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Discriminatory Value of the Low-Dose Dexamethasone Suppression Test in Establishing the Diagnosis and Differential Diagnosis of Cushing's Syndrome

ANDREA M. ISIDORI, GREGORY A. KALTSAS, SHAHID MOHAMMED, DAMIAN G. MORRIS, PAUL JENKINS, SHERN L. CHEW, JOHN P. MONSON, G. MICHAEL BESSER, AND ASHLEY B. GROSSMAN

Department of Endocrinology, St. Bartholomew's Hospital, London ECIA 7BE, United Kingdom

Cushing's syndrome requires a screening test of high sensitivity, followed by biochemical evaluation of the source of the tumor when the cause is ACTH dependent. The high-dose dexamethasone suppression test is still in common use as an aid in differential diagnosis, although its value has been queried. We have routinely used the low-dose dexamethasone suppression test for many years in the diagnosis of Cushing's syndrome but noticed that patients with pituitary-dependent Cushing's syndrome or Cushing's disease, usually showed some degree of suppression of their serum cortisol, compared to those with the ectopic ACTH syndrome. We therefore analyzed retrospectively the serum cortisol responses during the low-dose dexamethasone suppression test and the high-dose dexamethasone suppression test in 245 patients with ACTH-dependent Cushing's syndrome and compared the diagnostic utility of each test either alone or in combination with a standard test using CRH. Evaluation of the serum cortisol response at 24 and 48 h during the low-dose dexamethasone suppression test correctly identified 98% of patients with ACTH-dependent Cushing's syndrome and distinguished between pituitary and ectopic causes with a sensitivity of 82% and a specificity of 79%. In the same patients, the serum cortisol response to the high-dose dexamethasone suppression test had a slightly higher sensitivity (91%) and specificity (80%). However, the combined criteria of a more than 30% suppression of serum cortisol during the low-dose dexamethasone suppression test and/or a more than 20% increase in the CRH test had a significantly higher sensitivity (97%) and specificity (94%) than either the high-dose dexamethasone or the CRH tests alone in the differential diagnosis of ACTH-dependent Cushing's syndrome. It produced equivalent information to that when high-dose and CRH test results were combined. We therefore conclude that in our patient series, the serum cortisol response during the low-dose dexamethasone suppression test is highly sensitive in diagnosing Cushing's syndrome and, combined with the results of the serum cortisol response to the CRH test, offered a safe and cost-effective test in the differential diagnosis of ACTH-dependent Cushing's syndrome. There does not appear to be any necessity for retaining the high-dose dexamethasone suppression test in this diagnostic work-up. (*J Clin Endocrinol Metab* 88: 5299–5306, 2003)

ACTH-DEPENDENT CUSHING'S syndrome (CS) results from excessive cortisol secretion characterized by the loss of the normal feedback mechanisms and circadian rhythm of the hypothalamo-pituitary axis due to inappropriate secretion of ACTH from a pituitary tumor or an ectopic source (1). The clinician investigating patients with Cushing's syndrome has to be prepared to deal with equivocal test results: the first challenge the clinician encounters is to discriminate between mild Cushing's syndrome and a pseudo-Cushing's state such as certain patients with obesity or depressive illness; the second challenge in the differential diagnosis of ACTH-dependent hypercortisolism is identifying and locating the ACTH-secreting tumor (2–6).

Because increasing attention has been placed on early diagnosis of CS and the number of patients screened for hypercortisolism increases steadily, the clinician dealing with the work-up of these patients requires a simple and convenient test to screen a large number of patients and one that is also highly sensitive in identifying patients with very mild or preclinical Cushing's disease (CD).

The high-dose dexamethasone suppression test (HDDST) has long been one of the most useful tests of biochemical differential diagnosis of CS, based on the observation that in pituitary-mediated disease, ACTH secretion tends to retain some degree of responsiveness to glucocorticoid negative feedback, whereas tumors responsible for ectopic production of ACTH tend not to do so. However, the accuracy of the HDDST test has been reported to remain unsatisfactory (2, 5–19). Furthermore, it requires subjecting the patient with cortisol excess to the further burden of high doses of a drug with predominant glucocorticoid, but also some mineralocorticoid, activity. Over the last few years, the CRH stimulation test has gradually gained momentum as a useful dynamic test in the differential diagnosis of CS (11, 20–23). However, diagnostic errors using the CRH test occur with a frequency of 7–15% (2, 14, 21–26). Therefore, neither the HDDST nor the CRH stimulation tests alone consistently distinguish CD from the ectopic ACTH syndrome. For this reason, direct sampling from the venous drainage of the pituitary adenoma (the inferior petrosal sinuses), bilateral inferior petrosal sinus sampling (BIPSS), has been developed (27, 28). Although the BIPSS is an extremely powerful tech-

Abbreviations: AUC, Area under the curve; BIPSS, bilateral inferior petrosal sinus sampling; CD, Cushing's disease; CI, confidence interval; CS, Cushing's syndrome; EAS, ectopic source of ACTH; HDDST, high-dose dexamethasone suppression test; LDDST, low-dose dexamethasone suppression test; ROC, receiver operating characteristic curve.

nique for establishing the central origin of ACTH, it is an invasive technique that requires expertise, is expensive, and may not be free of hazard.

Because of the continuing need for improved noninvasive means of establishing endogenous CS to distinguish pituitary from ectopic ACTH-producing tumors, we reevaluated retrospectively the usefulness and accuracy of the HDDST, compared with the CRH test. In addition, we investigated whether combining the results of the low-dose dexamethasone suppression test (LDDSTs) with results form the CRH-stimulation test would achieve the same or a higher diagnostic accuracy than the HDDST in the differential diagnosis of ACTH-dependent CS.

Patients and Methods

Patients

Cases were drawn from those entered in the St. Bartholomew's Hospital Cushing's Database. Between 1964 and 2001, 413 patients with CS were investigated in the Department of Endocrinology of St. Bartholomew's Hospital, and the case records were entered in the database according to the etiology of hypercortisolism. Of these 413 patients, 60 had an adrenal adenoma, 30 had an adrenal carcinoma, five had macronodular adrenal hyperplasia, and 318 patients were classified as having ACTH-dependent CS: 274 of pituitary origin (CD) and 44 from an ectopic source of ACTH (EAS). Of the 318 patients with the ACTH-dependent CS who were potential candidates for this study, 73 were not entered because full results of serum cortisol levels during the LDDST, HDDST, or CRH test performed within the single institution of St. Bartholomew's Hospital were not available. Of the 245 patients entered into the study, 119 have been included in our previous publications (11, 16, 26, 29–34).

The diagnosis of CS was based on clinical features of hypercortisolism, absence of a circadian rhythm of serum cortisol in terms of an elevated midnight sleeping cortisol level, and lack of suppression of cortisol after an oral 2-mg 48-h LDDST for serum cortisol, according to a published departmental protocol (1, 35). Test results obtained in clinical centers other than St. Bartholomew's Hospital were taken into account in the clinical decisions but were not included in the current analysis. All biochemical investigations were assayed within the same institution. The decision to treat CS according to the suspected etiology represented a collective clinical judgment by the endocrinologists, radiologists, and surgeons on the basis of all investigations. A confirmed diagnosis of CD was made on the basis of demonstration of a corticotroph adenoma at pathological examination after surgery, resolution of clinical and biochemical abnormalities after pituitary surgery or pituitary irradiation, and/or pituitary tumor growth documented by pituitary scanning after medical treatment or bilateral adrenalectomy (36). All patients with EAS reported in this study had histological confirmation on tissue obtained either from primary lesions or metastases during surgical removal or diagnostic biopsies or autopsy pathology specimens. The study was approved as an institutional case-note review subject to departmental authorization. Informed written consent for the use of spare plasma for research samples was obtained from every volunteer participating in the study.

Diagnostic tests

All tests were performed according to an established departmental protocol (35).

LDDSTs and HDDSTs. A blood sample for serum cortisol measurement was obtained at 0900 h on d 0 (basal value). Dexamethasone orally at a dose of 0.5 mg for the LDDST and 2 mg for the HDDST was administered strictly every 6 h (at 0900, 1500, 2100, and 0300 h) for 48 h, commencing immediately after the first blood sample was taken. Blood samples at 0900 h after 24 and 48 h after the first dose of dexamethasone were collected for serum cortisol measurement. A serum cortisol value of less than 50 nmol/liter during the LDDST was taken as indicative of complete cortisol suppression (1). In 164 patients, the HDDST followed the LDDST immediately after the 48-h blood sample was obtained; in these cases the basal serum cortisol value of the LDDST was considered the basal value for the HDDST (2).

CRH test. A blood sample for serum cortisol measurement was taken at −15 and 0 min before and 15, 30, 45, 60, 90, and 120 min after the administration of 100 μg of human-sequence CRH iv. These data have recently been published in full (33).

Assays

All hormonal assays were performed at the Department of Chemical Endocrinology at St. Bartholomew's Hospital. Serum cortisol was measured by an in-house unextracted nonchromatographic RIA from 1982 until 2000: The coefficient of variation at 100 nmol/liter and 1000 nmol/liter was 6%. From the year 2000, serum cortisol was measured by competitive immunoassay format on the fully automated Technicon Immuno-1 analyzer (Bayer, Newbury, Berks, UK), using an immunomagnetic particle separation step and alkaline phosphatase for enzymatic generation of a colored complex quantified using absorbance at 405 nm. The lower limit of detection of serum cortisol concentration was set at less than 50 nmol/liter. These two assays were validated against each other using control sera and found to be equivalent. Before 1982, a fluorometric assay had been used for a few years after replacement of urinary steroid profiles. This assay proved to be equivalent to subsequent assays down to 150 nmol/liter, but below this level there was nonspecific fluorescence. Only a very small number of patients were assessed with this assay, and none was used to validate the absolute threshold criterion of less than 50 nmol/liter.

Statistical analysis

Estimates of sensitivity [true-positive results/(true-positive results + false-negative results)], specificity [true negative results/(true-negative results + false-positive results)], and diagnostic accuracy were determined for serum cortisol at various response levels. In the differential diagnosis, the presence of pituitary disease was considered to be a positive response (disease), and the presence of ectopic ACTH production was considered to be a negative response (nondisease).

To compare individual end points of the two tests at multiple levels of steroid stimulation or suppression without the bias of predetermined criteria, we constructed univariate curves of the receiver operating characteristic curves (ROCs) by plotting the sensitivity against (1 − specificity) at each stimulation or suppression level using dedicated software. We calculated the area under the curve (AUC) by interpolating the point of each response level into the best-fitting hyperbola by means of the least square method and calculated the integral of the curve falling between 0 and 1. The AUC represents the inherent accuracy of the test end point independent of the criteria; the chosen cut-off values were those offering highest specificity and sensitivity. The Kruskal-Wallis test was used for comparisons between independent groups. The Wilcoxon matched-pairs signed-rank sum test was used to compare within-subjects data. Correlation between variables was described using Spearman's rank correlation coefficients. All values quoted are means ± SD. Significance was taken as $P < 0.05$.

Results

Two hundred forty-five patients with hypercortisolism were unequivocally diagnosed as having CD (n = 209) or EAS (n = 36). The frequency of EAS in the entire series was 36 of 245 (15%). The availability of cortisol levels at each time point of the tests is reported in Table 1. All the patients included in this study presented at least one detectable midnight serum cortisol value measured when the patient was asleep in the hospital. The 24- and 48-h response of serum cortisol to the LDDST was also evaluated in 30 normal healthy volunteers (M:F, 13:17; mean age, 35 ± 6 yr; mean body mass index, 27 ± 2.1).

TABLE 1. Availability of test results in various patients according to the etiology of CS

Test	CD (n = 209)	EAS (n = 36)
LDDST (24 h)	159	30
LDDST (48 h)	194	33
HDDST (24 h)	156	29
HDDST (48 h)	187	32
CRH test	123	20

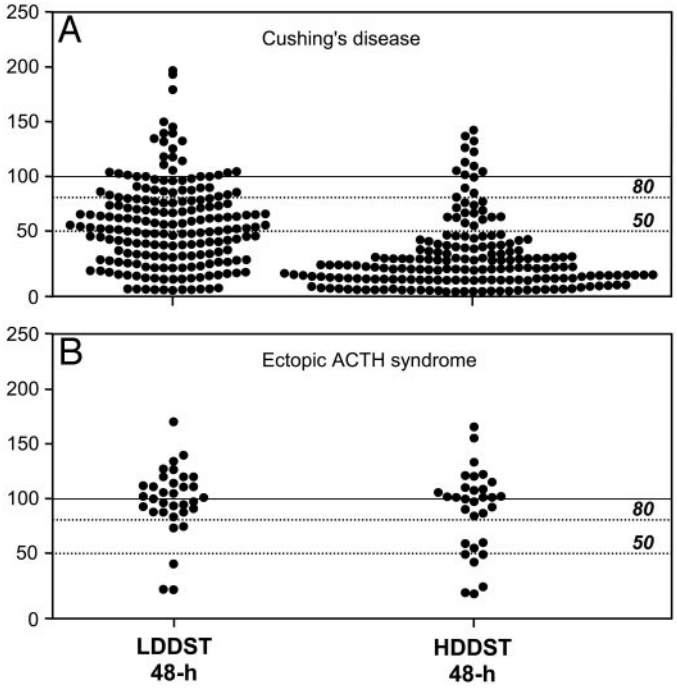

FIG. 1. Percentage of baseline of serum cortisol levels 48 h after 2 mg/d dexamethasone (LDDST 48 h) or 8 mg/d dexamethasone (HDDST 48 h) according to the etiology of CS.

The LDDST

In patients with CD, baseline serum cortisol values were 629 ± 225 nmol/liter, which decreased to 387 ± 276 nmol/liter 24 h after commencement of the LDDST [0 h *vs.* 24 h, $P < 0.001$; mean suppression, 39.4%; confidence interval (CI), 33.5–45.2%] and remained suppressed at 387 ± 290 nmol/liter after 48 h of 2 mg dexamethasone/d (24 h *vs.* 48 h, $P > 0.05$) (Fig. 1). Overall, 67% (107 of 159) of the patients with CD obtained maximal suppression of baseline serum cortisol levels at 24 h rather than 48 h, with the remaining 33% of patients showing a further suppression (>5% of the 24-h value) at 48 h (Fig. 2).

Patients with EAS had significantly higher mean serum cortisol values, compared with patients with CD ($P < 0.001$), and showed no suppression during the LDDST. Serum cortisol levels were 1221 ± 864, 1147 ± 950, and 1229 ± 1008 nmol/liter, respectively, at 0, 24, and 48 h of the LDDST. The mean percentage change from baseline was, respectively, 5.1% (CI, −3.0 to 13.13%) and 3.5% (CI, −8.7 to 15.7%) (Figs. 1 and 2).

As noted above, all patients with EAS failed to suppress serum cortisol levels during a LDDST, whereas 14 patients with CD suppressed baseline serum cortisol levels to undetectable levels (<50 nmol/liter) at 48 h. In addition, six patients with CD suppressed serum cortisol to an undetectable level at 24 h; among these, two patients had detectable cortisol levels at 48 h. Overall, the sensitivity of the LDDST to diagnose hypercortisolism in patients with ACTH-dependent CS who showed failure to suppress cortisol levels at either 24 or 48 h during a LDDST was 98%, which is significantly higher ($P < 0.05$) than that obtained considering the 48-h value alone (94%). Of the four patients who had suppressed cortisol to an undetectable level at both 24 and 48 h during the LDDST, three had positive histology for an ACTH-staining pituitary adenoma at transsphenoidal surgery, whereas one was symptomatically and biochemically cured after transsphenoidal hemihypophysectomy. Of the 10 patients who had detectable cortisol levels at 24 h but undetectable levels at 48 h, six patients had positive histology for an ACTH-staining pituitary adenoma, whereas the remaining four patients resolved clinical and biochemical abnormalities after pituitary surgery and pituitary irradiation.

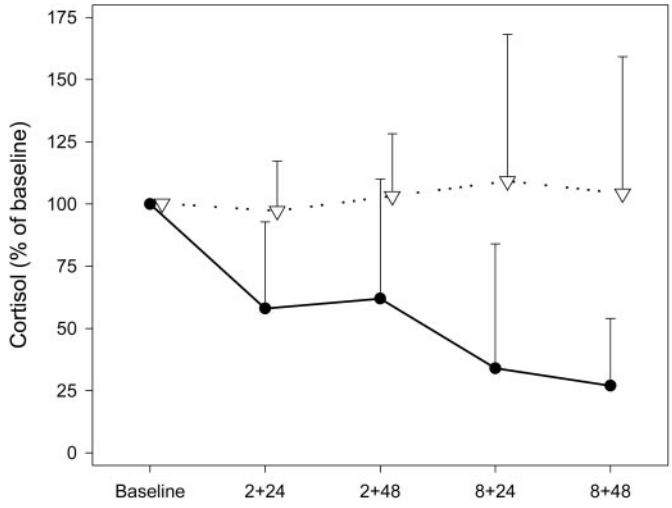

FIG. 2. Percentage change of serum cortisol levels during the consecutive LDDST+HDDST in 135 patients with CD (●) and 29 patients with ectopic ACTH-syndrome (△). The time of blood sample collection is shown as 2 + 24: at 0900 h after 24 h of 2 mg/d; 2 + 48: at 0900 h after 48 h of 2 mg/d; 8 + 24: at 0900 h after 24 h of 8 mg/d; 8 + 48: at 0900 h after 48 h of 8 mg/d.

All control subjects suppressed baseline serum cortisol values (273 ± 85 nmol/liter) to an undetectable level at both 24 and 48 h of the LDDST.

The HDDST

In patients with CD, baseline serum cortisol values were 588 ± 337 nmol/liter, which decreased to 220 ± 299 nmol/liter 24 h after commencement of the HDDST [0 *vs.* 24 h, $P < 0.001$; mean suppression, 72% (CI, 68.1–76.6%)] and remained suppressed at 215 ± 323 nmol/liter after 48 h of 8 mg dexamethasone/d (24 h *vs.* 48 h, P = NS). Patients with EAS had significantly higher mean baseline serum cortisol values, compared with patients with CD (Fig. 1, $P < 0.001$), and showed no suppression during the HDDST. Serum cortisol levels were 1266 ± 881, 1119 ± 813, and 1232 ± 973 nmol/liter, respectively, at 0, 24, and 48 h of the HDDST. The mean percentage change from baseline was 4% at 48 h (CI, −24 to

16%). In patients with CD but not in patients with EAS, the degree of cortisol suppression achieved with the HDDST was significantly higher than that obtained with the LDDST ($P < 0.001$). Figure 2 shows the percentage change from baseline of serum cortisol in patients with CD and EAS who underwent a consecutive LDDST and HDDST.

In patients with CD, a significant fall in serum cortisol was observed after 24 h of 2 mg dexamethasone/d ($P < 0.001$), with no substantial change at 48 h ($P > 0.1$); a further reduction was observed when the dose was increased to 8 mg/d during the HDDST, but, similar to the LDDST, no significant change was observed between the 24- and the 48-h values of the HDDST (Fig. 2). In patients with EAS, the percentage change from baseline was not statistically different at any time.

In patients with CD, the degree of cortisol suppression obtained during the LDDST was highly correlated with that obtained during the HDDST ($r = +0.54$, $P < 0.01$; Fig. 3). A greater than 30% suppression during the LDDST predicted a greater than 50% suppression during the HDDST in 98% of cases, and a greater than 50% suppression during the LDDST predicted such a response in the HDDST in 99% of cases (Fig. 3).

The CRH stimulation test

The response to the CRH test was analyzed as the percentage change of the mean cortisol values at −15 and 0 h, compared with the mean cortisol values at 15 and 30 h after administration of human-sequence CRH on the basis of recently published data (26). The mean percentage changes were 54% (CI, 45–63%) and 3% (CI, −1–7%) in patients with CD and EAS, respectively.

The majority of patients with CD (99 of 123, 80.5%) exhibited a 20% or more increase above baseline in serum cortisol levels after CRH administration, compared with 1 of 20 (5%) patients with EAS (95% specificity for the diagnosis of CD).

The differential diagnosis of the ACTH-dependent CS

ROCs were developed for the LDDST, HDDST, and CRH test for the differential diagnosis between CD and EAS. Because in patients with CD suppression of serum cortisol levels occurs at both 24 and 48 h, ROC curves were developed for the 24-h value (Fig. 4A), the 48-h value (Fig. 4B) and the mean of 24 and 48 h values of the LDDST (Fig. 4C). The ROC generated using individual cut-off values were then regressed to the best-fitting hyperbola ($y = y_0 + ax/b + x$). The area under each curve was calculated resolving the integral of hyperbola for the interval between 0 and 1; the calculated value of the AUC was then used as estimate of the accuracy of the test. The best cut-off values to discriminate between different etiologies are reported for each time point of the LDDST. A 15% or more suppression at 24 h of the LDDST had a sensitivity of 77% and specificity of 79% for the diagnosis of CD. A 20% or more suppression at 48 h of the LDDST had a sensitivity of 74% and specificity of 84% with an ROC-AUC of 0.80; suppression of 15% or more measured on the mean of the 24 and 48 h values had a sensitivity of 79%, specificity of 82%, and AUC of 0.85.

Analysis of the ROC curve of the HDDST revealed that, with respect to the standard criterion of 50% or more suppression, the sensitivity was 85% with a specificity of 81% (Fig. 4D); however, the optimal cut-off value was a greater than 60% suppression, which had a sensitivity of 80% and a specificity of 90% in the diagnosis of EAS. The AUC for the ROC of HDDST (0.89) was significantly higher than that of LDDST (0.80, $P < 0.05$), showing that the HDDST was slightly superior to the LDDST alone in the differential diagnosis of ACTH-dependent CS.

To establish whether the combination of the LDDST and CRH achieved the same accuracy as the HDDST, we selected from the ROC curves a cut-off value of the LDDST that had a better specificity than the best cut-off of the HDDST (Fig. 4F). This cut-off was a suppression of more than 30% measured on the mean of the 24- and 48-h values (sensitivity 65%, specificity 94%). We then combined the two criteria of a greater than 30% suppression and/or a greater than 20% increase during the CRH test and evaluated the ability of such criteria to differentiate the causes of ACTH-dependent CS. The combination of tests had a sensitivity of 94% and a specificity of 97%, with a diagnostic accuracy significantly higher than that of the HDDST alone. The combination of the results of the LDDST plus the CRH test correctly identified 94% of patients who have had both tests (121 of 129), with a

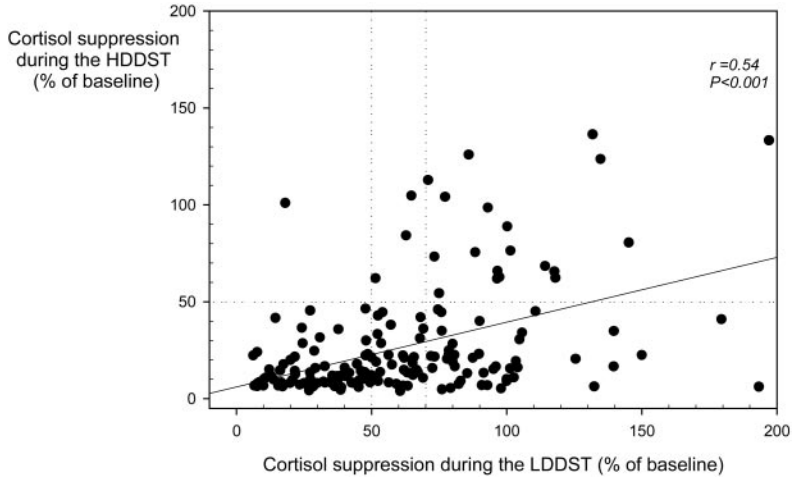

FIG. 3. Correlation between the degree of suppression during the LDDST and the HDDST in the 185 patients with CD.

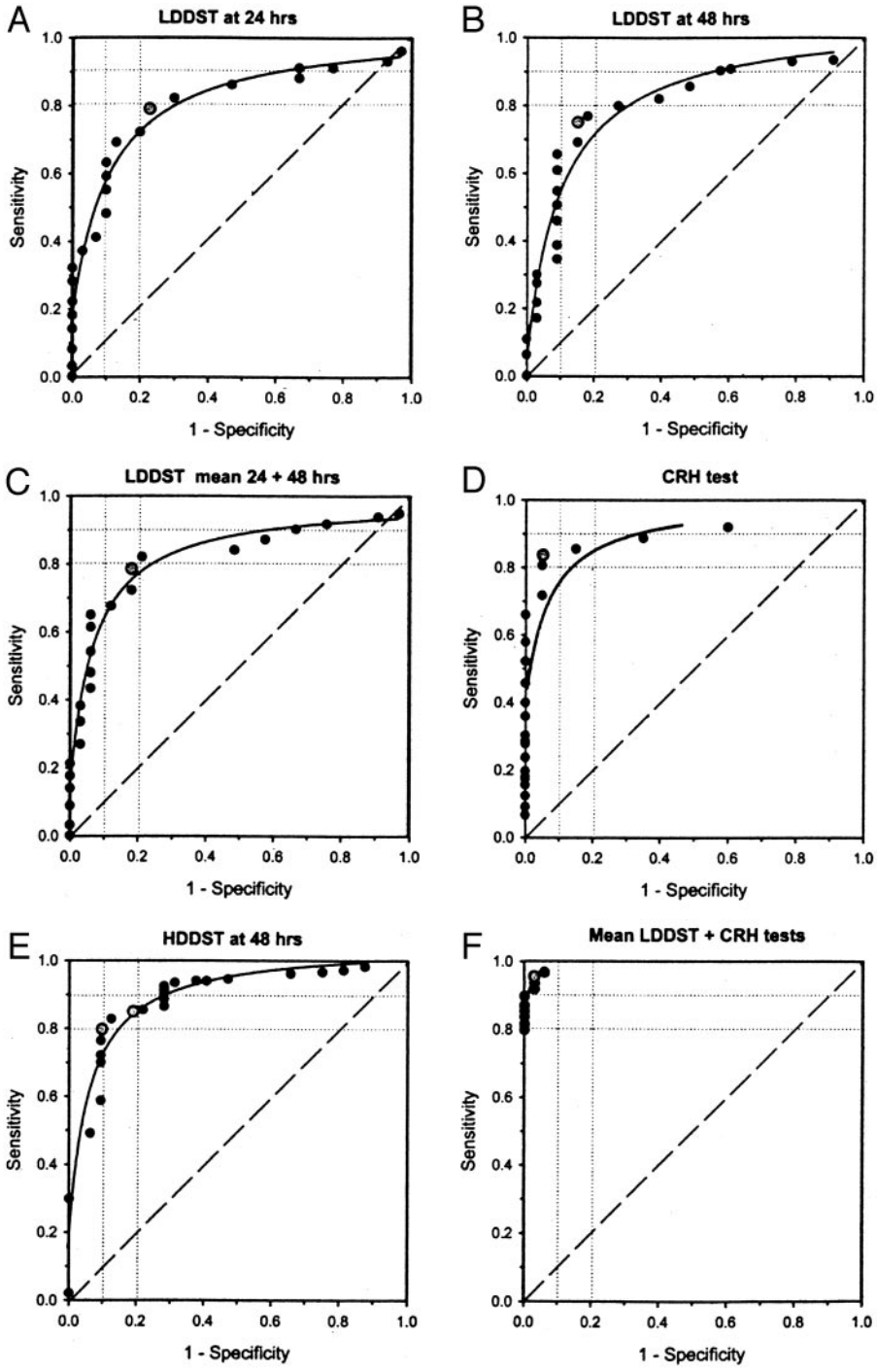

FIG. 4. ROCs for the LDDST (A–C), the CRH (D), the 48 h of the HDDST (E), and the combined response of the LDDST and CRH (F) for all data.

positive predictive value of greater than 99% and a negative predictive value of 82%. Finally, we investigated whether the combination of the HDDST plus the CRH was more accurate than the combination of the LDDST plus the CRH. Overall, the HDDST plus the CRH test had a sensitivity of 95% and a specificity of 93%. The CRH test associated with either the LDDST or the HDDST resulted in seven or six false negative diagnoses, respectively, and only one false positive result; thus, the LDDST and HDDST were equivalent when the results were used in conjunction with the CRH test in identifying patients with CD.

Discussion

The data provided in this study show that the formal 48-h LDDST based on measurement of serum cortisol at 24 and 48 h, after administration of 0.5 mg dexamethasone every 6 h for 48 h, remains a highly sensitive test for the diagnosis of endogenous hypercortisolism; in addition, when combined with the result of the CRH test, it provides a diagnostic accuracy higher than the HDDST alone in the differential diagnosis of ACTH-dependent CS.

Since the original description by Liddle (7) in 1960 of the

formal LDDST (37), a variety of protocols exist for the dexamethasone administration, and a range of diagnostic cut-offs that classify responses has been developed. Measurement of urinary steroids has been substituted in several instances by the measurement of serum cortisol (1). The 1-mg overnight dexamethasone suppression test, because of its ease of administration as an outpatient test, has been widely advocated as a screening test (1). However, to achieve a suitable level of sensitivity, up to 98% (10), the cut-off criteria for the overnight dexamethasone suppression test have had to be traded off against specificity. In the largest prospective series, the specificity of the overnight test was 87.5% (38); this may be compared with the specificity of the 2 mg/d 48-h test previously reported and confirmed in the present study, in the range 97–100% (39).

We have reviewed the response of serum cortisol in 245 patients with ACTH-dependent CS diagnosed, treated, and followed up in our department over the past 25 yr. In particular, we investigated the sensitivity of the test based on either the serum cortisol value obtained at 24 h or the mean of the 24- and 48-h values. Analysis of these end point values significantly improved the sensitivity of the test to 97% and 98%, respectively, when compared with the traditional 48-h value that has a sensitivity of 94%. Recently validated screening procedures, such as measurement of salivary cortisol (6, 40), have been advocated in place of the traditional 2-d or the overnight dexamethasone suppression tests as a first-line screening of patients with CS. Because assessment of patients with probable CS is currently performed at an earlier stage, in which the biochemical alterations in cortisol dynamics can be more subtle, earlier screening requires more sensitive testing. Our findings support the use of the LDDST at 24 h as a sensitive tool to assess such patients, particularly in identifying patients with CS who may be missed if analysis is limited to the 48-h value only. It is also conveniently performed on an outpatient basis. Indeed, using the 24-h value may make it possible to identify those patients with CD who suppress cortisol to normal at 48 h because of a decreased clearance of dexamethasone (41, 42). The findings of this study also suggest that the LDDST could be simplified further by shortening the test to the 24-h end point, although with a slight decline in sensitivity. However, the 24-h value needs to be validated in a group of patients with pseudo-Cushing's state to ensure that this is not associated with an undue loss of specificity.

In addition, we found that the majority of patients with CD exhibit some degree of cortisol suppression to the administration of low doses of dexamethasone, and a particular step-shaped pattern of response was observed in those patients who underwent the consecutive low-dose/high-dose suppression tests. Although originally designed for different purposes, the LDDST to diagnose endogenous CS and the HDDST to discriminate between different etiologies, we were interested in seeing whether the two tests elicit the same pattern of biological responsivity in ACTH-secreting cells either in the pituitary or ectopic tumors. If so, the LDDST could also be used in place of the high-dose test in the differential diagnosis of ACTH-dependent CS. Indeed, we showed that more than 30% suppression during the LLDST almost invariably predicted full suppression (>50%) during the HDDST. This step-wise response to two doses of dexamethasone is further evidence that corticotroph tumors have shifted their corticosteroid feedback curves to the left. Most current work suggest that this is not due to inherent changes in expression or structure of the glucocorticoid receptor, but the precise molecular pathology remains unclear (43).

High-dose dexamethasone suppression testing has previously been the mainstay of biochemical differential diagnosis between the pituitary and ectopic ACTH-dependent syndrome (33), although recently its utility has been disputed (44). This test is based on the observation that in pituitary-mediated disease, ACTH secretion tends to retain some degree of responsiveness to both hypophysiotropic factors and glucocorticoid negative feedback, whereas tumors responsible for ectopic production of ACTH tend not to do so. Although the HDDST has been used extensively, not all series have produced similar sensitivity and specificity. The number of patients with EAS in most series is limited, making the assessment of the efficacy of the test difficult (6, 17, 44). Attempts to develop increasingly sophisticated cut-off criteria to maximize specificity have not been uniformly reproduced and will eventually result in a fall in sensitivity. This is more evident in series including a considerable number of patients with EAS, in which the HDDST can be inaccurate in 20–30% of cases (2, 44). In the present series, the EAS accounted for 15% of patients with ACTH-dependent CS: Using the previously validated criteria, we found that 7 of 36 patients with EAS (19%) suppressed to more than 50% baseline cortisol levels during the HDDST. Reviewing these criteria, we found that more than 60% suppression of baseline cortisol levels had the highest accuracy in excluding EAS, with 80% sensitivity and 90% specificity for the diagnosis of CD. In addition, the present study shows that most of the diagnostic information provided by the HDDST could be predicted from the LDDST. Figure 2 shows that good discrimination between patients with pituitary and ectopic tumors can be achieved as early as the 24-h cortisol level of the of LDDST. In particular, the HDDST adds no further diagnostic value when a 30% degree of cortisol suppression is obtained during the LDDST. Thus, the LDDST appears to be a highly cost-effective test because it aids not only in the diagnosis of CS but can provide information on the etiology of ACTH-dependent CS with an accuracy only slightly inferior to the HDDST.

In view of the fact that the pretest probability of having CD in the presence of ACTH-dependent CS is significantly high, it has been claimed that the HDDST with its relatively low accuracy adds little to the work-up of patients with ACTH-dependent hypercortisolism (44). It is therefore still necessary to employ other diagnostic tests to improve diagnostic accuracy further. Although initially the CRH-stimulation test was hoped to reliably distinguish among the different causes of CS, combined analysis of all published series has revealed 7–15% false results (positive and negative) even if the best criteria are applied (33, 45–50). Most errors are false negative results in patients with CD who have no increase in plasma ACTH or serum cortisol after CRH administration, plus a very few false positive results of patients with an ectopic ACTH tumor who respond to CRH. The finding of this study show that the combined criteria of a greater than 30% cortisol

suppression during the LDDST, and/or a greater than 20% cortisol increase during the CRH test, have a sensitivity of 94% and a specificity of 97% in distinguishing between patients with CD from those with EAS. Therefore, the combined criteria present in this study achieved a diagnostic accuracy only marginally inferior to that reported in the literature for the BIPSS (1). This may be of substantial importance in the management of patients with ACTH-dependent CS when facilities for the performance of the BIPSS are not readily available. The data presented here suggest that the LDDST may be used for the initial confirmation of CS, and, in cases of confirmed hypercortisolism, the combined cortisol response to the LDDST and the CRH-test can be used to establish the source of ACTH, whether eutopic or ectopic, with a valuable degree of precision. However, it is important to note that the accuracy of any diagnostic test depends on the referral population, which in our case is that of a highly specialist center. It is likely, but requires validation, that this will also apply to less specialized referral services. Furthermore, there is variability in cortisol assays that, although less relevant when looking at percentage changes, may be important when considering absolute levels of cortisol as diagnostic criteria.

In brief, we suggest that the formal 2 mg/d, 48-h dexamethasone suppression test (LDDST) should be the investigation of choice with cortisol suppression evaluated at 24 and 48 h. The 24-h value is more sensitive in identifying mild forms of CS that may be missed if relying only on the 48-h value, but its specificity requires further investigation. A serum cortisol value derived from the mean of the 24- and 48-h cortisol values of the LDDST provides the highest sensitivity. In addition, in the presence of significant suppression (>30%) during the LDDST and/or a good response (>20%) to the CRH test, the diagnosis of CD is highly probable. Because a greater than 30% serum cortisol suppression to the LDDST invariably predicts cortisol suppression to the HDDST, our data further suggest that this test is of no additional value and can be abandoned. It is still our opinion that the BIPSS remains one of the most accurate single investigations in the differential diagnosis of CS, particularly in cases in which there are discordant biochemical and/or imaging findings; it is also useful in providing information on the lateralization of the tumor, especially in childhood (51). In summary, the differential diagnosis between CD and EAS can be performed with high accuracy by combining the results of the formal 2 mg/d 48-h LDDST and the CRH test for serum cortisol.

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Address all correspondence and requests for reprints to: Prof. A. B. Grossman, Department of Endocrinology, St. Bartholomew's Hospital, London ECIA 7BE, United Kingdom. E-mail: a.b.grossman@qmul.ac.uk.

Present address for A.M.I.: Cattedra di Andrologia, Dipartimento di Fisiopatologia Medica, Univerisità "La Sapienza" di Roma, Rome 00161, Italy.

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