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ORIGINAL ARTICLE

Maximizing the accuracy of Inferior petrosal sinus sampling: validation of the use of Prolactin as a marker of pituitary venous effluent in the diagnosis of Cushing's disease

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Objective This study aimed to increase the accuracy of the inferior petrosal sinus sampling (IPSS) procedure and reduce the false-negative rate through the addition of prolactin as a marker of pituitary venous outflow as well as validate this adjunct to the test process.

Context Inferior petrosal sinus sampling (IPSS) for ACTH is the current gold standard test for the differentiation of pituitary Cushing's disease from the ectopic ACTH syndrome. Although early studies with IPSS reported a diagnostic sensitivity and specificity approaching 100%, additional experience has revealed a false-negative rate of 1–10%. This has been attributed to either technical problems with unsuccessful petrosal sinus catheterization or anomalous venous drainage of the pituitary. Previous studies have suggested that the measurement of other anterior pituitary hormones may be useful during IPSS as a guide to the effectiveness of cannulation and to improve the diagnostic accuracy of the procedure.

Design We reviewed the data, in this retrospective cohort study, for all patients who had undergone IPSS for the investigation of ACTH-dependent hypercortisolism.

Patients The study included 83 patients who underwent IPSS at St. Thomas's hospital between 2005 and 2010.

Measurements Plasma ACTH and prolactin levels were measured both centrally and peripherally. The normalized ACTH/Prolactin inferior petrosal sinus/peripheral ratio was then calculated to assess the accuracy of the sampling procedure.

Results A total of 83 patients with confirmed ACTH-dependent cortisol excess underwent investigation with IPSS during the study period. Sixty-seven patients initially had a positive IPSS result (i.e. a basal central/peripheral ACTH ratio >2·0 and >3·0 post-CRH). However, when the concurrent prolactin data were used, six patients were additionally found to have positive results suggestive of pituitary Cushing's. The Prolactin normalized ACTH IPS/Peripheral ratios were all >0·8 in patients with proven Cushing's disease, whereas they were all <0·6 in proven ectopic ACTH syndrome. The diagnosis was subsequently confirmed histologically in 72 of the patients.

Conclusions Using Prolactin as a concurrent index of pituitary venous effluent helps us recognize whether pituitary venous blood has been accurately sampled. Normalizing the IPS/peripheral ratios with Prolactin helps to improve the accuracy of the result and reduces the false-negative rate. With regards to the usefulness/validity of this test in clinical practice, it is relevant, reproducible and is easily adaptable from the existing diagnostic sequence.

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Introduction

Inferior petrosal sinus sampling (IPSS) for ACTH (adrenocorticotrophin) differentiates pituitary-dependent Cushing's disease from the ectopic ACTH syndrome. The procedure is now widely available and highly accurate but it is technically demanding and it is generally recognized that the procedure should be performed in dedicated centres with a bulk of experience.[1,2]

Petrosal sinus to peripheral (IPS/Peripheral) ACTH ratios >2·0 in the basal state or a post-corticotrophin-releasing hormone (CRH) peak/peripheral ratio >3·0 is considered diagnostic of Cushing's disease in patients with ACTH-dependent cortisol excess.[3–5]

Inferior petrosal sinus sampling carries the risk of brain stem vascular damage, venous thrombosis, pulmonary embolus and cranial nerve palsies.[6] It is best used when there is proven ACTH-dependent Cushing's that may be due to ectopic ACTH secretion or where there is doubt as to whether a pituitary tumour is functioning. Contrary to previous claims, it is generally unreliable in lateralizing the site of ACTH production.[7] Although early studies with IPSS reported a diagnostic sensitivity and specificity approaching 100%, additional experience has revealed false-negative results (rates of 1–10% have been reported).[8,9] These false negatives have been attributed to either technical problems because of unsuccessful catheterization, a corticotroph adenoma not responsive to

CRH, atrophic inferior petrosal sinuses or anomalous venous drainage (such as filling defects of the vertebral venous plexuses).

Even before one starts investigation, the pretest probability that the patient with ACTH-dependent Cushing's syndrome has Cushing's disease is very high, and any investigation must improve on this pretest likelihood. However, as transsphenoidal pituitary surgery is widely accepted as the primary treatment for Cushing's disease, testing should be designed to avoid inappropriate pituitary surgery in patients with ectopic ACTH production. Thus, any test should ideally be set with 100% specificity for the diagnosis of Cushing's disease.

Previous studies have suggested that the measurement of other anterior pituitary hormones may be useful during IPSS, although this remains controversial.[10,11] It is thought to improve the centralizing ability of IPSS by correcting for the influence of nonpituitary blood draining into the inferior petrosal sinus.[2] Our current study uses Prolactin as an index of pituitary venous drainage to confirm correct catheter placement and assist in the identification of a pituitary source of ACTH hypersecretion. On reviewing the literature, this does not appear to have been previously validated as a test method in itself.

Although the majority of patients with noncentralizing inferior petrosal sinus to peripheral ratios have nonpituitary ACTH-secreting tumours, there has been a failure to localize ectopic ACTH producing tumours in a few patients.[12] In 2004, Findling *et al.*[13] described three patients who had surgically proven pituitary ACTH-dependent Cushing's whose peak petrosal sinus to peripheral ratio was <3·0 after CRH, which suggested that they instead had occult ectopic ACTH syndrome. When no clear ectopic source was discovered, they demonstrated that it was possible to diagnose the pituitary Cushing's correctly using a retrospective analysis of the archived samples when adding in the prolactin calculation. They compared the results of IPSS plus Prolactin (IPSS–PRL) in the three patients with those of 44 other patients with surgically proven Cushing's and five subjects with ectopic ACTH-secreting tumours. They were able to show that pituitary venous effluent had not been properly obtained. Using the IPSS–PRL as an index of pituitary venous drainage, they were able to confirm that there was in fact a source of ACTH excess from the pituitary and all the patients were subsequently offered corrective pituitary neurosurgery. In his group's analysis, Findling was able to demonstrate that the peak central/peripheral prolactin measurement is an accurate marker of IPS catheter placement; with a ratio of >1·8 confirming correct placement and a ratio of <1·3 identifying a misplaced catheter.

Our present study seeks to follow on from such work and clarify the utility and validity of the routine addition of prolactin when undertaking IPSS and explore its usefulness in clinical practice.

Methods

We analysed the retrospectively collated data on all patients who had undergone IPSS over a 5-year period at St. Thomas' Hospital between 2005 and 2010 (n = 83). With regards to demographics, the gender breakdown was 22% men, 78% women and the mean age of male patients was 48·1 and 55·2 years for females. The indication for IPSS in this patient group was biochemically proven ACTH-dependent hypercortisolism.

All patients had a clinical history, examination findings and initial biochemical investigations (elevated urinary-free cortisol, overnight Dexamethasone suppression test, low-dose Dexamethasone suppression test or CRH stimulation test) suggestive of Cushings' disease.

The technique at St. Thomas' for IPSS uses catheter insertion under fluoroscopic guidance followed by digital subtraction angiography at the start of the procedure to ensure correct catheter placement and to evaluate intracranial venous anatomy. This is also repeated at the end of the process to ensure that the catheters have not moved out of position. Plasma sampling occurs at fixed points throughout the procedure and includes concurrent measurement of prolactin from the right and left inferior petrosal sinuses during the standard test. This is part of routine clinical practice at our institution. The assumption is that prolactin secretion from the pituitary will be consistent so there should be no differences between prolactin levels on the right and left sides. This allows for enhanced interpretation of the ACTH results because one of the technical issues with IPSS is that unsuccessful blood vessel/sinus cannulation on one or other side can lead to false calculation of central/peripheral and/or right- or left-sided ACTH excess. Venograms are reviewed by a neuro-radiologist both at the time of the study and retrospectively as part of the routine of the multidisciplinary team results meeting to ensure correct placement of the catheter. As part of this current study, all venograms (including ones which would have been classed as negatives) were reviewed by another (independent) neuroradiologist.

ACTH and Prolactin levels were recorded. Basal and post-CRH IPS and peripheral samples were analysed for each patient. The standard criterion for the differential diagnosis of ACTH-dependent Cushing's syndrome is the dominant (peak) post-CRH IPS to peripheral ratio. The dominant ratio was defined as the highest ACTH IPS/Peripheral ratio at 2, 5 or 10 min after the injection of CRH. The basal (pre-CRH) ipsilateral IPS/Peripheral prolactin (PRL) ratios are calculated. Then, the post-CRH dominant petrosal sinus to peripheral ratio is divided by the basal ipsilateral IPS to peripheral PRL concentration.

The normalized ACTH/PRL IPS/peripheral ratio is calculated as follows;

$$\frac{\text{Dominant peak post-CRH IPS:ACTH/Peripheral post-CRH ACTH}}{\text{Ipsilateral basal IPS Prolactin/Peripheral basal Prolactin}}.$$

The analysis undertaken was a validation of the calculation developed in Findling's 2004 work.[13] We are essentially evaluating a previously suggested ratio in a real-world setting within a large patient group. Much of the work performed around the accuracy of IPSS appears to be related to the attempt to lateralize the pituitary tumour/source of ACTH excess and make subsequent pituitary surgery less radical; however, this was not the focus of our work. Our use of the Findling ratio builds on this earlier work and was the basis of our validation study. The calculation appeared to be a logical approach to controlling for central to peripheral discrepancies. It does not make significant assumptions about how

pituitary venous effluent is distributed.[13–15] If we look at selective venous sampling in other situations such as the adrenal gland in the context of hyperaldosteronism, there we see that there are similar, recognized methods for normalizing such ratios.[16,17] The St. Thomas's method collects plasma simultaneously for ACTH and prolactin, it does not take any extra time compared with the standard procedure.

Pituitary surgery was carried out by one of three experienced neurosurgeons, using the transsphenoidal approach. Pituitary adenoma tissue removed during surgery was then processed for pathological and immunohistochemical studies. Cushings' disease was classified as having pituitary tissue, which stained positive for ACTH on immunohistochemical tests and/or biochemical remission of cortisol excess.[1]

Results

Over a 5-year period, 83 patients with biochemical hypercortisolaemia secondary to ACTH excess underwent inferior petrosal sinus sampling to assess for a pituitary source.

Using the traditional data calculation with ACTH alone, 67 patients were found to have a dominant peak ACTH IPS/Peripheral ratio >3·0 (i.e. positive for Cushing's disease) and 16 were believed to be negative.

When the prolactin normalization calculation (ACTH/PRL IPS/Peripheral ratio) was used, six patients who had previously been disconfirmed as having pituitary Cushing's were reassigned as testing positive (ratio >0·8) and this was subsequently confirmed on postoperative histology in five cases (along with biochemical and clinical remission of cortisol excess). One case was indeterminate. Of the remaining 10 cases, these patients showed a ratio <2·0 on the initial analysis and <0·6 with the prolactin normalization calculation. They were subsequently diagnosed with the ectopic ACTH syndrome or in one case cyclical Cushing's.

Given that the presumed gold standard diagnostic test appeared to be inferior to the augmented test method, we chose to calculate the sensitivity and specificity of IPS with and without Prolactin *vs* histological diagnosis of pituitary Cushing's. Tables 1 and 2 outlines the results and the following calculations provide further information on the validity of the test methods.

Table 1. Comparison of inferior petrosal sinus sampling (IPSS) with Histological diagnosis of Cushing's disease

	Histologically proven Cushing's	
	Positive	Negative
IPSS alone		
Positive	66	1
Negative	6	10
Total	72	11

Sensitivity = 66/66 + 6 = 0·916 = 92%. Specificity = 10/10 + 1 = 0·909 = 91%. Positive predictive value = 66/66 + 1 = 0·985. Negative predictive value = 10/10 + 6 = 0·625.

Table 2. Comparison of inferior petrosal sinus sampling (IPSS) plus prolactin *vs* histology for the diagnosis of Cushing's disease

	Histologically proven Cushing's	
	Positive	Negative
IPSS with prolactin		
Positive	72	1
Negative	0	10
Total	72	11

Sensitivity = 72/72 + 0 = 1·0 = 100%. Specificity = 10/10 + 1 = 0·909 = 91%. Positive predictive value = 72/72 + 1 = 0·986. Negative predictive value = 10/10 + 0 = 1·0. The results demonstrate that the addition of prolactin correction calculation reduces the false-negative rate and as such increases the sensitivity of the test as well as the negative predictive value. The one false-positive patient in the IPSS with Prolactin group proceeded to have a pituitary exploration following MDT discussion of his imaging and biochemistry, but his histology yielded negative for ACTH staining.

Discussion

Diagnosis of Cushing's can be a difficult and controversial subject; it could broadly be based on positive histology or on clinical and biochemical improvement after surgery. Even before one starts investigation, the pretest probability that the patient with ACTH-dependent Cushing's syndrome has Cushing's disease is very high, and any investigation must improve on this pretest likelihood. However, as transsphenoidal pituitary surgery is widely accepted as the primary treatment of Cushing's disease, testing should be designed to avoid inappropriate pituitary surgery in patients with ectopic ACTH production. Thus, any test should ideally be set with 100% specificity for the diagnosis of Cushing's disease.

The technique of IPSS and its interpretation can be challenging.[18] It is now clear that false-negative test and to a smaller-degree false-positive test results do occur. This represents a small but meaningful limitation.[10] To minimize this, it is important to ensure the patient is actively hypercortisolaemic at the time of the study and that catheter position is confirmed as bilateral and any anomalous venous drainage noted by venography before sampling.

For some time, researchers have attempted to refine the IPSS process, through comparison with other pituitary hormones. One example includes the use of TRH to stimulate both prolactin and TSH from the pituitary with the intent to prove the adequacy of sampling.[15] Although this technique does not seem to have been widely adopted, it is worthy of note.

Using prolactin as a marker of pituitary venous effluent helps us to recognize that pituitary venous blood has not been sampled accurately. Dilution of petrosal venous blood by a nonpituitary source, depending on how aggressively the catheters are advanced into the petrosal sinus may account for differences in sensitivity. To overcome possible venous drainage or suboptimal catheterization, the peak dominant IPS/Peripheral ACTH ratios were each normalized to the basal IPS/PRL ratios from the corresponding anatomic sites. The PRL-normalized ACTH IPS/peripheral ratios were all >0·8 in patients with proven Cushing's disease, whereas they were all <0·6 in cases of ectopic ACTH secretion.

Despite radiological evidence of appropriate catheter placement, concurrent IPS/Peripheral prolactin ratios indicate that successful IPS venous sampling is not achieved in some cases.

Recently published studies by Mulligan *et al.*[10] and Daousi *et al.*[1] have demonstrated similar results to our own, showing that there is a reduction in false-negative results in IPSS sampling when prolactin and ACTH are measured simultaneously. Cases that would have otherwise been identified as false positives or negatives would have led to misdiagnosis and potentially undergone pituitary surgery unnecessarily. Although dealing with patients in just one major south London teaching hospital setting, our results reflect the consistency and accuracy of the augmented test method when compared with the most reliable, fully diagnostic test that we have – the histology and immunocytochemistry from excised pituitary tissue.

There was one patient in our study group who was thought to be a false positive for IPSS with prolactin as they had negative histology. In the context of biochemical evidence of cortisol excess (in the absence of an ectopic or adrenal source), the patient underwent three transsphenoidal pituitary operations with complete clearance of the fossa followed by radiotherapy treatment. This led to only a partial biochemical cure so arguably represents an indeterminate case and perhaps highlights the difficulty of establishing a final diagnosis in some challenging cases of Cushing's.[24]

Ultimately, in terms of whether IPSS with prolactin is a valid investigation, the answer is subject to several tests; one has to consider whether the test is relevant to clinical practice and this relates to the utility of the test.[20] We have shown that because of its greater sensitivity and the fact it only requires further analysis of the samples already taken during IPSS, then it could be used preferentially to the current form of IPSS.

When compared with the gold standard, IPSS has been viewed as the current best test for the differentiation of Cushing's disease from ectopic ACTH. IPSS plus Prolactin comes out better in our analysis in terms of significantly greater sensitivity, which therefore will reduce the false-negative rate. As a validation study, we were able to include an appropriate spectrum of subjects. We had data on a large number of patients who had already been through several screening tests for ACTH-dependent cortisol excess. Workup and expectation bias has been avoided as this was a retrospective analysis of all patients undergoing the procedure. One difficult point is with regards to the reproducibility of the test. IPSS represents a difficult, expensive, time-consuming procedure associated with some risks and does not tend to be repeated. Patients did not undergo the procedure more than once, so reproducibility per se is hard to confirm. The ultimate aim of the IPSS prolactin variant is to increase the accuracy of the results when the test is performed. The features of the test as derived from this validation study are; sensitivity = 100%, specificity = 91%, positive predictive value = 98%, negative predictive value = 100%.

Conclusions

Given this information, how should IPSS with Prolactin be placed in the context of other potential tests in the diagnostic sequence? IPSS only usually occurs after the confirmation of ACTH-dependent hypercortisolism. The test is traditionally used to confirm the presence of a pituitary source, and the prolactin variant described in this study actually represents only a minor addition to the standard testing procedure.

In conclusion, this study supports the value of measuring prolactin as a surrogate of pituitary venous drainage and demonstrates that it is a valid test, with greater sensitivity and specificity than the current gold standard test, which is IPSS sans prolactin, as in the words of Findling 'it confirms the fidelity of the sampling procedure'.[2,13] It can reduce the false-negative rate in patients with Cushing's disease who do not demonstrate an appropriate central to peripheral ACTH gradient. We therefore propose from the results of this analysis (and others) that this minor variant of the standard test is useful, reliable and applicable to clinical practice.

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Competing interests

Nothing to declare.

Conflict of interests

All authors declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work.

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