampled during an off phase. Finally, problems with the assay or sample processing could also lead to a false negative result. Assay variability has been estimated at 10%. Because the threshold criterion is a ratio, variability can be magnified if the numerator and denominator are affected in opposite directions (29). Thus, ratios near the threshold criterion need to be viewed as inconclusive, especially when the original ACTH measurements are low. Incorrect sample handling may lead to both falsely elevated and lowered IPS:P ratios, if proteolytic destruction of ACTH is allowed to occur by failure to appropriately chill the samples after withdrawal.

The IPSS procedure has been reported to be more sensitive than MRI in the diagnosis of Cushing’s disease (30). Positive or suggestive pituitary imaging was found in only 47% (64 of 136) of those patients with proven pituitary disease whose imaging results are available (true positive MRI) and in 22% (two of nine) of those patients with proven ectopic disease (false positive MRI). However, of the seven patients with false negative IPSS procedures whose MRI results are available, six (86%) had positive or suggestive MRI imaging. These data suggest that a discrepancy between the MRI and IPSS results should lead to consideration of pituitary exploration if no other source of ACTH overproduction can be demonstrated.

Our data also indicate that although the predictive value of a positive test is high, false positive results do occur. Three of the five patients with false positive IPSS had low peripheral ACTH levels, with the IPS:P ratio reaching threshold at only one time point, making the results difficult to interpret. Two patients, however, had results indistinguishable from those expected with a pituitary source. False positive IPSS results may have multiple causes. They can be seen in normal subjects, and we unknowingly catheterized one patient who on further review had factitious hypercortisoluria, although the IPS results met the criteria for a pituitary tumor (31). This patient was excluded from our analysis. Ectopic CRH-producing tumors can stimulate endogenous ACTH production by the pituitary, with a positive IPS:P ratio (32, 33). CRH-positive tumor staining was found in one of the two patients with bronchial carcinoids who had false positive results (and both patients responded with improvement in their hypercortisolism after surgical therapy.) Theoretically, ectopic tumors with intermittent ACTH secretion or adrenal tumors with intermittent cortisol production could incompletely suppress endogenous ACTH production and allow an IPS:P gradient to persist (34). To minimize the risk of obtaining a false positive result in a patient with periodic hormonogenesis from an ectopic tumor, 24-h UFC samples were requested on the day before the planned IPSS. Depending upon the periodicity of the cyclic production, however, a single elevated UFC on the day of the sampling may not be sufficient to exclude inadequate suppression of pituitary corticotrophs, and more frequent sampling may be required.

These data also suggest that analysis of the peripheral ACTH response to CRH, although not a replacement for formal peripheral CRH stimulation testing, may provide useful information in addition to that obtained from the IPSS itself (12, 35). All of the patients with a false negative IPSS response exhibited a robust increase in peripheral ACTH level after CRH, suggesting that the production of ACTH by the pituitary adenoma was not adequately sampled and perhaps either was not draining into the petrosal venous system or had been diluted by nonpituitary venous blood. When the results of the CRH stimulation and the IPSS agreed (either both positive or both negative), the site of the tumor was correctly predicted in every case. When there was no peripheral rise in ACTH level, but the IPS:P threshold ratio was still reached, the results were indeterminate. Although we have too few nonpituitary tumors in our series to make concrete recommendations regarding a diagnostic percent increase in peripheral ACTH levels, both an increase of 35% [as reported for formal CRH stimulation testing (12)] and even 5% above baseline gave useful information. In those studies that have compared formal peripheral CRH stimulation testing to the IPSS, the results have been mixed, because not all pituitary tumors met ACTH or cortisol criteria after CRH stimulation (36, 37). It is interesting to note that the four false negative results reported on the basis of a hypoplastic IPS all exhibited a positive ACTH rise in response to CRH (5).

Clinical implications

The following clinical implications of these findings are important to consider.

Sampling adequacy. It is important to be certain that the IPS sampling procedure was truly successful, with normal pituitary venous drainage and correct handling of samples, before concluding that a negative result is predictive of an ectopic source, because anatomical abnormalities are not uncommon, and the ability to obtain adequate samples may...
vary with experience. An experienced team of radiologists is critical to the success of the procedure.

**Imaging studies.** Radiographic evaluation for ectopic disease has been recommended in only those cases where the IPSS is consistent with this diagnosis (38). It is unclear whether routine radiographic screening (chest and abdominal computed tomography or MRI) in all patients with negative MRI imaging of the pituitary, before IPSS, would add to the diagnostic evaluation. Any additional diagnostic accuracy gained by this approach remains to be determined and would also be a function of the sensitivity and specificity of chest and abdominal imaging.

**Surgical referral.** Transsphenoidal exploration should be considered in all patients with unsuccessful IPS catheterizations after negative body imaging if other endocrine evaluation is consistent with a pituitary source. In addition, transsphenoidal exploration should be considered in those patients with a negative IPSS in whom no ectopic source can be found, especially where the pituitary MRI is positive or suggestive, or there is a robust increase in peripheral ACTH levels after CRH administration.

**Presumed ectopic tumors.** Those patients currently being managed on the basis of a negative IPSS as if they had presumed ectopic tumors, where no ectopic source has yet been found, should at least be periodically reimaged with pituitary MRIs to monitor for the enlargement of an undetected pituitary adenoma, and consideration should be given to transsphenoidal exploration. This may be even more important in those patients who underwent adrenalectomy for control of their hypercortisolemia, because it may not be appreciated that they are at risk for Nelson’s syndrome.

**Correlation of IPSS with peripheral ACTH levels after CRH stimulation.** Our data suggest that useful additional information can be gained by correlating the results of the IPS:P ratios with the percent increase in peripheral ACTH levels.

The ultimate differentiation of ectopic from central sources of ACTH overproduction remains a diagnostic dilemma (39). Although positive IPS sampling is beneficial in confirming a pituitary source, the results of negative IPS procedures, especially, must be interpreted with caution.

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Address all correspondence and requests for reprints to: Dr. Brooke Swearingen, ACC 331, Massachusetts General Hospital, Fruit Street, Boston, Massachusetts 02114. E-mail: bswearingen@partners.org.

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