CLINICAL STUDY

Biochemical screening for subclinical cortisol-secreting adenomas amongst adrenal incidentalomas

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Abstract

Objective: Biochemistry and 131I-6β-iodomethyl norcholesterol scintigraphy (IMS) have both been used to assess cortisol secretion by adrenocortical incidentalomas. However, which biochemical abnormalities indicate subclinical corticoid excess is still debatable whilst IMS is expensive and cumbersome. The aim of the study was to evaluate prospectively patients with adrenal incidentalomas using both IMS and biochemical methods to examine whether the IMS pattern is associated with biochemical abnormalities and, if this is so, to find a biochemical parameter that could be used as a screening test to identify a subset of patients on whom IMS could subsequently be performed.

Methods: Thirty-one patients with benign cortical adenomas were recruited from 43 consecutive patients with adrenal incidentalomas. All 31 patients underwent IMS and measurement of (i) 0800 h serum cortisol, ACTH, dehydroepiandrosterone and 17-hydroxyprogesterone; (ii) midnight serum cortisol; (iii) 2400 h excretion of urinary free cortisol; (iv) cortisol after the overnight 1 mg dexamethasone (DEX) suppression test; (v) cortisol after an i.v. 4 mg DEX test; (vi) determination of the diurnal variation in serum cortisol.

Results: Sixty-one per cent of patients displayed unilateral uptake during IMS and 39% showed bilateral uptake. Patients with unilateral uptake exhibited significantly lower ACTH concentrations (P < 0.005), higher midnight cortisol concentrations (P = 0.02), disrupted diurnal variation of serum cortisol (P = 0.02) and higher cortisol concentrations after DEX suppression tests (P = 0.01). Cortisol concentrations following the two DEX suppression tests correlated closely (r = 0.80, P = 0.0001). The i.v. 4 mg DEX test was clearly more sensitive for the diagnosis of unilateral uptake than the overnight 1 mg DEX test (76 vs 52%). Using various thresholds of cortisol concentration following the overnight 1 mg DEX test, it was found that the sensitivity of the test could be improved to 100% if the threshold was set at 60 nmol/l rather than the classical value of 138 nmol/l. All patients but one with post-test serum cortisol concentrations above 60 nmol/l as against none of patients with cortisol below 60 nmol/l exhibited at least one associated biochemical abnormality indicating subclinical glucocorticoid excess.

Conclusion: In adrenocortical incidentalomas, unilateral uptake during IMS suggests subclinically excessive and/or autonomous cortisol secretion. A cortisol concentration above 60 nmol/l following the overnight 1 mg DEX test is highly correlated with unilateral uptake and is associated with biochemical abnormalities indicating subclinical glucocorticoid excess. Our results favour the use of the 1 mg overnight DEX test with revised criteria of interpretation as a screening test for subclinical hypercortisolism among patients with adrenocortical incidentalomas.

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Introduction

The widespread application of non-invasive high resolution imaging techniques has led to the increased detection of incidentally discovered adrenal masses (1, 2). Most of these are benign adrenocortical adenomas (1). Recent studies have shown that, although not associated with the specific clinical symptoms of Cushing’s syndrome, a number of incidentalomas produce excessive amounts of cortisol and can suppress the activity of the hypothalamo–pituitary–adrenal (HPA) axis at various intensities. It is not yet clear
whether secretory autonomy in these subclinical cortisol-secreting adenomas (SCSA) leads to clinical morbidity. This uncertainty questions the need to treat and hence to diagnose SCSA amongst adrenal incidentalomas (3). It is likely that a minority of these tumours will lead to overt Cushing’s syndrome with time. However, data from the literature suggest that some patients with SCSA exhibit a predisposition to obesity, hypertension, osteoporosis and diabetes mellitus and may benefit from adrenal surgery (4–12). While waiting for long-term prospective studies evaluating precisely the outcome of patients with this condition and how aggressively we should diagnose SCSA, it seems reasonable to evaluate glucocorticoid secretion in patients with adrenal incidentalomas with a diameter above 1 cm.

The diagnostic strategy to be used to identify SCSA amongst adrenal incidentalomas remains controversial. Various biochemical criteria alone or in association have been employed across different studies whilst what constitutes subclinical corticoid excess has not yet been accurately defined (3, 5, 6, 8, 10–15). A number of arguments favour the use of 131I-6β-iodomethyl norcholesterol scintigraphy (IMS) to diagnose SCSA. From a historical point of view, IMS is the tool that allows recognition of this entity before exclusive radiocholesterol (RC) uptake by the mass (16, 17). Two studies have pointed out that this uptake pattern during IMS is associated with a greater degree of functional autonomy (18, 19). In two other recent studies, exclusive uptake by the mass or an asymmetrical concordant pattern appear to indicate a risk of disease progression and adrenal insufficiency after removal of the tumour (5, 10). In addition, IMS provides useful information about the likelihood of the malignancy of incidentalomas (18, 20). However, IMS is cumbersome and its cost precludes its widespread use in the investigation of adrenal incidentalomas that display the features suggestive of benign cortical adenomas during computed tomography (CT) scanning.

The aim of the present study was, therefore, to evaluate prospectively patients with adrenal incidentalomas using both scintigraphic and biochemical methods and to correlate the results of both approaches in order to: (i) examine if the pattern of RC uptake is associated with the level of hormonal dysfunction and/or autonomy and, if this is so, (ii) find a biochemical parameter that could be used in a screening test, further investigations being subsequently performed on a subset of selected patients.

Subjects and methods

Subjects

Forty-three consecutive patients (24 women and 19 men; mean age 58 ± 13.8 years, range 24–80) with adrenal incidentalomas of more than 10 mm (range 12–52 mm) in diameter were recruited over a 22-month period. Among these, 31 (19 women and 12 men) had abdominal CT scan features suggesting benign cortical adenomas (size <50 mm, homogeneous content, density on unenhanced CT scan less than 10 Hounsfield Units). Two patients had bilateral masses. None of the patients displayed specific clinical features of Cushing’s syndrome. Hypertension, obesity and diabetes were highly prevalent among the patients (48, 51 and 16% respectively) but were not considered to be specific symptoms of hypercortisolism.

The diagnosis of benign adenoma was confirmed in three cases by surgery. The aetiology of the remaining tumours, based on the results of biopsy, surgery or endocrine investigations, was: phaeochromocytoma (two cases), haematoma (three cases), benign cyst (two cases), schwannoma (one case), metastasis of kidney or gastric cancer (two cases), leiomyosarcoma (one case) and cystic lymphangioma (one case). One patient refused further investigation.

Methods

Scintigraphy The IMS was performed in basal conditions without administration of dexamethasone (DEX). In all cases, 37 MBq (1 mCi) of 131I-6β-iodomethyl norcholesterol (Norchol-131; CIS Bio International, Gif sur Yvette, France) was injected intravenously. Lugol’s solution was administered daily, 2 days prior to and throughout the week of scanning in order to suppress thyroid accumulation of 131I. Adrenal imaging was performed using a gamma camera equipped with a high-energy, parallel-hole collimator. Posterior and anterior abdominal images (15 min/image) were obtained on day 5 after the tracer injection. A mild laxative (Colopeg, Macrogol, Nicholas Gaillard, France) was given (173 g daily) beginning 2 days before imaging and on the day of imaging. Scan interpretation was done qualitatively by a nuclear medicine physician well experienced in the field of adrenal imaging. Two types of interpretation were defined: unilateral uptake concordant with the adrenal mass, with no visualisation of the contralateral gland; bilateral uptake whether it was symmetrical or prevalent on one side.

Hormonal evaluation Patients with suspected benign cortical adenomas underwent the following hormonal evaluation: (i) measurement of the 0800 h serum cortisol, adrenocorticotrophin (ACTH), dehydroepiandrosterone sulphate (DHEAS) and 17-hydroxyprogesterone (17-OHP) concentrations; (ii) measurement of the midnight serum cortisol concentration; (iii) measurement of the 2400 h excretion of urinary free cortisol (UFC); (iv) determination of the diurnal variation in serum cortisol concentration by the ratio of the mean of four measurements at 30-min intervals around
DHEAS concentrations in serum was 2–14 pmol/l respectively. The lower limit of normal for concentrations at 0800 h were 200–700 nmol/l and commercially available kits: serum cortisol (Coat a Count; DPC, Los Angeles, CA, USA); urinary cortisol (Cis Bio International); serum ACTH (Brahms, Berlin, Germany); serum DHEAS (Immunotech, Marseille, France); serum 17-OHP (Biomerieux, Lyon, France).

The normal range for serum cortisol and ACTH concentrations at 0800 h were 200–700 nmol/l and 2–14 pmol/l respectively. The lower limit of normal for DHEAS concentrations in serum was >1 μmol/l for subjects younger than 60 years and >0.5 μmol/l for subjects older than 60 years. The normal range for serum 17-OHP concentrations was 1.8–6.2 nmol/l in males and 0.5–2.6 nmol/l in postmenopausal females or during the follicular phase. The upper limit for normal for midnight cortisol concentration was set at the 95th percentile of values measured in 30 controls (228 nmol/l). The normal range for 2400 h UFC was 20–100 μg/24 h. A per cent ratio for cortisol of less than 50% between the concentration recorded around midnight and around 0800 h was considered to be a normal diurnal rhythm. The classical 138 nmol/l threshold was used in the first instance to analyse the results of the overnight 1 mg DEX suppression test (22). In accordance with the original study of Abou Samra et al. (21), the response to the i.v. 4 mg DEX test was evaluated on the 0800 h serum cortisol concentration following the infusion of DEX (normal range obtained in 12 control subjects <27–66 nmol/l).

### Statistical analysis
Results are given as the means ± s.d. For statistical purposes, the value corresponding to the limit of detection of assays was used for undetectable concentrations of several endocrine parameters (e.g. 27 nmol/l for serum cortisol; 1.1 pmol/l for ACTH; 0.27 μmol/l for DHEAS; 0.3 nmol/l for 17-OHP). Comparison between variables was performed using the Mann–Whitney test. Regression analysis was performed using Spearman rank’s test. The level of statistical significance was set at \( P < 0.05 \).

### Results
Among the 31 patients studied, 19 cases (61%) demonstrated unilateral uptake on the side of the adrenal mass (group I) and 12 cases (39%) demonstrated bilateral uptake (group II) during IMS. The two patients with bilateral tumours exhibited bilateral uptake. Tumour diameter was greater in group I than in group II (30.5 ± 8.6 vs 24.1 ± 9.2 mm; \( P = 0.04 \)) (Table 1).

A number of biochemical parameters were similar within patients of the two IMS groups (Table 1) such as the 2400 h UFC, and 0800 h serum cortisol, 17-OHP and DHEAS concentrations.

In contrast, a number of biochemical parameters differed significantly between the two groups (Table 1): (i) Patients in group I displayed lower 0800 h serum ACTH concentrations (2.4 ± 1.3 vs 4.9 ± 1.9 pmol/l; \( P = 0.0005 \) (Fig. 1a). Eight patients in group I (42%) but none in group II had low 0800 h serum ACTH concentrations. (ii) Midnight serum cortisol concentrations were higher in group I than in group II (234.5 ± 134.5 vs 139.3 ± 69.4 nmol/l; \( P = 0.02 \) (Fig. 1b). Seven patients in group I (36%) and only one patient in

### Table 1 Characteristics of patients (group I, unilateral uptake during IMS; group II, bilateral uptake during IMS) with adrenal incidentalomas according to the scintigraphic pattern. Values are means ± S.D.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group I</th>
<th>Group II</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59.8 ± 13.2</td>
<td>61.1 ± 12.7</td>
<td>( P = 0.91 )</td>
</tr>
<tr>
<td>Tumour size (mm)</td>
<td>30.5 ± 8.6</td>
<td>24.1 ± 9.2</td>
<td>( P = 0.04 )</td>
</tr>
<tr>
<td>UFC (μg/24 h)</td>
<td>72 ± 85.8</td>
<td>39 ± 21.0</td>
<td>( P = 0.63 )</td>
</tr>
<tr>
<td>Cortisol at 0800 h (nmol/l)</td>
<td>496.2 ± 145.3</td>
<td>459.0 ± 99.6</td>
<td>( P = 0.54 )</td>
</tr>
<tr>
<td>17-OHP at 0800 h (nmol/l)</td>
<td>2.2 ± 1.5</td>
<td>2.0 ± 1.2</td>
<td>( P = 0.87 )</td>
</tr>
<tr>
<td>DHEAS at 0800 h (μmol/l)</td>
<td>1.6 ± 2.7</td>
<td>1.8 ± 1.4</td>
<td>( P = 0.18 )</td>
</tr>
<tr>
<td>ACTH at 0800 h (pmol/l)</td>
<td>2.4 ± 1.3</td>
<td>4.9 ± 1.9</td>
<td>( P = 0.0005 )</td>
</tr>
<tr>
<td>Cortisol at 2400 h (nmol/l)</td>
<td>234.5 ± 134.5</td>
<td>139.3 ± 69.4</td>
<td>( P = 0.02 )</td>
</tr>
<tr>
<td>Midnight–morning cortisol ratio (%)</td>
<td>0.51 ± 0.30</td>
<td>0.29 ± 0.11</td>
<td>( P = 0.02 )</td>
</tr>
<tr>
<td>Cortisol post</td>
<td>191.5 ± 164.9</td>
<td>64.2 ± 52.0</td>
<td>( P = 0.0009 )</td>
</tr>
<tr>
<td>1 mg DEX (nmol/l)</td>
<td>160.6 ± 143.9</td>
<td>52.4 ± 32.5</td>
<td>( P = 0.01 )</td>
</tr>
<tr>
<td>4 mg DEX (nmol)</td>
<td>160.6 ± 143.9</td>
<td>52.4 ± 32.5</td>
<td>( P = 0.01 )</td>
</tr>
</tbody>
</table>
group II (8%) had elevated midnight serum cortisol concentrations. (iii) The mean midnight to morning serum cortisol concentration ratio was significantly higher in group I than in group II (195.1 ± 164.9 vs 64.2 ± 52.0 nmol/l; P = 0.0009) (Fig. 1c). Similar findings were also observed with the i.v. 4 mg DEX suppression test (160.6 ± 143.9 vs 52.4 ± 32.5 nmol/l; P = 0.01). The serum cortisol concentrations following the two DEX suppression tests correlated closely (r = 0.80; P = 0.0001). Thirteen patients (76%) in group I and only three patients in group II (25%), including the two patients with a bilateral adenoma, did not suppress adequately following the i.v. 4 mg DEX test. Using the classical 138 nmol/l threshold for cortisol serum concentrations following the overnight 1 mg DEX suppression test (22), 52% of patients in group I and 17% of patients in group II (corresponding to the two patients with a bilateral adenoma) exhibited non-suppressibility. The sensitivity of the i.v. 4 mg DEX test for the diagnosis of unilateral uptake was therefore 76% and 52% for the 1 mg DEX suppression test.

The difference in sensitivity between the two DEX suppression tests prompted us to re-examine the characteristics of the overnight 1 mg DEX suppression test using different thresholds for post-test serum cortisol concentrations (Table 2). A 100% sensitivity was obtained with a 60 nmol/l threshold. Using this cut-off value, the specificity of the test decreased from 83 to 67%. However, the series included two patients with bilateral tumours of roughly similar size that were therefore likely to display bilateral uptake. When taking into consideration (i) patients with unilateral tumours at CT scanning and (ii) the ten patients with non-adenomatous incidental adrenal tumours who also underwent overnight 1 mg DEX suppression test, the specificity of the overnight 1 mg DEX suppression test for the prediction of unilateral uptake using the 60 nmol/l threshold increased to 85% (Table 2).

Patients with serum cortisol concentrations below 60 nmol/l after the overnight 1 mg DEX suppression test had no associated specific biochemical abnormality of the HPA axis (e.g. abnormal suppressibility to the i.v. 4 mg DEX suppression test, elevated UFC, elevated midnight serum cortisol concentrations, disrupted diurnal rhythm of serum cortisol concentrations or low 0800 h ACTH concentrations). On the contrary, among patients with post-test serum cortisol concentrations above 60 nmol/l only one patient (4%) had no associated abnormality while 57% of these displayed one additional biochemical abnormality of the HPA axis and 39% of patients displayed at least two additional biochemical abnormalities.

The serum cortisol concentration after the overnight 1 mg DEX suppression test correlated positively with the midnight serum cortisol concentration (r = 0.54; P = 0.003) and correlated inversely with the morning serum ACTH concentration (r = -0.55; P = 0.003). The size of the tumour correlated positively with the

![Figure 1](a) Individual serum ACTH concentrations (note the logarithmic scale) in patients with unilateral and bilateral IMS uptake. Four patients in the group had values below the limit of detection of the assay. Horizontal bars indicate mean values. (b) Individual midnight serum cortisol concentrations in patients with unilateral and bilateral IMS uptake. Horizontal bars indicate mean values. (c) Individual 0800 h serum cortisol concentrations following the overnight 1 mg DEX suppression test in patients with unilateral and bilateral IMS uptake. Horizontal bars indicate mean values. The closed circles correspond to patients with bilateral tumours.
midnight serum cortisol concentration ($r = 0.45; P = 0.01$). By contrast, the correlation between the size of the tumour and the morning serum ACTH concentration indicated no statistical significance ($r = -0.34; P = 0.07$).

**Discussion**

Studies show that the prevalence of SCSA among adrenal incidentalomas varies from less than 5% to approximately 50% (5, 6, 8, 12–15, 18, 19, 23, 24). Although SCSA comprise a heterogeneous group with a wide spectrum of hypercortisolism and autonomy, the variability in diagnostic criteria used among studies may account mainly for the variability in the estimated prevalence of these tumours. Both biochemical and scintigraphic methods can be used to evaluate the HPA axis among patients with adrenocortical incidentalomas.

Various biochemical criteria have been used to try to define what constitutes abnormal cortisol secretion among adrenal incidentalomas. For example, Terzolo et al. (14) required the association of an elevated UFC and a serum cortisol level above 138 nmol/l after an overnight 1 mg DEX suppression test to define subclinical hypercortisolism. Reincke et al. (12) defined subclinical hypercortisolism as the presence of serum cortisol concentrations greater than 80 nmol/l after a short 3 mg DEX suppression test and confirmation with a high-dose DEX (8 mg) suppression test. Tsagarakis et al. (15) identified SCSA with serum cortisol concentration above 70 nmol/l following a low-dose DEX suppression test. Clearly, the main limitation of biochemical diagnosis is the absence of a gold standard test and consensus about which test (or tests) should be performed in clinical practice.

From a historical point of view, IMS is the tool that allowed the identification of SCSA amongst adrenal incidentalomas. Bierwaltes et al. (16) first suggested that the pattern of unilateral uptake corresponded to partial suppression of the HPA axis by the adenoma and showed that ACTH administration could elicit normal iodocholesterol uptake in the contralateral adrenal. This was confirmed by Charbonnel et al. (17), Gross et al. (25), using adrenal venous sampling, demonstrated a larger cortisol output from the tumour side with frequent contralateral adrenal suppression. More recently, two studies suggested that IMS is useful in characterising the functional status of adrenocortical incidentalomas (18, 19). The results of our study confirm and expand the data of Bardet et al. (19) and Barzon et al. (18). Although various degrees of hyperfunction were found within each group, our data suggest that patients with unilateral uptake, taken as a whole, have a higher degree of cortisol secretion and autonomy than those with bilateral uptake.

In our study, serum DHEAS and 17-OHP concentrations did not correlate with the scintigraphic pattern. The relationships between DHEAS and 17-OHP concentrations on the one hand and between these two parameters and the aetiology of adrenocortical incidentalomas (non-functioning vs cortisol-secreting) on the other hand remain controversial (3, 5, 19, 26–29). Indeed, low DHEAS concentrations are not restricted to patients with silent hypercortisolism and are frequently associated with benign adrenocortical tumours irrespective of their functional status. Therefore, the reduction in serum DHEAS concentrations may not be fully explained by blunted ACTH secretion (5, 14, 23). Furthermore, low DHEAS levels are difficult to interpret in patients older than 60. These steroid abnormalities may be the consequence of complex dysregulation of intra-tumoural enzymes that are not at present fully understood (29). Similar conclusions have been reached with 17-OHP (5, 24). In our study, UFC was elevated in a minority of patients (21%) with unilateral uptake. Although UFC is the gold standard for the diagnosis of Cushing’s syndrome, our study confirms its lack of sensitivity for the detection of subclinical disorders of the HPA axis (12, 30).

Apart from its usefulness for the assessment of the likelihood of malignancy of incidentalomas (1), the investigation of the functional status of adrenocortical incidentalomas and the identification of patients at risk of progression of cortisol secretion are two major arguments in favour of the use of IMS among patients with incidentalomas. More recently, patients without biochemical evidence of subclinical Cushing’s syndrome.
syndrome but unilateral uptake at IMS and/or a bilateral asymmetrical concordant pattern, were reported to exhibit adrenal insufficiency following adrenalectomy (5). However, the usefulness of IMS in predicting evolution towards complete hyperfunction has to be confirmed (10). Furthermore, IMS is cumbersome, exposes patients to a significant level of irradiation and is expensive. These major drawbacks preclude its use in a systematic way for screening patients with adrenocortical incidentalomas. The second aim of our study was thus to correlate biochemical abnormalities to scintigraphic patterns in order to identify a single convenient and inexpensive endocrine investigation that could be used in clinical practice to screen patients with adrenal incidentalomas.

On first analysis, the suppressibility of serum cortisol after the i.v. 4 mg DEX test was the biochemical parameter which correlated most closely with the scintigraphic pattern. These findings are in accordance with those of Tsagarakis et al. (15) which emphasised the usefulness of the low-dose DEX suppression test (LDDST) for the assessment of subtle glucocorticoid autonomy in patients with adrenal incidentalomas by linking the response to this test to that of other indicators of the status of the HPA axis. The convenience of the LDDST as well as the i.v. 4 mg DEX test in out-patients (0.5 mg DEX every 6 h for 48 h) is, however, questionable. The overnight 1 mg DEX suppression test was introduced in 1965 by Nugent et al. (31) for the ambulatory screening of Cushing’s syndrome. In our study, there was a close correlation between the serum cortisol concentrations after the i.v. 4 mg DEX and overnight 1 mg DEX suppression tests. However, the sensitivity of the overnight 1 mg DEX suppression test for the prediction of unilateral uptake using the ‘classical’ cut-off value for serum cortisol concentrations (e.g. 138 nmol/l) was clearly lower than that of the i.v. 4 mg DEX test. Recent controversy has emerged with regard to the cut-off values to be used, since the criteria of what constitutes an abnormal response following the test have been largely anecdotal (11, 15, 29, 30). Indeed, the ‘classical’ 138 nmol/l cut-off value was elaborated in the 60s, based on the Mattingly fluorometric assay, which overestimates serum cortisol concentrations as compared with current immunoassays (32). Using current immunoassays, serum cortisol concentrations after LDDST are clearly lower than the values quoted in earlier reports (11, 15). This is also true for the overnight 1 mg DEX test (30). Huizenga et al. (33) have shown recently that among 216 elderly individuals, only 9.3% had serum cortisol concentrations above 50 nmol/l, and 2.3% above 140 nmol/l. Similar results have been published by several groups (34, 35). These data clearly indicate that with currently used immunoassays, and in the absence of drugs or pathological conditions that can alter DEX suppression, a serum cortisol concentration above 50–60 nmol/l is likely to indicate subtle alterations of the HPA axis (30, 33–36). In the present study, lowering the cut-off value for post-DEX serum cortisol concentration to 60 nmol/l increased the sensitivity for the prediction of unilateral uptake to 100%. As expected, lowering the cut-off level decreased the specificity of the test. However, two patients with bilateral uptake also had bilateral tumours, a condition frequently associated with alterations in cortisol secretion (18). Indeed, when taking into consideration (i) patients with unilateral tumour at CT scanning and (ii) the ten patients with non-adenomatous adrenal tumours who also underwent overnight 1 mg DEX suppression test, the specificity of the overnight 1 mg DEX suppression test for the prediction of unilateral uptake increased to 85%. Since maximum sensitivity is the aim of a screening strategy, and assuming that patients do not take drugs or have associated pathological condition known to induce spurious lack of suppression (30), the characteristics of the 1 mg overnight DEX suppression test with revised criteria of interpretation are, in our hands, compatible with its use as a first line of investigation in patients with adrenocortical incidentaloma. Due to the adrenal origin of the pathological cortisol secretion and since the overnight 1 mg DEX test has shown low specificity in some studies, other investigators recommend the use of a higher dose of DEX (3 mg instead of 1 mg) to reduce false positive results (6, 12, 29). However, even with the use of a 3 mg DEX dose, there is still a need for confirmatory investigations, including a high-dose DEX suppression test (12).

The small sample size of the groups in our series is another important limitation and a major concern is how arbitrary is the 60 nmol/l cut-off limit. However, one must note that serum cortisol concentration following the 1 mg DEX suppression test correlated with several other parameters of HPA function. Contrary to patients with adequate suppressibility to 1 mg DEX, all but one patient with post-test serum cortisol concentration above 60 nmol/l had at least one additional biochemical abnormality of the HPA axis. Therefore, using our assay, a serum cortisol concentration above 60 nmol/l after the overnight 1 mg DEX suppression test is highly suggestive of adrenal hyperfunction and may help to identify an early stage of functional autonomy.

Several studies have found significant correlation between the size of tumours and post-DEX serum cortisol concentrations and/or other biochemical parameters of autonomy (3, 10, 19). This suggests that tumoral cells have an intrinsically limited secretory activity and that the overall cortisol production and degree of autonomy depend, at least in part, on the size of the tumour. Consistent with this hypothesis, the mean of the size of tumours in group I of our study was significantly higher than in group II. We found borderline but non-significant correlation between the size of tumours and the morning ACTH concentration,
a finding that may be due to the small number of patients studied. However, the size of the tumour correlated positively with the midnight serum cortisol concentrations. These findings, together with the recent demonstration of increased risk of progression of endocrine abnormalities in patients with adrenocortical adenomas >30 mm in size (10), suggest that the size of the incidentaloma is an important parameter in considering the extent of additional investigations.

In conclusion, assuming that patients do not take drugs or have associated pathological conditions known to affect the activity of the HPA axis and/or the metabolism of DEX (30), our study shows that a serum cortisol concentration above 60 nmol/l following the overnight 1 mg DEX suppression test is highly correlated with unilateral uptake during IMS and with other associated biochemical abnormalities of the HPA axis. These findings support the use of the overnight 1 mg DEX suppression test with this threshold as a screening test in patients with adrenocortical incidentalomas in order to select patients eligible for further evaluation of the HPA axis. Which confirmatory tests should be performed in clinical practice is an important and controversial issue. In our opinion, IMS is not mandatory in this complementary work-up: the malignancy of the incidentaloma can often be excluded with the use of CT scanning only (37); IMS is an expensive and complex procedure that exposes the patient to significant irradiation and does not add significant information to a well-done biochemical work-up. With regard to the low specificity of the 1 mg DEX suppression test reported in some studies, we recommend the performance of a confirmatory suppression test using higher doses of DEX (LDDST, 8 mg suppression test or i.v. 4 mg DEX test) (11, 12, 21, 30).

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