Bilateral sequential inferior petrosal sinus sampling with corticotrophin-releasing hormone stimulation in the diagnosis of Cushing’s disease

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Abstract

Objective: The demonstration of a central to peripheral ACTH gradient in a hypercortisolaemic patient is diagnostic of Cushing’s disease. We tried to determine whether single blood samples for ACTH obtained sequentially from each of the inferior petrosal sinuses following human corticotrophin-releasing hormone (hCRH) stimulation can reliably establish such a gradient.

Design: Prospective study.

Patients: Seventeen patients with clinical and biochemical features of Cushing’s syndrome.

Methods: After the administration of hCRH, the patients underwent bilateral sequential inferior petrosal sinus sampling, with a single blood sample obtained from each of the inferior petrosal sinuses sequentially, along with a peripheral venous sample. The petrosal sinus catheter was withdrawn immediately after obtaining a blood sample. Patients did not require indwelling catheters in the petrosal sinuses, nor heparinisation.

Results: Bilateral sequential inferior petrosal sinus sampling correctly identified a pituitary source of ACTH, as shown by a central to peripheral ACTH ratio >2, in all patients in whom the procedure was successfully carried out. All patients underwent transsphenoidal pituitary surgery resulting in remission.

Conclusions: The simplified method of inferior petrosal sinus sampling, using a single sequential sample from each of the inferior petrosal sinuses, following initial hCRH stimulation, is as accurate as the more complex test using multiple bilateral simultaneous inferior petrosal sinus samples. It avoids the use of indwelling cerebral venous catheters and is therefore unlikely to cause brain stem damage.

Introduction

The differential diagnosis of Cushing’s syndrome, in particular the differentiation of pituitary-dependent adrenocorticotrophin (ACTH) hypersecretion from ectopic ACTH secretion, is often difficult. Pituitary imaging with computed tomography or magnetic resonance scanning sometimes identifies an adenoma but the results may be misleading due to high false positive and false negative results. Identifying an ACTH gradient between inferior petrosal sinuses and the peripheral blood is at present the most accurate method of identifying a pituitary source of ACTH hypersecretion (1). This is done by sampling both inferior petrosal sinuses simultaneously for ACTH, using pre-placed catheters, before and after the administration of corticotrophin-releasing hormone (CRH). The demonstration of a central to peripheral gradient in the concentration of ACTH in a hypercortisolaemic patient is diagnostic of a pituitary source of ACTH hypersecretion. Bilateral simultaneous inferior petrosal sinus sampling (BSimIPSS) as presently carried out is technically demanding in time and materials and carries a small risk. Cavernous sinus sampling (2, 3), developed to improve diagnostic accuracy, is even more invasive and complex but provides no advantage over inferior petrosal sinus sampling (4). Jugular venous sampling is less invasive but also less sensitive (5). We have simplified inferior petrosal sinus sampling to make it easier, cheaper and potentially a safer procedure, without any loss in diagnostic accuracy.

Patients and methods

Patients

Seventeen consecutive patients referred to the endocrine service were studied prospectively. There were 4 males and 13 females with a mean age of 38 years (range 22–73) (Table 1). The patients had history and clinical findings typical of Cushing’s syndrome. After 2
days of familiarisation with the metabolic ward and before the initiation of any treatment, they underwent biochemical and dynamic tests as in-patients. This included the measurement of 24 h urinary cortisol, a dexamethasone suppression test, a CRH test, and pituitary and/or adrenal imaging studies. All patients were hypercortisolaemic for at least 2 days preceding petrosal sinus sampling. One patient was excluded as she had no detectable ACTH. Three patients underwent BSimIPSS as previously described (1) and were sampled for 1 h. Thirteen patients underwent bilateral sequential inferior petrosal sinus sampling (BSeqIPSS) with human CRH (hCRH) stimulation. All patients were subsequently shown to have pituitary-dependent ACTH hypersecretion, as shown by cure or remission following pituitary surgery.

Methods

**Inferior petrosal sinus sampling** BSeqIPSS was carried out in the neuro–angiographic suite with minimal or no sedation. hCRH (100 μg) was administered through an indwelling cannula in an antecubital vein at the beginning of the procedure (i.e. at the time of femoral puncture). Under local anaesthesia, a standard femoral vein puncture was made. A 5 French catheter with guide wire was advanced into the right inferior petrosal sinus, guided by digital subtraction imaging with the injection of small quantities of non-ionic water soluble contrast (Lopamidol or Lohexol). Prior to sampling, a venogram was done with gentle injection of contrast material taking particular care to avoid reflux of contrast as far as the cavernous sinus. Blood (4–6 ml) was withdrawn from the petrosal sinus. A check venogram was then done, this time including the ipsilateral cavernous sinus but avoiding the contralateral cavernous sinus if it had yet to be sampled. The venous anatomy was defined at this stage to avoid inadvertent sampling of a large clival vein. The catheter was then withdrawn into the superior vena cava and the procedure repeated on the left side. Finally the catheter was withdrawn and firm pressure applied at the groin puncture site. A peripheral blood sample was taken each time an inferior petrosal sinus was sampled. BSimIPSS was carried out in a similar way but hCRH was given only after both inferior petrosal sinus sampling catheters were in place. Samples were taken simultaneously from both inferior petrosal sinus sampling and a peripheral vein at 0, 2, 5, 10, 20, 40 and 60 min.

**ACTH assay** Samples for ACTH were collected in EDTA tubes on ice, and plasma separated by centrifugation within 30 min and stored at −20 °C until assayed. The ACTH assay used was a pre-extracted radioimmunoassay with an inter- and intra-assay coefficient of variation of 15–19% and 7.5%, using National Institute for Biological Standards and Control (London, UK) ACTH standard 74/555.

Results

Both inferior petrosal sinuses were successfully sampled in all but one patient. All had a central to peripheral gradient of >2.0, and went into remission following transphenoidal pituitary surgery. Eight out of thirteen patients in whom a central to peripheral gradient was demonstrated had a ratio of central to peripheral <2 in one of the petrosal sinuses (Table 1). Patient No. 1 was a technical failure because it was not possible to catheterise her left inferior petrosal sinus. Samples were obtained from the left jugular bulb and the right inferior petrosal sinus, neither of which showed a central to peripheral gradient. She underwent pituitary surgery resulting in cure of her Cushing’s disease.

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Table 1 Patient details with the results of BSeqIPSS following the i.v. administration of 100 μg hCRH. Bold numbers show the higher of the two central to peripheral ratios. Inter-sinus ratio is the ratio of the right and left central to peripheral ratios.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex/age</th>
<th>Right IPS</th>
<th>Peripheral</th>
<th>Left IPS</th>
<th>Peripheral</th>
<th>Central to peripheral ratio</th>
<th>Inter-sinus ratio</th>
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<tbody>
<tr>
<td>1</td>
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<td>86</td>
<td>82</td>
<td>117*</td>
<td>114</td>
<td>1.05</td>
<td>1.04</td>
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<td>2</td>
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<td>177</td>
<td>162</td>
<td>2087</td>
<td>166</td>
<td>1.09</td>
<td>12.55</td>
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<td>2225</td>
<td>184</td>
<td>1987</td>
<td>270</td>
<td>12.2</td>
<td>1.66</td>
</tr>
<tr>
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<td>35</td>
<td>91</td>
<td>32</td>
<td>0.94</td>
<td>2.21</td>
</tr>
<tr>
<td>5</td>
<td>F/60</td>
<td>279</td>
<td>78</td>
<td>63</td>
<td>60</td>
<td>3.56</td>
<td>3.44</td>
</tr>
<tr>
<td>6</td>
<td>M/26</td>
<td>117</td>
<td>121</td>
<td>683</td>
<td>114</td>
<td>0.96</td>
<td>6.21</td>
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<td>F/34</td>
<td>1118</td>
<td>119</td>
<td>69</td>
<td>71</td>
<td>9.04</td>
<td>9.32</td>
</tr>
<tr>
<td>8</td>
<td>F/33</td>
<td>315</td>
<td>201</td>
<td>1000</td>
<td>199</td>
<td>1.57</td>
<td>5.03</td>
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<tr>
<td>9</td>
<td>F/44</td>
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<td>577</td>
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<td>4.48</td>
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<tr>
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<td>1849</td>
<td>179</td>
<td>279</td>
<td>315</td>
<td>10.31</td>
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<tr>
<td>11</td>
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<td>110</td>
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<td>14.62</td>
<td>12.08</td>
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<tr>
<td>12</td>
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<td>4000</td>
<td>720</td>
<td>3970</td>
<td>780</td>
<td>5.56</td>
<td>1.09</td>
</tr>
<tr>
<td>13</td>
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<td>320</td>
<td>167</td>
<td>530</td>
<td>149</td>
<td>1.9</td>
<td>3.56</td>
</tr>
</tbody>
</table>

*Represents a jugular sample as the left inferior petrosal sinus could not be catheterised. IPS = inferior petrosal sinus.
BSimIPSS showed that there was a central to peripheral gradient at baseline and throughout the procedure which peaked at 2 to 10 min after hCRH (Table 2 and Fig. 1).

**Lateralisation of the source of ACTH**

A direct inter-petrosal sinus ratio could not be determined due to the time interval between collection of the two samples. Instead, a ratio between the right inferior petrosal sinus to peripheral, and the left inferior petrosal sinus to peripheral gradient was calculated, and this showed a gradient >1.38 in all cases. The accuracy and usefulness of such lateralisation in this series could not be determined due to the inability to localise or detect an ACTH-secreting adenoma during surgery or on histological examination of the excised pituitary tissue in 6 out of 17 patients.

After the administration of hCRH, it took a mean time of 23 min (range 8–32 min) to obtain a sample from the right inferior petrosal sinus, and a mean time of 50 min (range 30–94 min) to sample the left.

**Discussion**

Our results show that high central to peripheral ACTH ratios are seen at baseline and throughout the sampling period, making it feasible to sample the inferior petrosal sinuses sequentially and demonstrate a central to peripheral gradient. This was confirmed in every patient studied with BSeqIPSS.

The differential diagnosis of Cushing’s syndrome is often difficult and selective venous sampling to detect a source of ACTH hypersecretion has been employed for many years. The earliest attempts at sampling were made by obtaining blood from the jugular vein (6–8) to

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**Table 2** Results of BSimIPSS (ACTH ng/ml) in individual patients following the i.v. administration of 100 μg hCRH at time 0.

<table>
<thead>
<tr>
<th>Sex/age</th>
<th>Site</th>
<th>0</th>
<th>2</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>F/73</td>
<td>Right IPS</td>
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<td>1230</td>
<td>1510</td>
<td>1600</td>
<td>940</td>
<td>590</td>
<td>370</td>
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<tr>
<td></td>
<td>Left IPS</td>
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<td>75</td>
<td>246</td>
<td>174</td>
<td>80</td>
<td>81</td>
<td>88</td>
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<tr>
<td></td>
<td>Peripheral</td>
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<td>43</td>
<td>47</td>
<td>63</td>
<td>82</td>
<td>58</td>
<td>42</td>
</tr>
<tr>
<td>F/32</td>
<td>Right IPS</td>
<td>36</td>
<td>50</td>
<td>56</td>
<td>28</td>
<td>24</td>
<td>26</td>
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</tr>
<tr>
<td></td>
<td>Left IPS</td>
<td>189</td>
<td>780</td>
<td>490</td>
<td>360</td>
<td>232</td>
<td>247</td>
<td>272</td>
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<tr>
<td></td>
<td>Peripheral</td>
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<td>48</td>
<td>37</td>
<td>26</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>F/34</td>
<td>Right IPS</td>
<td>940</td>
<td>2900</td>
<td>5400</td>
<td>5100</td>
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<td>4900</td>
<td>2090</td>
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<tr>
<td></td>
<td>Left IPS</td>
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<td>1980</td>
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<td>5400</td>
<td>4300</td>
<td>3300</td>
<td>2130</td>
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<td>39</td>
<td>58</td>
<td>61</td>
<td>217</td>
<td>120</td>
<td>265</td>
<td>109</td>
</tr>
</tbody>
</table>

IPS = inferior petrosal sinus.

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Figure 1 Ratio of right (R) and left (L) inferior petrosal sinus to peripheral venous ACTH concentrations following the i.v. administration of 100 μg hCRH at time 0. A ratio of 2 or more was considered to indicate a pituitary source of ACTH.
demonstrate a central to peripheral ACTH gradient. It was shown that there was no jugular vein to peripheral blood ACTH gradient in patients with ectopic ACTH production (9) in marked contrast to patients with Addison’s disease or following adrenalectomy for Cushing’s disease (10). Selective sampling of the jugular vein showed evidence of a pituitary source of ACTH in 90% of patients with Cushing’s disease (5). Sampling from one or both inferior petrosal sinuses, but not the jugular bulb, showed a gradient of >2.0 in all patients with Cushing’s disease but not in those with ectopic ACTH secretion (11), thus allowing a clear distinction to be drawn between these two groups (12). However, sampling from a single inferior petrosal sinus can be misleading (13). Unilateral sampling in our series could have been misleading in 6 out of 13 patients.

BSimIPSS avoids the problems associated with jugular vein or unilateral inferior petrosal sinus sampling and invariably identifies a pituitary source of ACTH when the procedure is technically successful (14–17). The petrosal sinus to peripheral gradient may be increased by the administration of CRH (18–21). In a large series, a petrosal sinus to peripheral gradient of 3·0 after CRH administration showed a 100% sensitivity and specificity in those patients in whom Cushing’s disease could be confirmed by surgery (1).

Using BSeqIPSS with hCRH, we were able to demonstrate a pituitary source of ACTH (a central to peripheral ACTH ratio >2) in all patients where the procedure was technically successful. In these cases, the BSimIPSS would have provided no further diagnostic advantage. The three patients who had BSimIPSS had central to peripheral ACTH gradients diagnostic of Cushing’s disease at baseline and throughout the sampling period, with a brief increment following CRH administration. Sequential sampling at any of these time periods would provide the same diagnostic information. As this series did not include any patient with ectopic ACTH syndrome, the specificity of the test could not be validated.

The technique of BSimIPSS is technically difficult and may be complicated by catheter displacement during the procedure, which can invalidate the test. This is avoided in the modified sequential sampling method described as the catheters are not left in situ. A serious potential complication of leaving a cannula in situ in both inferior petrosal sinuses is cavernous sinus thrombosis, a condition which carries a high morbidity and mortality (22, 23). Although BSimIPSS has been performed without heparinisation (21), most groups use heparin to avoid this dangerous complication. However, heparinisation may itself be hazardous in hypercortisolaemic patients, and in the event of perforation of the petrosal sinus, a complication that has occurred during petrosal sinus catheterisation (24) for other indications, anticoagulation may prove catastrophic. Since BSeqIPSS does not require an indwelling catheter in the inferior petrosal sinus, heparinisation is unnecessary. It is of interest that none of our patients developed groin haematoma, unlike those in the reported series in which anticoagulation was used (1). Pulmonary embolism has been reported after BSimIPSS (25) and this may be related to the development of an inguinal haematoma (26). Brain stem injuries that have occurred during BSimIPSS (27, 28) are thought to be due to localised venous hypertension (27) resulting from the temporary venous occlusion due to the indwelling catheter. These injuries include damage to the pons, IVth ventricle, medulla (27) and the pontomedullary junction probably due to venous infarction (29). Although uncommon, the risk of catastrophic brain stem damage cannot be justified for a procedure used to confirm Cushing’s disease in a population in whom the prior probability of this diagnosis is very high, and it does not completely rule out a false positive (30) or negative result (31). Further, in patients with early (32), mild, or pseudo Cushing’s (33), petrosal sinus sampling has poor diagnostic value.

We could not determine the value of inter-sinus ACTH gradient in our series due to the inability to identify and orientate accurately a pituitary adenoma during surgery and on histology in some patients, a problem encountered in other studies (34). Nevertheless, all achieved remission. Several studies have shown that using BSimIPSS with CRH stimulation, lateralisation of the adenoma within the pituitary gland may be achieved (1, 14, 16, 35) in some or all patients, and hemihyphysectomy of the suspected side has been advised if an adenoma cannot be identified during surgical exploration. Others, however, have found lateralisation by inferior petrosal sinus sampling to be misleading (17, 21), and in one report the intra-operative measurement of ACTH in the peri-pituitary blood was used to correct the misleading results from inferior petrosal sinus sampling (36). The value of inferior petrosal sinus sampling in the lateralisation of a pituitary source of ACTH is uncertain. It is likely to remain so due to the frequent asymmetry (seen in 35 to 39%) of pituitary venous drainage (37–39), although this does not impede successful catheterisation (37). Further, lateralisation of secretion of ACTH, arginine vasopressin and oxytocin is seen in the petrosal sinus of normal volunteers. ACTH and arginine vasopressin responses to CRH were similarly lateralised, implying the normal occurrence of asymmetry in the concentrations of ACTH in the petrosal sinus (40). Lateralisation may not be so important as the cure rate is unaffected by the nature and extent of the pituitary lesion or the surgical procedure used (41).

In summary, inferior petrosal sinus sampling can identify a pituitary source of ACTH in patients with clear clinical and biochemical evidence of Cushing’s syndrome. Complications are infrequent but can produce crippling brain stem damage. BSeqIPSS with CRH stimulation is as effective as BSimIPSS in the
diagnosis of Cushing’s disease. This is due to the fact that an elevated central to peripheral ACTH ratio is present at baseline and persists during the sampling period, with a further brief increment following CRH administration. It is technically easier and does not need heparinisation or indwelling cerebral venous catheters. It thus avoids the problems of catheter displacement and local venous hypertension, making brain stem damage unlikely. It is cheaper, requires fewer blood samples, and takes up less procedure time, but should only be done when other investigations prove inconclusive.

References
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