

Dynamic testing in Cushing's syndrome

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Abstract Endogenous Cushing's syndrome (CS) results from chronic exposure to excess glucocorticoids. CS can be ACTH-dependent, caused by ACTH-secreting pituitary or ectopic tumours, or ACTH-independent, caused by cortisol-secreting adrenal tumours. CS can be an extremely difficult diagnosis to make, and assessment will include clinical, biochemical and radiological evaluation. Several screening tests are used for the confirmation of hypercortisolaemia and its differentiation from other, more frequent, clinical abnormalities, such as simple obesity, hypertension, depression etc. Other dynamic tests are useful for establishing the aetiology. We have reviewed the current literature on the diagnosis of CS, and based on these data and our own experience, suggest the most useful tests and diagnostic criteria to be used. We conclude that even though laboratory testing is a fundamental part of the investigation of patients with CS, the interpretation of the tests should always be performed with extreme care, as none of the tests has proven fully capable of distinguishing all cases of CS. The biochemical results should be interpreted jointly with the clinical aspects and the radiology findings in a probabilistic matrix, and not as part of a uniform algorithm.

Keywords Hypercortisolaemia · Urinary free cortisol · Dexamethasone test · BIPSS

Introduction

Cushing's syndrome (CS) results from chronic exposure to hypercortisolaemia. Even though it is a rare disease, with an estimated incidence of 5–6 cases per 10⁶ population per year [1], it is characterised by high morbidity: in its severe form and when untreated, it is associated with a high mortality, approximately 50% at 5 years [2]. Therefore, early diagnosis and establishing the exact aetiology of CS, and prompt management, are of fundamental importance. The diagnosis of CS remains challenging for the clinician as some of the most typical symptoms and signs of CS, such as obesity, hypertension and depression, are non-specific and thus many patients may require screening investigation.

CS can be divided into two categories: ACTH-dependent and ACTH-independent. Cushing's disease, caused by hypersecretion of ACTH from a pituitary adenoma, is the most frequent of the ACTH-dependent forms, accounting for 60–80% of all cases. The remaining cases of ACTH-dependent forms are due to ectopic secretion of ACTH from multiple tumour types, the most frequent being small-cell lung carcinoma. Adrenal adenomas or carcinomas with excessive autonomous cortisol secretion account for 15–20% of the cases of CS. In addition, rare forms of CS include ectopic CRH production, macronodular adrenal hyperplasia, including adrenal hyperplasia secondary to abnormal hormone receptor expression (e.g. gastric inhibitory polypeptide, catecholamines, vasopressin, GnRH), and primary pigmented nodular adrenocortical disease [3–5].

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Biochemical diagnosis of hypercortisolaemia and differential diagnosis

The presence of certain clinical symptoms and signs (central obesity, facial rounding and plethora, wasting of the limbs, spontaneous bruising, hirsutism, muscle weakness, vertebral fractures, hypertension, diabetes mellitus, acne, loss of libido and menstrual irregularity) [6] provide the stimulus for further biochemical and radiological investigation of possible CS. It is then necessary to perform biochemical tests to confirm or refute the diagnosis.

Hypercortisolaemia as revealed by the loss of the normal circadian rhythm of cortisol secretion, and disturbed feedback of the hypothalamo–pituitary–adrenal axis (HPA), are the cardinal biochemical features of CS. The diagnosis is based upon these principles, and confirmation of hypercortisolism must be established before any attempt at differential diagnosis, since the reported sensitivity, specificity and diagnostic accuracy of all tests currently used are valid only during periods of sustained hypercortisolism [7]. The diagnosis of CS is a two-step process; once the hypercortisolaemia is established, the precise cause may be investigated.

To screen for CS, tests of high sensitivity should be used initially, so as to avoid missing milder cases. Tests of high specificity can then be employed to exclude false positives.

A recent international consensus on the diagnosis of CS discussed the use of first-line screening tests and included urinary free cortisol, low-dose dexamethasone suppression tests and late-night salivary cortisol [7].

Urinary free cortisol

Measurement of urinary free cortisol (UFC) has been widely used as a screening test for CS. Approximately 10% of serum cortisol is unbound and physiologically active, and free cortisol is excreted by the kidney. Nevertheless, the majority is then reabsorbed in the renal tubules and only a small percentage, approximately 1%, is excreted unaltered in the urine. Excessive cortisol secretion saturates circulating cortisol binding globulin (CBG), resulting in an increase in UFC [8].

A 24-h UFC collection produces an integrated measure of serum cortisol, smoothing out the variations in cortisol during the day, and it is not affected by factors that influence CBG levels. If the index of suspicion is high and the first result is normal, up to three 24-h urine collections should be performed [7]. If cortisol excretion results are normal in three collections, then CS is highly unlikely, except in the case of cyclical CS [7]. Conversely, UFC values more than 3–4-fold greater than the upper limit of normal are virtually diagnostic of CS [9]. However, the test

requires an adequate 24-h urine collection, possibly with contemporaneous creatinine measurement. In children, the urinary cortisol excretion should be corrected for body surface area/1.72 m². UFC can be measured both by radioimmunoassay (RIA) and high performance liquid chromatography (HPLC) methods, with the latter representing the method of choice. The sensitivity and specificity of this test have been reported to range from 95 to 100%, and 98%, respectively [10]. However, in patients with mild CS, UFC levels may be consistently normal [6], whereas elevated UFC levels may also be found in pregnant women, in 40–60% of depressed patients, in polycystic ovarian syndrome (PCOS), and in other “pseudo-Cushing’s” states, thus reducing its specificity [11, 12]. In our opinion, the published data do not support the use of UFC as a sensitive and specific screening test for CS. Nevertheless, several completely normal collections of UFC, if collected correctly, render the diagnosis of CS very unlikely. Patients with marginally elevated levels require further investigation.

Low-dose dexamethasone suppression tests

Patients with CS have impaired feedback regulation of the HPA-axis. Dexamethasone is a potent glucocorticoid which is not measured in the routine cortisol assays, and its administration results in suppression of the HPA axis in normal individuals and a fall in plasma and urinary cortisol. The overnight dexamethasone suppression test (DST) involves the oral administration of 0.5–2 mg of dexamethasone (most commonly 1 mg) at 23.00 h–24.00 h and measurement of serum cortisol at 08.00 h or 09.00 h the next morning; there appears to be no better discrimination using 2 mg rather than 1 mg of dexamethasone [13]. Due to its ease of administration in an outpatient setting, the 1 mg DST has been advocated by many as the screening test of choice for the demonstration of endogenous hypercortisolism [7]. However a variety of diagnostic cut-offs have been proposed: a 09.00 h serum cortisol of >50 nmol/l (1.8 µg/dl) is suggestive of CS with a sensitivity of between 88 and 100% [14, 15], but probably approaching 100%. Unfortunately, when used in this way the specificity is relatively low, of the order of 80%. In some centres, the 2-day low dose DST (LDDST) (0.5 mg of dexamethasone every 6 h) is used as a first-line screening test instead of the overnight DST. A similar cut-off of an 09.00 h serum cortisol of <50 nmol/l at both 24 and 48 h is used for the 2-day LDDST, which in our experience provides this test with higher sensitivity (98%) than the overnight 1 mg DST [16]. Using the serum cortisol at 48 h alone proves a sensitivity of 95% [10], although others have been less sanguine regarding the

sensitivity of these criteria. False positives can be due to decreased dexamethasone absorption or accelerated hepatic metabolism due to drugs such as phenytoin, carbamazepine, aminoglutethimide, phenytoin, phenobarbitone and rifampicin [17], increased concentration of CBG (pregnancy or estrogen treatment), depression and alcoholism. Nevertheless, in our experience these alternate diagnoses have rarely been proven to be a problem, and we have found its specificity to be extremely high.

Cortisol circadian rhythm

Late-night salivary cortisol

Several studies have documented that failure to decrease serum cortisol at midnight, while the patient is asleep, is associated with a sensitivity approaching 100% in identifying patients with CS; the concentration of cortisol in saliva correlates highly with free plasma cortisol and may be used as an alternative to serum cortisol [18–20], but optimal results have required hospitalisation for 48 h, and thus may offer no advantages over the midnight serum cortisol (see below) [16, 21]. A late night (23.00 h) home-collected salivary cortisol is a simple way to screen for CS and could become increasingly used if it indeed proves to have a sustained high diagnostic sensitivity and specificity [18, 19, 22]. It could be particularly useful in investigating patients with cyclical CS with repeated evening measurements over time. Nevertheless, there is considerable assay variability, no single diagnostic threshold has been validated, and experience in its use is still limited to specialist centres [23]. As it is difficult to sample salivary cortisol in sleeping patients, measurement of morning salivary cortisol following an 1 mg DST may further improve the accuracy of the late night salivary cortisol [24]. Again, we advise against the uniform take-up of this test unless a given centre has achieved its own normal ranges and utility for the particular assay utilised.

The midnight serum cortisol, and the low-dose dexamethasone test if the overnight test has proven positive (see above), and the combined DST–CRH test, are considered only as second-line screening tests in many centres [7].

Midnight serum cortisol

Patients with CS show either a loss of the normal circadian rhythm of cortisol or a rhythm which is set at a higher level [25, 26]. A random serum cortisol measurement is unlikely to be helpful in the diagnosis. Similarly, there is considerable overlap in 09.00 h serum cortisol values in patients with CS and the normal range [27]. This overlap

diminishes throughout the day, such that at 23.00 h only 3.4% of patients with CS have values within the normal range [27]. After excluding any confounding factors such as drugs [28, 29], a single sleeping midnight cortisol was found to be >50 nmol/l (1.8 µg/dl) in all 150 patients with CS, and substantially less in all controls, thus achieving a sensitivity of 100% [16]. Others have adopted a higher cut-off value (7.5 µg/dl, 207 nmol/l) to achieve 100% specificity [18]. The test requires inpatient admission for a period of at least 48 h and it is important that the test is performed in the correct environment. False positive results can occur in the critically ill, in acute infection, heart failure, and in the pseudo-Cushing's state associated with depression [6]. However, it has been reported that the midnight cortisol can be of diagnostic value also in non-sleeping patients, where a cut-off value of 8.3 µg/dl (229 nmol/l) gave a sensitivity of 91.8% and specificity of 96.4%, and was more accurate than the UFC [30].

The combined dexamethasone–CRH (DST–CRH) test

The DST–CRH test was originally designed to distinguish CS from 'pseudo-Cushing's states, and initially a sensitivity and specificity of 98–100% and 96–100%, respectively were reported [12]. Two hours after the last dexamethasone administration of a standard LDDST, ovine-sequence CRH (1 µg/kg) is administered intravenously at 08.00 h; a serum cortisol level greater than 1.4 µg/dl (38 nmol/l) measured 15 min after CRH administration was reported to correctly identify patients with CS [12]. Nevertheless, a series of new studies, performed in different centres, have shown that the addition of CRH to the standard LDDST protocol does not confer any additional diagnostic benefit, because while it has similar sensitivity to LDSST it has lower specificity [31–34]. Overall, in 92 patients without CS in these reports, the specificity of the classical LDDST was 79% (95% confidence interval, 70–86%) compared with a 70% specificity for the DST–CRH test (confidence interval, 60–78%) [35]. In 59 patients with Cushing's syndrome, the sensitivity was 96% for the LDDST and 98% for the DST–CRH test [35]. The diagnostic criteria used differed among the different studies. Overall, the original cortisol criterion of 1.4 µg/dl (38 nmol/l) performed poorly compared with higher cut-off points. Erikson et al. identified ACTH levels at 15 min post-CRH of more than 27 pg/ml (5.9 pmol/l) as a cut-off which would give the best diagnostic accuracy compared to other time-points ACTH or cortisol values [34]. In general, it would appear that there is little significant advantage in the use of DST–CRH test which would compensate for its increased complexity and cost. Its use with human-sequence CRH also requires validation.

Differential diagnosis of ACTH-dependent CS

Following the diagnosis of CS, the next step is to establish the aetiology. Plasma ACTH values greater than 20 ng/l (4 pmol/l) suggest an ACTH-dependent cause. For values between 10 and 20 ng/l (2–4 pmol/l), we have found that a CRH stimulation test with measurement of plasma ACTH is very useful, as patients with ACTH-dependent CS exhibit a peak ACTH response to CRH exceeding 20 ng/l (4 pmol/l) [7, 36]. Imaging of the adrenal glands without further biochemical testing has been advised when an ACTH level of <5 ng/l at 09.00 h is found [36]. Cushing's disease (CD) accounts for the majority of cases of ACTH-dependent CS, between 85 and 90% in most series. Nevertheless, the differential diagnosis from ectopic CS (ECS) remains a challenge, and it is necessary to avoid unnecessary pituitary surgery. Levels of serum cortisol and plasma ACTH tend to be higher in the ectopic ACTH syndrome, but there is considerable overlap of values, producing poor discrimination [10, 37]. In addition, although the presence of POMC precursors, due to partial processing to ACTH, is indicative of the ECS, it is also found in many corticotroph macroadenomas and occasionally in microadenomas, thus limiting its diagnostic accuracy [8]. Hypokalaemia and an associated alkalosis are present in the majority of cases of ectopic ACTH syndrome, but are also seen in 10% of cases of cases of CD [38]; therefore, it has a high sensitivity but low specificity for the ECS. Generally, lower albumin levels, due to very high serum cortisol levels, are found in the ECS [39]. Several tests are used in the differential diagnosis of CD and ECS.

High-dose dexamethasone test (HDDST)

There are a number of versions of the HDDST, including the standard 2-day oral high dose (2 mg every 6 h for eight doses), the 8 mg overnight oral, and the iv 4–7 mg tests [10]. The standard HDDST test consists of administering 2 mg dexamethasone every 6 h for 48 h with measurement of serum cortisol at 09.00 h at 48 h. It is based on the fact that the corticotroph tumour cells in CD retain *some* responsiveness to the negative feedback effects of glucocorticoids, while tumours ectopically secreting ACTH generally do not [37]. A fall of over 50% in the post-dexamethasone serum cortisol compared to the basal value is indicative of CD, with a sensitivity varying from 60–80% and a high specificity, which can be improved further if a cut-off of cortisol suppression of greater than 80% is used [7, 40]. A simplified overnight test has been proposed, using a single dose of 8 mg of dexamethasone at 23.00 h, and comparing serum cortisol levels taken on the morning

before and after administration. Various criteria for suppression have been applied, but this alternative HDDST appears to have poorer discrimination than the original test [41–43]. Lately, the effectiveness of the HDDST has been questioned as on several occasions the sensitivity and the specificity of the test appear to be less accurate than the pre-test statistical likelihood of CD [44]. Recent studies in our institution, comparing directly the performance of the LDDST and HDDST using serum cortisol measurements, both in adult and paediatric patients, revealed that most of the diagnostic information provided by the HDDST could be predicted from the LDDST; if serum cortisol at 09.00 h at 48 h was suppressed by more than 30% in the LDDST, this almost invariably predicted 'adequate' suppression (>50%) to the HDDST (Fig. 1) [45, 46]. Due to these inherent limitations of the test, some authors have suggested the abandonment of the HDDST as it provides little diagnostic advantage in the differential diagnosis of ACTH-dependent CS in relation to other tests.

The corticotrophin releasing hormone (CRH) stimulation test

Non-pituitary tumours, in contrast to the normal pituitary corticotrophs, usually do not express CRH receptors and exhibit an absent ACTH response to CRH [36]. There is no

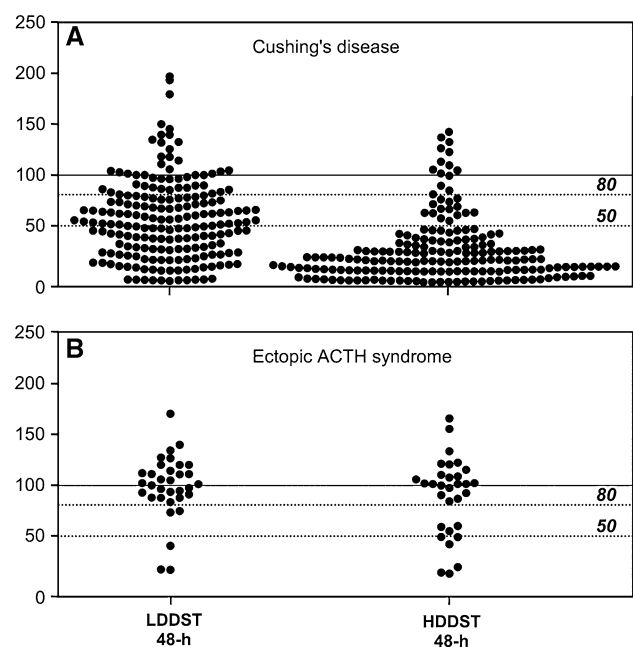


Fig. 1 Percentage of baseline of serum cortisol levels 48 h after 2 mg/d dexamethasone (LDDST 48 h) or 8 mg/d dexamethasone (HDDST 48 h) according to the etiology of CS. (Reproduced with permission from Isidori AM et al. (2003) J Clin Endocrinol Metab 88:5299–5306)

consensus as yet on the criteria for interpreting the response to CRH test. Variability in the interpretation depends on the type of CRH used (human [h] versus ovine [o]), biochemical parameters considered (increase above baseline in ACTH, 35–50%; versus cortisol, 14–20%) and the time points evaluated (ACTH, 15–30 min; cortisol, 15–45 min) [47–49]. With the oCRH test, blood for serum cortisol and plasma ACTH is taken at –15, –10, –1, 5, 15, 30, 45, 60 min after an injection of 1 µg/kg oCRH at 09.00 h. The best differentiation between CD and ECS is obtained with a maximum increase in ACTH of at least 35% [50]. This gives the test a sensitivity of 93% and a specificity of 100%. A maximum serum cortisol increase of at least 20% produced a sensitivity of 91% and a specificity of 88% [50]. hCRH has qualitatively similar properties to oCRH, although it is shorter-acting and it elicits a smaller rise in cortisol and plasma ACTH [47]. hCRH 100 µg is injected over 30 s, with blood for plasma ACTH and serum cortisol taken at –15, 0, 15, 30, 45, 60, 90, 120 min. A maximal rise in cortisol above baseline of at least 14% showed a reported sensitivity of 85% with 100% specificity (Fig. 2) [48]. In a study from Italy, oCRH was compared to hCRH test and a maximal 50% increase in ACTH and cortisol levels gave a 100% specificity. The sensitivity for the ACTH response was comparable for the two types of CRH (85 and 87% for oCRH and hCRH respectively); the sensitivity for the cortisol response was significantly greater with oCRH than with hCRH (67 versus 50%) [49]. However, because ectopic ACTH-producing tumours can also respond to CRH, increasing the cut-off level of the response will not produce 100% specificity, thus preventing complete reliance on this test. It should also be noted that many normal subjects will show little or no response to

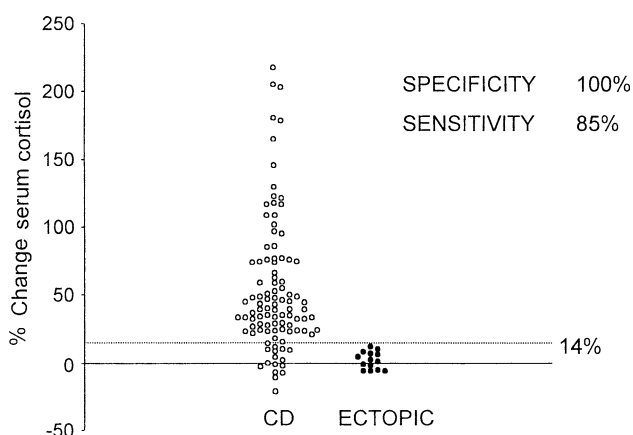


Fig. 2 Percentage change in serum cortisol from a mean basal at –15 and 0 min to a mean value calculated from the levels at 15 and 30 min after the administration of hCRH (100 µg iv) in 100 patients with CD and 14 patients with the EC. (Reproduced with permission from Newell-Price J et al. (2002) J Clin Endocrinol Metab 87:1640–1645)

CRH if their basal levels are sufficiently high, such that the test cannot be used to diagnose ECS unless the *prior* diagnosis of CS has been established without doubt.

Vasopressin, desmopressin, hexarelin tests

Lysine or arginine vasopressin (AVP) stimulate ACTH release via the specific corticotroph AVP receptor, the V3 receptor, particularly from ACTH-secreting pituitary tumours. It has been used for the differential diagnosis of ACTH-dependent CS; however, this test appears to be inferior to the CRH stimulation test [51] and has significant adverse effects including abdominal pain, nausea and flushing. Desmopressin (DDAVP, Ferring), a preferential vasopressin receptor V2 and V3 agonist, causes a significant rise in ACTH and cortisol in most patients with CD and only in occasional patients with the ECS [52]. Nevertheless, its overall sensitivity and specificity are lower than with the CRH test. It has also been used in combination with CRH and appeared to increase the discriminatory value above either test alone [53], but more recent data have cast doubt on the clinical value of the combined test [54]. It therefore remains to be seen whether the co-administration of CRH and desmopressin presents an improvement over the standard CRH stimulation test. Nevertheless, desmopressin might be useful in the discrimination between CD and pseudo-CS [55] where it seems to have a higher diagnostic effectiveness with respect to the DST-CRH test [33]. Hexarelin is a growth hormone secretagogue that also stimulates ACTH release, possibly through vasopressin secretion in normal subjects [56]. In a small series it was shown to be able to distinguish CD from adrenal or ectopic cases [57]. Interestingly, there is a response to hexarelin only in microadenomas, but not in macroadenomas [58]. Growth hormone secretagogue receptors have nevertheless been demonstrated in ectopic ACTH-secreting tumours [59, 60] and GH secretagogues can also stimulate ACTH secretion in these tumours [61]. Therefore, it is doubtful whether hexarelin and other GH secretagogues can be recommended for standard clinical practice.

Bilateral inferior petrosal sinus sampling (BIPSS)

None of the non-invasive tests mentioned above seems to achieve the desirable diagnostic accuracy approaching 100%. Bilateral simultaneous inferior petrosal sinus sampling (BIPSS) with CRH stimulation provides the most accurate and reliable means of distinguishing pituitary from non-pituitary ACTH-dependent CS, and currently represents the ‘gold standard’ in the differential diagnosis

of CS [62, 63]. The procedure should be performed when cortisol levels are elevated, indicating currently active secretion of ACTH by the tumour, and avoiding testing during an inactive cycled-out phase of CS. BIPSS is practised to confirm a central source of ACTH, and also plays some role in the lateralization of the site of ACTH hypersecretion [36].

In practice, after the radiologist catheterises both inferior petrosal sinuses (IPSs), blood samples for ACTH are obtained in the basal state and at 3–5 min (and at 10 min in some centres) after iv oCRH (1 µg/kg) or hCRH (100 µg) simultaneously from both IPSs and a peripheral vein. A central (inferior petrosal) to peripheral plasma ACTH gradient (IPS/P), usually taken as 2:1 basally or 3:1 after CRH, is consistent with CD. In addition, the sensitivity of the test can be increased by the measurement of intravenous-CRH-stimulated plasma ACTH levels [64]. A review of all major published series analysing the results of BIPSS revealed an overall sensitivity of 96% and specificity of 100% when applying the criteria of a basal and CRH stimulated ratio of greater than 2 and 3, respectively, in distinguishing CD from the ECS [10]. Thus, overall, BIPSS is probably the most sensitive tool in the investigative armoury for the diagnosis of CD, and appears to have a specificity approaching 100%, using appropriate criteria [65]. It should be noted, however, that false positive tests in patients with ectopic ACTH syndrome have been reported, possibly due to cyclicity [66]. A combination of CRH and desmopressin has been used to improve the diagnostic accuracy of BIPSS; while a previous study reported a false positive response during this procedure [62], a more recent study from Kaskarelis et al. reported a sensitivity of 97.9% and specificity of 100% when a cut-off value for IPS/P gradient greater than 2 was chosen, and the test was 100% accurate in a subgroup of patients with previous contradictory HDDST/CRH test results [63]. Similar results have been published by Castinetti et al., reporting a sensitivity of 95% [67]. A slightly lower sensitivity (92.1%), with the same specificity (100%), was recently reported by Machado et al. [61].

The usefulness of BIPSS for lateralizing a pituitary adenoma secreting ACTH is limited and has been a matter of controversy [68–70]. A gradient of 1.4 or greater between both sides of pituitary has been used as a predictor of tumour location; however, in the same combined literature review, the diagnostic accuracy of BIPSS was found to range between 50 and 100% [10]. The reliability of this technique may be improved by venography at the time of catheterisation. We have found it to be particularly helpful in children, when imaging is rarely of great use [71].

Although BIPSS is well-tolerated, very occasional side effects have been reported including brainstem vascular damage and haematomas, deep vein thrombosis and

pulmonary emboli [13]. Since the results of BIPSS as well as the incidence of these adverse events are related to the experience of the radiology team, this procedure should be performed only in specialised centres. In recent years, different sites of venous sampling (cavernous and jugular veins) have been used. If one or both IPS are not adequately catheterised, an ACTH ratio of greater than 2 obtained between the high internal jugular to peripheral veins can be associated with 100% specificity in diagnosing CD [62]. It is unlikely that cavernous sinus catheterisation is worth the extra cost and potential risk.

Conclusions

The diagnosis of CS is a complex process and remains a challenge for the endocrinologist. CS is a relatively rare disease and because of the lack of specificity of many of its symptoms, efficient screening procedures are necessary. Demonstration of pathological hypercortisolaemia is required with first-line screening tests such as the UFC, dexamethasone tests or midnight salivary cortisol, although we recommend the standard 48 h low-dose dexamethasone test; in many cases confirmatory tests such as midnight serum cortisol will be necessary. The establishment of the differential diagnosis of CS is best performed in specialised centres: this will require assessment of plasma ACTH and one or other dynamic test such as the CRH test, but we currently recommend the use of BIPSS in all cases except (possibly) in the presence of an obvious pituitary macroadenoma. Laboratory testing is a fundamental part of the investigation of patients with CS, but the interpretation of the tests should always be done carefully as none of the tests has proven fully capable of distinguishing all cases of CS, and the biochemical results should be interpreted jointly with the clinical aspects and the radiology findings.

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