Transsphenoidal Surgery for Cushing Disease After Nondiagnostic Inferior Petrosal Sinus Sampling

**BACKGROUND:** Inferior petrosal sinus sampling (IPSS) is a useful technique for confirming a pituitary source of adrenocorticotropic hormone (ACTH) overproduction in Cushing disease. Uncertainty remains regarding the appropriate course of therapy when an ectopic tumor is predicted by IPSS but none can be found and in circumstances when the procedure cannot be successfully completed owing to technical or anatomic limitations.

**OBJECTIVE:** To determine an appropriate course of action after nondiagnostic IPSS.

**METHODS:** We reviewed 288 IPSS procedures in 283 patients between 1986 and 2010 at our center. An IPS:peripheral ACTH ratio ≥ 2 at baseline or ≥ 3 after corticotrophin-releasing hormone was considered predictive of a pituitary source of ACTH. A procedure was considered nondiagnostic if the procedure was successfully performed and the results predicted an ectopic source but none could be found despite extensive imaging or if the IPS could not be bilaterally cannulated because of technical difficulties or anatomic variants.

**RESULTS:** The sensitivity, specificity, positive predictive value, and negative predictive value of IPSS for detecting a pituitary source in Cushing disease were 94%, 50%, 98%, and 29%, respectively. We identified 3 categories of nondiagnostic IPSS comprising 44 of the total procedures. These patients underwent exploratory transsphenoidal surgery, and in 42 of these patients (95%), a pituitary source was surgically proven, with a remission rate of 83%.

**CONCLUSION:** Transsphenoidal surgery should be considered in cases of ACTH-dependent Cushing disease and noncentralized or technically unsuccessful IPSS without evidence of ectopic tumor.

**KEY WORDS:** ACTH, Cushing disease, Inferior petrosal sinus, Pituitary gland, Transsphenoidal surgery

Cushing syndrome (CS) refers to the clinical and metabolic effects of chronic systemic glucocorticoid excess, including centripetal fat distribution, skin fragility, muscle wasting, osteoporosis, impaired immunity, decreased glucose tolerance, dyslipidemia, hypertension, and increased risk of cardiovascular events. Endogenous CS is typically caused by overproduction of adrenocorticotropic hormone (ACTH) by a pituitary tumor or an ectopic tumor producing ACTH or peptides causing ACTH release or by overproduction of cortisol by the adrenal glands. Cushing disease (CD), or overproduction of ACTH by the pituitary gland, is the most common of these.

The morbidity and mortality of uncontrolled CS are significant, largely as a result of cardiovascular complications. Epidemiological studies have identified an increased risk of coronary artery disease, heart failure, and myocardial infarction in both CD and CS patients, contributing to a mortality rate in active disease up to 5.5 times that of matched control subjects. It is therefore imperative to identify CS, to determine its cause, and to initiate treatment expeditiously.
If the evaluation suggests a pituitary adenoma, the treatment of choice is transsphenoidal surgical resection, with reported remission rates of 60% to 90%. Arriving at the diagnosis of CD in the absence of an obvious tumor on magnetic resonance imaging (MRI) requires demonstration of a pituitary source producing ACTH. This determination may be difficult because endocrine testing may yield confusing or contradictory results. In such cases, inferior petrosal sinus sampling (IPSS) has emerged as a useful technique for confirming a central source of ACTH overproduction before proceeding with transsphenoidal exploration. Although early descriptions reported high sensitivity and specificity, recent studies have identified a low but significant false-negative rate. In particular, uncertainty remains regarding the appropriate course of therapy when an ectopic tumor is predicted by IPSS but none can be located and in circumstances when the procedure cannot be successfully completed because of technical or anatomic limitations. To address this question, we reviewed the results after transsphenoidal exploration in cases in which IPSS predicted an ectopic source but none was found despite extensive investigation and in those cases in which technical difficulties and/or anatomic variants prevented the acquisition of predictive data.

PATIENTS AND METHODS

Patient Population

The records of all patients undergoing IPSS for CD at the Massachusetts General Hospital between 1986 and 2010 were reviewed. The IPSS results of a number of these patients have previously been reported. The present study reviews additional data from patients studied from 2002 to 2010, focusing on those patients in whom considerable diagnostic uncertainty remains, and represents an analysis of data including patients whose treatment recommendations were based on the results of this previous report.

Endocrine and Imaging Evaluation

All patients had proven hypercortisolemia on the basis of multiple assays, including 24-hour urine free cortisol (UFC) levels and, in later years, late-night salivary cortisol levels. An ACTH-producing source was suspected on the basis of hypercortisolemia in association with nonsuppressed ACTH levels. All patients underwent MRI of the pituitary before proceeding to IPSS. In general, MRIs were either negative or equivocal before the IPSS was recommended. All patients with clearly discernible macroadenomas (>1 cm) were referred directly for surgery. Patients with subcentimeter abnormalities were considered equivocal and referred for IPSS. Almost all patients had a 24-hour UFC confirming hypercortisolism within 1 day of the IPSS.

IPSS Technique and Analysis

The IPSS was performed by a single group of vascular radiologists as described previously. Cannulation of the IPS was performed after systemic heparinization and confirmed by angiographic demonstration of cavernous sinus filling. Successful IPSS was defined as bilateral IPS cannulation. After bilateral cannulation, baseline samples were obtained simultaneously both from catheters and peripherally. Two baseline measurements were obtained before corticotropin-releasing hormone (CRH) administration. In patients receiving CRH stimulation, ovine CRH was administered at 1 µg (to a maximum dose of 100 µg), and sequential samples were obtained at 3, 5, 10, and 15 minutes after CRH injection. Samples were collected in prechilled tubes and transferred on ice to the endocrine laboratory for ACTH measurement immediately after the procedure.

The IPS:peripheral (IPS:P) ACTH ratio was calculated both at baseline and for all 4 post-CRH time points. The finding of any baseline IPS:P ratio ≥2 or poststimulation ratio ≥3 was considered predictive of a pituitary source. An IPSS was considered nondiagnostic (of a pituitary source) if the procedure was successfully performed and the results predicted an ectopic source but none could be found despite extensive imaging (type I; Table 1) or if the IPS could not be bilaterally cannulated because of either technical difficulties or anatomic variants, thereby precluding the calculation of true bilateral IPS:P ratios. In such cases, the IPS:P ratios were calculated from available data. We defined type II nondiagnostic procedures as those in which the ratios nevertheless centralized and type III as those in which ratios did not centralize (Table 1).

Diagnostic Determination

The results after transsphenoidal surgery (TSS) in these inconclusive categories were reviewed to determine the optimal therapeutic approach in the setting of diagnostic uncertainty. Those patients in whom IPSS predicted an ectopic source underwent extensive systemic imaging. Our routine sequence for peripheral imaging consisted of chest computed tomography (CT), followed by abdominal/pelvis CT, followed by neck CT (in some cases), positron emission tomography/CT (in some cases), and Octreoscan. If negative, we typically repeated imaging periodically so that occult ectopic tumors could be found.

The diagnosis of a pituitary source was considered confirmed if an ACTH-staining adenoma was found at operation and/or by the demonstration of remission as defined by a postoperative fasting serum cortisol level of <5 µg/dL and 24-hour UFC level of <20 µg/d.

Statistical Analysis

Descriptive statistics were calculated using the mean as a measure of central tendency and the standard error of the mean as a measure of variability. The results of this previous report.

| TABLE 1. Inferior Petrosal Sinus Sampling Categorization Based on Cannulation Success and Adrenocorticotropic Hormone Values |
|-------------|-----------------|
| Type       | IPSS Characteristics                                      |
| I          | Successful bilateral catheterization; values did not meet criterion for a central source; no ectopic source found |
| II         | Unsuccessful catheterization owing to technical difficulty (unilateral or bilateral jugular samples) or variant anatomy (plexiform or atretic sinus); values did meet criterion for a central source |
| III        | Unsuccessful catheterization owing to technical difficulty (unilateral or bilateral jugular samples) or variant anatomy (plexiform or atretic sinus); values did not meet criterion for a central source |

*IPSS, inferior petrosal sinus sampling.*
dispersion. Given the nonnormal distribution of IPS:P ratios, comparisons were made by use of nonparametric statistics and reported as median ± SE. We used the Mann-Whitney U test for unpaired comparisons and the Wilcoxon rank-sum test for paired comparisons.

RESULTS

Between 1986 and 2010, 283 patients underwent 288 IPSS procedures to aid in the diagnosis of CS (Figure 1). Five patients underwent 2 procedures. The mean ± SE age was 41.5 ± 0.9 years (range, 7-81 years), with a male:female ratio of 1:4. A final diagnosis was available in 249 patients (88%). Successful bilateral sampling was achieved in 240 procedures (83%) in 237 patients. Of these successful procedures, IPS:P ratios indicated a pituitary source in 200 procedures (83%) in 199 patients. Mean ± SE follow-up interval was 30 ± 6.5 months (range, 0.1-109 months).

Diagnostic Yield After Successful IPSS Procedures

Successful bilateral IPS sampling was achieved in 240 procedures in 237 patients. Sixteen of these patients (who had 16 IPSS procedures) either underwent further workup and surgery at other institutions or were lost to follow-up and therefore were excluded from further analysis. One patient who underwent a single IPSS spontaneously remitted with no further treatment.

In the remaining 223 procedures in 220 patients, IPSS results predicted a pituitary source in 200 procedures (199 patients) by either baseline or post-CRH ACTH levels (Figure 1A). CRH was unavailable in 43 procedures. The vast majority of these cases, 197 procedures in 196 patients, proceeded to TSS. A pituitary source was confirmed by either pathological identification or remission by laboratory value normalization in 194 patients (undergoing 195 procedures). In terms of the accuracy of the IPSS for predicting the location of the causative tumor, these procedures thus represented true positives.

The remaining 2 procedures in 2 patients predicted a central source, but adenoma was not identified histologically, and the patients did not undergo remission. In one of these, a 24-hour UFC near the time of the IPSS procedure was elevated at 575 mg. Hypercortisolism near the time of catheterization reduced the likelihood of cyclicity as a reason for the false-positive result. After extensive further evaluation, this patient was diagnosed with an...
occult CRH-secreting tumor, a rare but recognized cause for falsely centralized IPSS studies. The other patient’s UFC on the date of the IPSS was elevated at 82 μg (upper limit of normal, 70 μg), and previous UFC values over the preceding 7 weeks were similarly elevated (128 and 229 μg), thereby reducing the likelihood of cyclicity. Despite a thorough subsequent evaluation, however, his diagnosis remains unclear. These 2 cases therefore represent false positives.

Three patients with elevated UFC on the day of IPSS (130, 254, and 97 μg) underwent catheterization predicting a central source and were therefore referred for TSS. During the routine preoperative evaluation, however, suspicious lesions were identified. In 1 patient, a nodule was seen on the preoperative chest x-ray. In a second patient, an abdominal CT obtained during the workup for anemia revealed an adrenal lesion. Details of the third patient’s motivation for imaging are not available. Rather than undergoing TSS, therefore, these patients were referred for surgery on their candidate ectopic tumors. The first was found to have a bronchial carcinoid; the second, an adrenal carcinoma; and the third, an adrenal adenoma. All 3 patients were cured after resection of their respective lesions. These 3 cases were therefore also considered false positives.

Twenty-three successful IPSS procedures in 21 patients predicted an ectopic source by failing to meet either baseline or post-CRH threshold criteria (Figure 1B). In 5 of these 21 patients (representing 5 procedures), a candidate lesion was identified by imaging, and the patients were referred for resection. Four were bronchial carcinoids, and 1 was an intrasphenoid pituitary adenoma. These 5 cases therefore represent true negatives. In 5 additional procedures in 4 patients, a lesion was never found but presumed to be ectopic, and the patients’ symptoms were controlled with adrenalectomy.

In the remaining 13 noncentralized IPSS procedures in 12 patients, a peripheral lesion was never found despite extensive investigation. We refer to these as type I nondiagnostic IPSS procedures. Given the possibility of a false-negative IPSS result and the failure to document any other source of excess ACTH, these patients underwent exploratory TSS. A pituitary source was confirmed in 11 of these patients (representing 12 false-negative procedures). The single remaining patient remained undiagnosed, and the patient’s symptoms were controlled with adrenalectomy.

Diagnostic Yield With Unsuccessful IPSS Procedures

Forty-eight attempted IPSS procedures in 46 patients were unsuccessful because of either technical difficulties in cannulating 1 or both petrosal sinuses or anatomic abnormalities, including jugular occlusion or a plexiform or atretic sinus. Venous samples could sometimes be obtained from the jugular vein or from 1 IPS, even if both could not be cannulated. Twelve of these patients (13 procedures) were treated at another institution or lost to follow-up and therefore were excluded.

Despite the fact that the procedures were technically unsuccessful, the available sampling data could still be stratified by the above-mentioned pre-CRH and post-CRH threshold criteria in some of the remaining 34 patients. Twenty-three procedures in 22 patients (n = 10 because of technical difficulty; n = 12 because on anatomic variation) attained the criterion to predict a central source (Figure 1C). In 1 patient who underwent 2 unsuccessful IPSS procedures, an ectopic bronchial carcinoid was discovered. The remaining 21 patients (representing 21 procedures) underwent exploratory TSS. We refer to these procedures, in which bilateral IPSS was unsuccessful but available data nevertheless predicted a central source, as type II nondiagnostic IPSS procedures. All but one of these patients were proven to have a pituitary source. The single patient without a confirmed pituitary source remained undiagnosed.

Twelve of the unsuccessful procedures in as many patients (n = 8 owing to technical difficulty; n = 4 owing to anatomic variation) failed to meet the criterion for a pituitary source (Figure 1D) with available sampling. Imaging revealed a chest lesion in one of these, and the patient was cured after resection of a bronchial carcinoid. The remaining 11 patients with negative peripheral imaging studies underwent exploratory TSS. We refer to these procedures, in which bilateral IPSS was unsuccessful and available data were not predictive of a central source, as type III nondiagnostic procedures. All 11 had confirmed pituitary sources.

Nondiagnostic IPSS Procedures

Of the various clinical situations described above, we focused our attention on the 3 that represented particular diagnostic dilemmas. These “nondiagnostic” situations were those in which either a successful IPSS predicted an ectopic source but none was found despite thorough anatomic and functional imaging or the IPSS was not successfully completed because of either technical difficulties or anatomic abnormalities that precluded bilateral IPS cannulation.

This group consisted of 45 procedures in 44 patients. Mean age (range, 11-78 years) was similar between this group and the entire study population (41.5 and 40.0 years; P = .55, 2-tailed t test). To examine the diagnostic yield of our management strategy in these situations, we stratified the patients into 3 groups based on the particular category of IPSS result (Table 1). Type I procedures included those in which bilateral IPSS was successfully performed but predicted an ectopic tumor that could not be located. Type II procedures were those in which bilateral IPSS was unsuccessful because of either technical difficulties (inability to cannulate the IPS or occluded vessels) or anatomic variations (plexiform, atretic, or hypoplastic sinuses) during which ACTH levels (baseline or post-CRH) nevertheless met the criteria for a pituitary source. Type III procedures were those in which bilateral IPSS was similarly unsuccessful and in which ACTH levels did not meet centralizing criteria.

The 12 patients with 13 type I procedures underwent exploratory TSS given the lack of other candidate sources of ACTH excess. As mentioned above, 11 patients (92%) comprising 12 procedures had a proven pituitary source (false negatives), and 1
remains undiagnosed. Of these 11 patients with pituitary tumors, 9 remained in remission at last follow-up, 4 after a second TSS (Table 2). One of the remaining 2 had undergone adrenalectomy after 2 nondiagnostic IPSS procedures before TSS and returned with Nelson syndrome, and another had positive pathology but recurred.

In the 32 patients in whom bilateral sampling could not be performed, the attempted IPSS procedure nonetheless yielded at least 1 sample that met threshold criteria in 21 patients (type II). In 20 of these patients (95%), a pituitary source was proven surgically. Seventeen of these patients achieved remission after TSS. Of the remaining 3, 1 underwent adrenalectomy, 1 underwent proton beam therapy, and the third is being considered for the same.

In the 11 type III patients, successful bilateral IPSS was not achieved, and the available samples did not reach baseline or post-CRH criteria for a central source. After negative peripheral imaging for an ectopic tumor, a pituitary source was surgically confirmed in all 11 patients after exploratory TSS. Nine of these patients achieved remission after TSS, 2 of whom experienced recurrence. An additional 2 patients were in remission after further treatment, adrenalectomy in 1 and proton beam therapy in the other.

Of the total 44 patients (representing 45 procedures) who underwent exploratory TSS across all 3 nondiagnostic categories, 42 (95%) were determined to have a surgically proven pituitary tumor, and the majority achieved long-term remission with TSS. Surgical remission after TSS was achieved in 9 of 11 patients (82%) despite successful noncentralizing bilateral IPSS (type I) and in 26 of 31 patients (84%) after unsuccessful IPSS procedures (types II and III).

### Analysis of IPSS Results by Category

A summary contingency table of IPSS results compared with a surgically confirmed source is shown in Table 3. Among the total of 217 patients with successful IPSS procedures and surgically proven diagnoses, 207 had a pituitary source (195 true positives, 12 false negatives) and 10 had an ectopic source (5 true negatives, 5 false positives). The sensitivity, specificity, positive predictive value, and negative predictive value of IPSS for detecting a pituitary source in CS were thus 94%, 50%, 98%, and 29%, respectively.

To understand the differences between true and false IPSS results, we compared IPS:P ratios of true-negative and false-negative procedures, as well as true-positive and false-positive procedures (Figure 2). At baseline, the median ratio for true-negative procedures was 1.30 ± 0.05 and that for false-negative procedures was 1.28 ± 0.07, an insignificant difference (P = .31, Mann-Whitney U test). Baseline median ratios for the true positives (13.1 ± 2.4) were also similar to those for the false positives (5.0 ± 6.5; P = .56, Mann-Whitney U test).

After CRH stimulation, median ratios in true-negative cases rose very slightly from 1.30 ± 0.05 to 1.61 ± 0.12, an insignificant change (P = .13, Wilcoxon signed-rank test). Median ratios in false-negative cases, however, increased significantly from 1.28 ± 0.07 to 2.02 ± 0.12 (P = .007, Wilcoxon signed-rank test). Poststimulation ratios were higher in false-negative cases than in true-negative cases (P = .02, Mann-Whitney U test).

Similarly, median ratios in false-positive procedures rose insignificantly from 5.0 ± 6.5 to 12.0 ± 45.2 (P = .63, Wilcoxon signed-rank test). Median ratios in true-positive cases, in contrast, increased significantly from 13.1 ± 2.4 to 51.5 ± 13.2 (P < .001, Wilcoxon signed-rank test). The difference between poststimulation values in these 2 groups, however, did not achieve significance (P = .16, Mann-Whitney U test).

### Exclusion of Low ACTH Values

The lower limit of standard laboratory sensitivity for the measurement of ACTH levels is 1 to 4 pg/mL. Baseline measurements near this limit may be unreliable because even a small change after stimulation may reach threshold and thereby contribute to a false positive. We therefore calculated test performance separately after excluding any procedure in which any measured ACTH value was < 5 pg/mL.

With this minimum requirement, 11 overall procedures were excluded, providing a total of 277 for analysis. There was a new total of 188 true positives (instead of 195) and 3 false positives (instead of 5) and still 12 false negatives and 5 true negatives. The new sensitivity was still 94%, but the specificity was 64% (instead

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**TABLE 2. Inferior Petrosal Sinus Sampling Category-Specific Results**

<table>
<thead>
<tr>
<th>Type</th>
<th>n</th>
<th>Pituitary Source, n (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Remission, n (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>12</td>
<td>11 (92)</td>
<td>9 (82)</td>
</tr>
<tr>
<td>II</td>
<td>21</td>
<td>20 (95)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>III</td>
<td>11</td>
<td>11 (100)</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>42 (95)</td>
<td>35 (83)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number (percentage of per-category total) of patients in whom a pituitary source was determined by either pathology (identification of adenoma) or clinical remission (from laboratory values).

<sup>b</sup>Number (percentage of cases identified as pituitary source) of patients in remission after transsphenoidal surgery, including those undergoing a second operation after either recurrence or a noncurative first operation.

**TABLE 3. Contingency Table of Inferior Petrosal Sinus Sampling (IPSS) Results Compared With Proven Diagnosis**

<table>
<thead>
<tr>
<th>IPSS &lt;sup&gt;+&lt;/sup&gt;, n</th>
<th>IPSS &lt;sup&gt;−&lt;/sup&gt;, n</th>
<th>Total, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>195</td>
<td>12</td>
</tr>
<tr>
<td>Ectopic</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>*</sup>IPSS, inferior petrosal sinus sampling.
of 50%). The positive and negative predictive values were unchanged at 98% and 29%, respectively.

DISCUSSION

We examined our experience with IPSS as a diagnostic procedure in the evaluation of patients with CS. Comparing the results of 288 IPSS procedures in 283 patients with surgically proven diagnoses, we found a high positive predictive value (98%) and sensitivity (94%) but relatively low negative predictive value (29%) and specificity (50%). We paid particular attention to 3 categories of clinical situations that commonly present diagnostic dilemmas in the decision-making process: successful catheterizations that predict ectopic lesions that remain occult despite extensive imaging (type I), unsuccessful procedures that nevertheless predict a central source (type II), and unsuccessful procedures that are not predictive of a central source (type III).

Our results suggest that TSS should be strongly considered when surgeons are faced with a technically successful IPSS that predicts an ectopic source that cannot be located despite extensive imaging (type I). Although early reports of the sensitivity and specificity of IPSS for diagnosing CD were 100%, more recent studies have emphasized higher false-negative rates. Our observed sensitivity and negative predictive value reflect these higher false-negative rates. Given the high prevalence of CD in the subset of patients who have undergone an endocrine evaluation consistent with ACTH-dependent CS preceding IPSS, as well as the relatively low negative predictive value of the test, a negative test result with no other indication of an ectopic source may well reflect IPSS limitations rather than an alternative diagnosis. Earlier in our experience, some of these patients were
treated as harboring a presumptive ectopic source without exploratory TSS, and a number were referred for adrenalectomy. Other series have similarly reported cases that were undiagnosed after a noncentralized IPSS without imaging evidence of a lesion.\textsuperscript{16,28,29} Our results suggest that a substantial fraction of these may in fact represent false negatives and should therefore be strongly considered for exploration if a thorough search for an ectopic tumor is unrevealing. If surgical exploration is not performed, this group of patients should be monitored with serial MRIs for the possible future visualization of a previously undetected pituitary tumor, especially because they may be at risk for the Nelson syndrome if bilateral adrenalectomy is used to control hypercortisolism.

These results are somewhat at variance with previous studies. One reason for the discrepancy may be that previous studies eliminated those patients in whom the final diagnosis was unknown, which would tend to minimize the reported false-negative rate. In addition, advances in imaging and endocrine evaluation over the years may have increased the percentage of those patients with pituitary adenomas in the overall population of patients undergoing IPSS and decreased those with ectopic tumors. If the true-negative rate becomes lower in the sampled population (as those patients are eliminated from IPS sampling by other means) relative to the false-negative rate of the test, it becomes more likely that a negative result will nonetheless be consistent with a pituitary tumor. The low prevalence of ectopic ACTH-producing tumors in this cohort can be considered a major limitation because it may predispose to a favorable outcome of exploratory TSS despite a noncentralized IPSS.

Catheterizations in the 32 type II and III patients were unsuccessful as a result of either technical sampling complications or variant anatomy. The issue of the frequency of anatomically variant sinuses and their association with aberrant IPSS results was previously addressed by Doppman et al.\textsuperscript{22} In a sample of 100 catheterizations, they observed 25% with unilateral or bilateral atretic inferior petrosal sinuses. Reviewing 501 separate patients with surgically proven CD, they found 4 false negatives, all of whom had an anatomically variant sinus. These false-negative values resulted in a delay in therapy of 2 to 3.5 years in 3 of the 4 patients. Our fraction of 16% of patients (46 of 283) with an anatomically variant sinus is similar to their value. Of the 21 centralized procedures in our series with variant anatomy (type II), we observed 20 with surgically proven CD. This finding further supports the notion that the high positive predictive value of a successful IPSS also applies to instances in which samples were obtained from other or variant venous regions; ie, if the ACTH gradient is sufficient to obtain a positive result even at a distant or anomalous site, it must have been at least as steep in the IPS, and therefore TSS can be recommended.

Our results from the 11 type III patients also suggest how to proceed if an unsuccessful procedure results in subcriterion measurements. Our ability to identify a pituitary source remained high even in this group. Given the fact that type I catheterizations produce false negatives, it is not surprising that type III patients do so also because samples obtained from other venous regions or anatomic variants can be expected to demonstrate a lower baseline ACTH level and blunted response to CRH as a result of venous admixture. We therefore advocate strong consideration of surgical exploration in each of these situations to avoid unnecessary and potentially harmful delays.

In our previous examination of our results through 2002, we reported on 185 procedures, 145 of which were successful bilateral cannulations and 40 of which were complicated by technical problems or variant anatomy.\textsuperscript{18} Eight of the procedures (6% of successful procedures) in our previous report were false negatives. Our present series includes an additional 103 procedures, 95 of which were successful. Four of these were false negatives (4% of successful procedures), a similar proportion. Toward the end of the previous study period, we implemented our current practice of surgically exploring patients with CS in whom the imaging and IPSS evaluation are nondiagnostic. Our present results bear out the utility of this strategy in that only 2 of 44 explored patients in the present series did not have a surgically proven pituitary source.

There are several possible reasons for a false-negative IPSS result. As mentioned, nonpituitary venous admixture from an anatomically variant IPS or from a more distal site (owing to venous occlusion or other inability to cannulate the IPS) could dilute the sample, thereby artificially lowering measured ACTH levels. Even when radiographic confirmation of appropriate placement is obtained, however, it is possible that a nonpituitary source could dilute inferior petrosal venous blood. Some have therefore advocated confirming catheter position and pituitary sampling by measuring and normalizing ACTH levels against other pituitary hormones such as prolactin\textsuperscript{30} or thyrotropin-releasing hormone.\textsuperscript{31} False negatives can also occur because of variable responses of pituitary corticotroph adenomas to exogenous CRH, so the poststimulation ACTH levels may not reach the expected threshold. Indeed, false-negative IPSS procedures in this series demonstrated an increase in ACTH after stimulation but to a far lesser degree than did true positives (Figure 2). This finding also suggests that an analysis of the peripheral ACTH response to CRH may be useful because a rise in peripheral ACTH was seen in the false-negative cases but not in the true-negative cases. A further potential contributor to the false-negative rate is our MRI cutoff of 1 cm. Lesions larger that this threshold were referred directly for surgery, but small abnormalities were considered equivocal and referred for IPSS. Lowering this threshold may have reduced false negatives (but also may have led to negative explorations). Other reasons for false negatives include sampling cyclically productive adenomas during a low phase and mis-categorizing borderline ratios owing to random variation or inappropriate sample handling.

Using different IPSS threshold criteria would change the false-negative rate and therefore the sensitivity and specificity of the procedure. The data in Figure 2A suggest that lowering the post-CRH threshold to approximately 1.8, for example, would reduce the number of false negatives (making 6 of them true negatives).
without increasing the number of false positives. We previously performed a receiver-operating characteristic analysis to address this point and arrived at a similar conclusion.\textsuperscript{20} It would be worthwhile to validate alternative thresholds in other data sets, particularly because the composition of our patient population reflects referral bias to our institution, possibly accounting for the relatively small number of ectopic ACTH-producing tumors identified. The diagnostic performance of IPSS would certainly vary, depending on the pretest probability of a pituitary vs ectopic ACTH source.

The characteristics of the false-positive procedures also deserve some attention. We observed 5 in our present series: 1 with a bronchial carcinoid secreting CRH, an extremely rare condition\textsuperscript{21}; 1 each with an ACTH-secreting bronchial carcinoid, adrenal adenoma, and adrenal carcinoma, and 1 undiagnosed. Eleven of the 288 procedures were excluded when considering only those in which ACTH measurements were $\geq 5$ pg/mL. With this minimum requirement, the specificity of IPSS for predicting a central source was 64\% instead of 50\%.

Given our current strategy of surgically exploring patients with ACTH-dependent CS regardless of the success and/or resulting prediction of the IPSS when an ectopic tumor is not found on standard imaging, the utility of performing the procedure at all may be reasonably questioned. We continue to perform IPSS because a positive study has a very high predictive value (98\%), which legitimizes an aggressive surgical approach and confirms the need for pituitary-directed therapy if surgery is unsuccessful. In addition, extensive imaging to search for an ectopic tumor in all cases of ACTH-dependent CS before an IPSS is performed may incidentally uncover abnormalities found on imaging that may lead to morbidity from unnecessary procedures. Given the high positive predictive value of the IPSS and the morbidity of untreated CS, a centralized result provides the rationale for persistence and thoroughness during TSS.

**CONCLUSION**

We sought to determine the most appropriate course of action after nondiagnostic IPSS in patients with CS. We report a significant rate of false-negative IPSS procedures for predicting a pituitary source and suggest that exploratory TSS should be considered despite noncentralized or unsuccessful IPSS when an ectopic source cannot be found despite extensive evaluation and imaging. Recognition of this issue should help reduce deleterious delays in the treatment of patients with CD.

**Disclosures**

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**REFERENCES**

White Matter Fibers, HCP Projection Fibers

White matter fiber architecture from the Connectome Scanner dataset. The fibers are color-coded by direction: red = left-right, green = anterior-posterior, blue = ascending-descending (RGB = XYZ). Laboratory of Neuro Imaging, Jack Van Horn, PhD and Vaughan Greer.