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Dalia L. Batista, Jehan Riar, Meg Keil and Constantine A. Stratakis
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Diagnostic Tests for Children Who Are Referred for the Investigation of Cushing Syndrome

Dalia L. Batista, MD, Jehan Riar, BS, Meg Keil, RNP, Constantine A. Stratakis, MD, DSc

Section on Endocrinology and Genetics, Developmental Endocrinology Branch, and Pediatric Endocrinology Inter-Institute Training Program, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland

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ABSTRACT

OBJECTIVE. Endogenous Cushing syndrome in children is a rare disorder that is most frequently caused by pituitary or adrenocortical tumors. Diagnostic criteria have generally been derived from studies of adult patients despite significant differences in both the physiology of the hypothalamic-pituitary-adrenal axis and the epidemiology of Cushing syndrome in childhood. The purpose of this study was to identify the tests that most reliably and efficiently diagnose pituitary or adrenal tumors in a large cohort of pediatric patients with Cushing syndrome.

METHODS. A retrospective review of clinical data of children who were referred to a tertiary care center for evaluation for Cushing syndrome during the years 1997 to 2005 was conducted. A total of 125 consecutive children were studied retrospectively; 105 were found to have Cushing syndrome, which was confirmed histologically; and 20 children who did not have Cushing syndrome or any other endocrinopathy served as the control group. The following tests were performed in all children: midnight and morning cortisol, corticotropin hormone, urinary free cortisol and 17-hydroxycorticosteroid levels, ovine corticotropin-releasing hormone stimulation test, and overnight high-dosage dexamethasone suppression test. Imaging of the pituitary and adrenal glands was also obtained. The main outcome measure was the sensitivity of these parameters for the diagnosis and differential diagnosis of Cushing syndrome at 100% specificity.

RESULTS. A midnight cortisol value of $\geq 4.4 \mu g/dL$ confirmed the diagnosis of Cushing syndrome in almost all children, with a sensitivity of 99% and a specificity of 100%. Suppression of morning cortisol levels $>20\%$ in response to an overnight, high-dosage dexamethasone test excluded all patients with adrenal tumors and identified almost all patients with pituitary tumors (sensitivity: 97.5%; specificity: 100%).

CONCLUSIONS. Our study suggests that among children who were referred for the evaluation of possible Cushing syndrome, a single cortisol value at midnight followed by overnight high-dosage dexamethasone test led to rapid and accurate confirmation and diagnostic differentiation, respectively, of hypercortisolemia caused by pituitary and adrenal tumors.
ENDOGENOUS CUSHING SYNDROME (CS) is a rare disorder and is even more rare among children. Despite significant differences in the pathophysiology and epidemiology of hypercortisolism among age groups, criteria derived from studies on adult patients with CS have been used for the confirmation and differential diagnosis of this disorder in children.

CS may be caused by corticotropin-producing pituitary tumors, a disorder also known as Cushing disease (CD), or by corticotropin-independent, cortisol-producing adrenocortical tumors. In children, ectopic production of corticotropin is extraordinarily rare; it has been reported only in a handful of cases confined at the extremes of pediatric age, infants with neuroblastomas or other neuroendocrine tumors, and adolescents with carcinoids, sporadic or in the context of multiple endocrine neoplasia type 1. Conversely, bilateral adrenocortical hyperplasias are far more common as causes of CS in children than in older patients. Developmental differences in the regulation of the hypothalamic-pituitary-adrenal axis between young and older individuals, as well as other factors, such as exposure to steroid hormones, medications and other exogenous substances, stress, and chronic illness, may influence normal values for several of the tests used in the diagnostic testing of CS. In addition, the diagnosis of CS in children is facilitated by the inhibitory effects of hypercortisolism on height gain. Indeed, the deceleration of growth velocity with a concurrent and unabated weight gain is the hallmark of CS in childhood. In contrast, in lieu of these apparent signs, extensive biochemical investigation is needed for the confirmation of the diagnosis of CS in adult patients, especially when their situation is complicated by moderate weight gain and other conditions, which collectively have been called pseudo-Cushing states.

These differences suggest that the lengthy, costly, and often complicated algorithm that has been proposed for the confirmation and diagnostic differentiation of CS in adults may not be the most efficient way to identify causes of endogenous hypercortisolism in children. The purpose of this study was to identify the tests that most reliably and efficiently diagnose pituitary or adrenal tumors in a large cohort of pediatric patients who were referred to our center for evaluation for possible CS.

METHODS

Patients

The charts of a total of 125 consecutive children, 105 who had CS and 20 who served as a control group, who were studied at the National Institutes of Health Warren Magnuson Clinical Center between 1997 and 2005 were retrospectively analyzed (Table 1). The criteria that were used for the confirmation of CS were (1) elevated urinary free cortisol (UFC) and 17-hydroxycorticosteroid (17OHS) levels before treatment and their normalization after surgery and (2) histopathologic confirmation of a pituitary lesion leading to CD or adrenocortical lesion leading to CS. All children with CD received transsphenoidal surgery (TSS); patients with adrenocortical adenomas or other tumors and/or corticotropin-independent bilateral hyperplasia were treated by unilateral or bilateral adrenalectomy, respectively. The control group consisted of 20 children who were referred to our center for evaluation; they were not found to have CS, were followed up for a number of years, and continued to be normal. The National Institute of Child Health and Human Development/National Institutes of Health clinical center institutional review board approved these studies for all of our patients under protocols 95-CH-0059, 00-CH-0160, and 00-CH-0180. Informed consent from the patients’ parents (and assent from older children) was obtained in all patients.

Studies

Growth Parameters

BMI for age and gender was based on the National Center for Health Statistics data: overweight was defined as a BMI for age and gender >95th percentile. Height for

| TABLE 1 Baseline Characteristics of Children with CS and Control Subjects |
|-------------------------------|------------------|-------------------|-----------------|
|                               | Baseline Characteristics | CD               | Adrenocortical Tumors | Control        |
| Girls/Boys                    | 40/40              | 18/7              | 13/7               |
| Age, median (range), y        | 12 (5 to 17)       | 11 (3 to 18)      | 12 (3 to 17)       |
| Race                          |                   |                   |                  |
| Asian                         | 4                 | 2                 | 0                |
| Black                         | 2                 | 2                 | 1                |
| Hispanic                      | 21                | 5                 | 1                |
| White                         | 53                | 16                | 18               |
| BMI z-score, median (range)   | 2.10 (0.16 to 3.50)| 1.90 (0.06 to 4.50) | 2.50 (0.33 to 5.00) |
| HA z-score, median (range)    | −1.13 (−4.00 to 2.60) | −0.89 (−5.00 to 1.80) | −0.08 (−3.00 to 2.00) |
| UFC excretion, median (range), μg/m2 per 24 h | 161.0 (70.0 to 804.0) | 308.8 (71.0 to 810.0) | 24.1 (15.0 to 69.0) |
| 17OHS excretion, median (range), mg/g of creatinine per 24 h | 10.2 (1.4 to 56.0) | 14.3 (1.6 to 53.0) | 4.5 (2.8 to 8.1) |
| Midnight cortisol, median (range), μg/dL | 17.9 (3.2 to 102.0) | 25.4 (9.0 to 41.2) | 2.0 (1.0 to 4.3) |

HA indicates height for age and gender. To convert UFC (μg/dL) to Systeme International units (nmol/day), multiply by 2.759. To convert 17OHS (mg/g of creatinine) to Systeme International units (nmol/m of creatinine), multiply by 0.132, or to convert to μmol/day, multiply by 2.759. To convert midnight cortisol (μg/dL) to Systeme International units (nmol/L), multiply by 27.59.
age and gender was also based on the National Center for Health Statistics data. The following information was analyzed for each patient:

1. Twenty-four–hour UFC expressed per square meter of body surface area (µg/m² per 24 hours) and 17OHS expressed per grams of creatinine excreted in 24 hours (mg/g of creatinine per 24 hours). All 24-hour urine collections were obtained for at least 3 consecutives days while the children were inpatients. The mean rate of UFC and 17OHS excretion in normal children is <70 µg/m² per 24 hours (193 nmol/day) and <7 mg/g of creatinine per 24 hours (6.5 mmol/mol of creatinine), respectively.16

2. Diurnal plasma cortisol variation, including midnight cortisol values, as previously described.17 A venous sampling catheter was placed at least 2 hours before the test; cortisol levels were drawn at 11:30 PM and 12:00 AM and at 7:30 AM and 8:00 AM while the patient was lying in bed and asleep.

3. 8:00 AM plasma corticotropin and cortisol levels followed by ovine corticotropin-releasing hormone (oCRH) stimulation, as previously described.18 In brief, a venous catheter was placed in the forearm the night before testing; the patient was fasting and lying in bed, and oCRH was administered at a dosage of 1 µg/kg of body weight at 8:00 AM. Samples for cortisol and corticotropin were taken 5 minutes before administration; at the time of administration; and at 15, 30, and 45 minutes after the administration of oCRH. The response to the last was expressed as the percentage change from baseline by subtracting the pretest cortisol and corticotropin values from the posttest values and dividing by the former. The mean cortisol increase was estimated at 30 and 45 minutes from baseline. For corticotropin, the mean increase was estimated at 15 and 30 minutes after the administration of oCRH.

4. Overnight high-dosage dexamethasone suppression test (HDDST), as previously described.19 For all children, the 8-mg dexamethasone dosage was adjusted to 120 µg/kg per dose (maximum dosage was 8 mg) and a dexamethasone level was checked concurrent with the posttest cortisol levels, as previously described.19 Dexamethasone was given orally at 11:00 PM. Pretest cortisol levels were drawn at 8:00 AM in the morning of the day of the test; the postdexamethasone cortisol levels were drawn at 9:00 AM, in the morning of the next day. Cortisol suppression from baseline was determined using the formula 100 – [(postdexamethasone cortisol level in plasma/pre-dexamethasone cortisol level in plasma) × 100].19

5. Computed tomography (CT) scan of the adrenal glands, as previously described.20 The study was obtained using a single-slice scanner (General Electric Medical Systems, Milwaukee, WI) with a collimation of 3-mm, 7.5-mm/second table feed, and 3-mm increments with a scan delay of 70 seconds before and after intravenous bolus injection of nonionic contrast material (Omnipaque 300; Nycomed, Inc, New York, NY), which was injected at a rate of 2 mL/second (at a maximum dosage of 140 mL).

6. MRI of the pituitary gland, as previously described.21 All images were obtained using a 1.5 T scanner (Sigma: General Electric Medical Systems). Coronal precontrast T1-weighted spin echo images and precontrast spoiled gradient recalled acquisition in steady state images were obtained. Both spin echo and spoiled gradient recalled acquisition in steady state studies were repeated after intravenous administration of gadolinium contrast material.

7. The Liddle’s test was obtained in children, as previously described,22,23 who were suspected of having micronodular adrenal hyperplasia: low- and high-dosage dexamethasone was administered according to body weight (30 and 120 µ/kg per day). Mean values of urinary cortisol excretion at baseline (2 days) were compared with values on the second day of the high-dosage dexamethasone (day 6).

Hormone Assays
Plasma corticotropin and cortisol were measured, as previously described.16–19,22,23 Plasma corticotropin intra-assay and interassay coefficients of variation were 6.2% and 11%, respectively. UFC excretion was measured by direct radioimmunoassay (Smith Kline Bioscience Laboratories, King of Prussia, PA).16–19 The intra-assay and interassay coefficients of variation were 5.4% and 9.3% to 11.5%, respectively. Urinary 17OHS excretion was measured by a modification of the colorimetric method of Porter and Silber.22,23 The intra-assay and interassay coefficients of variation were 5.9% and 10.7%, respectively.

Statistical Analysis
Data are expressed as median (range) or mean ± SEM. For all statistical comparisons, P < .05 was considered significant. Data were analyzed using the Stata 8.0 statistical software (Stata Corp, College Station, TX). The 95% confidence intervals (CI) were determined for sensitivity and specificity values of all testing, as previously described.17–19 Repeated measures of analysis of variance were used to analyze data where appropriate. Friedman’s repeated measures analysis of variance was used initially within each group to assess any differences in cortisol and corticotropin levels in response to oCRH. Wilcoxon matched pair test within and the Mann-Whitney U test followed this between groups. Receiver operating characteristics (ROC) curves were constructed as...
previously described\textsuperscript{17,19} and used to assess the utility of each measure for differential diagnosis. The point on the ROC curve with specificity of 100\% provided the best cutoff value of each test.

**RESULTS**

**Clinical Presentation**

The sample consisted of 125 consecutive children: 80 children had a diagnosis of CD, 25 children had primary adrenal disease (4 had a unilateral adenoma, and 21 had micronodular adrenal disease), and 20 children who were found to be normal (albeit overweight or obese) served as the control group. There were no significant differences between the ages of the children with CS and the control group other than in their overall baseline UFC and 17OHS excretion (Table 1). Consistent with the overall higher incidence of adrenal tumors in girls,\textsuperscript{2} the female-to-male ratio was significantly higher in the adrenal tumor group. Median BMI SD score for the control group was 2.5 (range: 0.33–5.00), for children with CD was 2.1 (range: 0.16–3.50), and for children with adrenal tumors was 1.9 (range: 0.06–4.50). Accordingly, in the control group, children were overweight in 85\% (17 of 20) of the cases; 81\% (85 of 105) of the children with CS and/or CD were overweight ($P$ > .1). Overall height-for-age $z$ scores were lower in children with CS.

**Test Performance**

**Sensitivity**

**UFC and 17OHS**

These tests did not distinguish between the 2 diagnostic groups of CS, adrenal and pituitary tumors (Table 1). All patients with CS occasionally had UFC or 17OHS in the reference range in their 24-hour urine samples (Fig 1). Overall, 6\% (5 of 80) of the patients with CD had at least one 24-hour UFC collection in the reference range, whereas this was observed in 32\% (8 of 25) of the children with adrenocortical tumors. Sensitivity of UFCs for children with CS was 88\% (92 of 105; 95\% CI: 79\%–93\%); for the 17OHS, the sensitivity of the test was 81\% (85 of 105; 95\% CI: 72\%–88\%). Averaging UFC and 17OHS values over 3 days provided less overlap between groups as shown in Fig 2.

**Morning Corticotropin Level**

Patients with CD had the highest levels of morning corticotropin with a median of 18 pg/mL (4 pmol/L; range: 8–164 pg/mL or 2–36 pmol/L). All children with CD had a corticotropin level >5 pg/mL (1.1 pmol/L). There were statistically significant differences between the groups in morning corticotropin levels but also a great overlap between individual values. A 100\% specificity was achieved with a cutoff value of 29 pg/mL (6 pmol/L). This value gave the test a sensitivity of 70\% (56 of 80; 95\% CI: 59\%–79\%).

**Midnight Cortisol Level**

The results of this test distinguished patients with hypercortisolism from those of the control group accurately ($P$ = .001). Again, this test did not differentiate between the 2 diagnostic groups of patients with CS, those with pituitary (CD) and those with adrenal tumors. A single midnight cortisol level of $\geq$4.4 \textmu g/dL (121 nmol/L) provided the highest sensitivity of 99\% (104 of 105; 95\% CI: 94\%–100\%; Fig 3).

**oCRH Stimulation Test**

None of our children had ectopic CS, which seems to be an extraordinarily rare form of CS in childhood. Using previously published data from adult studies in our center, a corticotropin increase of 35\% from baseline after oCRH administration gave the test a sensitivity of 81\% (64 of 79; 95\% CI: 70\%–89\%) in identifying patients with pituitary tumors (CD). A cortisol increase of $\geq$20\% after oCRH administration gave the test a sensitivity of 74\% (59 of 80; 95\% CI: 63\%–83\%; Fig 4) for the same purpose. These data compare with the performance of the test for these cutoff points in adult patients with CD/CS.

**HDDST Test**

A decrease of morning cortisol value after the overnight HDDST of $\geq$20\% from baseline provided the highest sensitivity in distinguishing children with CD from children with primary adrenal disorders with a sensitivity of 97.5\% (77 of 79; 95\% CI: 90\%–99\%; Fig 5).

**Imaging Studies**

A lesion was seen by MRI of the pituitary gland in 63\% of children with CD (50 of 80; 95\% CI: 51\%–73\%). CT scanning of the adrenal gland showed bilateral nodules or a unilateral adrenal mass in 72\% (18 of 25; 95\% CI: 50\%–87\%) of children with CD and/or CD were overweight ($P$ > .1). Overall height-for-age $z$ scores were lower in children with CS.

**Specificity**

**UFC and 17OHS**

It is interesting that 10\% (2 of 20) and 25\% (5 of 20) of the children in the control group also had at least 1 24-hour urine collection in the abnormal range for UFC and 17OHS, respectively. Specificity of UFC was 90\% (18 of 20; 95\% CI: 67\%–98\%) and for 17OHS was 75\% (15 of 20; 95\% CI: 51\%–90\%). Again, averaging UFC and 17OHS values over 3 days provided less overlap between groups (Fig 2).

**Morning Corticotropin Level**

Children with adrenocortical tumors had corticotropin levels that were significantly lower than those in children with CD (but not undetectable). The median corticotropin value for children with adrenal disease was 2 pg/mL (0.44 pmol/L; range: 1–29 pg/mL or 0.22–6
pmol/L). A value of 29 pg/mL (6 pmol/L) gave the test a specificity of 100% (25 of 25; 95% CI: 83%–100%).

**Midnight Cortisol and HDDST**

These 2 tests gave the highest specificity: for midnight cortisol, the specificity was 100% (20 of 20; 95% CI: 80%–100%) for ruling out CS (Fig 3); for the differential diagnosis of CS between patients with CD and those with adrenal tumors, the HDDST gave a specificity of 100% (23 of 23; 95% CI: 82%–100%).

**Imaging Studies**

All children with CD underwent adrenal CT scanning: bilateral adrenal hyperplasia was observed in 53% (42 of 80) of children with CD, but none of these children had the nodular adrenal findings that were observed in children...
with adrenal adenomas or micronodular or macronodular hyperplasia, giving the test 100% specificity (95% CI: 94%–100%). All children with adrenal disease had their pituitary gland imaged by MRI; 2 children with adrenal tumors had pituitary MRI findings, which gave the MRI a specificity of 92% (23 of 25; 95% CI: 73%–99%).

**False-Negative Test Results**

**Biochemical Testing**

The tests with the lowest false-negative results were the serum midnight cortisol and the HDDST: 5% and 8%, respectively. False-negative tests for UFC and 17OHS were reported in 42% and 57% of children with

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**FIGURE 2**

Average 24-hour excretion (mean: 3 days collection) of UFC (A) and 17OHS (B) in patients with CD, adrenocortical tumors, and controls. The upper normal value is indicated by the dotted line.

**FIGURE 3**

Midnight cortisol values in children with CS (n = 80) and adrenocortical tumors (n = 25) and in control subjects (n = 20). Significant overlap occurred between individual points.

**FIGURE 4**

Corticotropin (A) and cortisol (B) responses to oCRH test in children with CD (n = 80), adrenocortical tumors (n = 25), and control subjects (n = 20). CRH corticotropin level was not available for 1 patient with CD. Significant overlap occurred between individual points.

**FIGURE 5**

Cortisol response to an overnight HDDST in patients with CD (n = 79) and adrenocortical tumors (n = 23) and in control subjects (n = 20). For 1 patient with CD, HDDST test results were not available. Two children with adrenal disease did not undergo HDDST but underwent Liddle’s test. Significant overlap occurred between individual points.
CS, respectively, whereas for morning corticotropin, they were observed in 49% of the children.

**Imaging Studies**

False-negative results were observed in 57% of MRI studies in children with CD. Among CT scans of the adrenal glands, a false-negative result was observed in 8% of children with adrenal tumors.

**False-Positive Test Results**

**Biochemical Testing**

False-positive test results were observed in children of the control group: for the UFCs, the rate of false-positive test results was 2%, and for the 17OHS, it was 6% for single-day collections. No child with adrenal disease had elevated morning corticotropin above the cutoff value of 29 pg/mL (6 pmol/L); no false-positive results were observed for the midnight cortisol test and the HDDST.

**Imaging Studies**

No false-positive results were reported for scans of the adrenal glands, whereas for the MRIs of the pituitary gland, false-positive results were obtained in 4% of the children. It is interesting that 1 of the 2 children with adrenal disease did in fact have an adenoma of the pituitary gland that was producing growth hormone and prolactin (data not shown).

**Positive Predictive Value (PPV)**

**Biochemical Testing**

As discussed, from the total of 125 children who were tested for CS in our center, 105 indeed had this diagnosis. Midnight cortisol and HDDST had the highest PPV. Midnight cortisol PPV of the test was 100% (95% CI: 96%–100%), and for the HDDST, the PPV was 100% (94%–100%). The PPV for morning corticotropin was 100% (95% CI: 92%–100%), for UFC was 98% (95% CI: 92%–100%), and for 17OHS was 94% (95% CI: 87%–99%).

**Imaging Studies**

The CT scans of the adrenal glands had a high PPV at 100% (95% CI: 78%–100%); MRIs of the pituitary gland had a PPV of 96% (86%–99%).

**Negative Predictive Value (NPV)**

**Biochemical Testing**

The NPV for UFC was 58% (95% CI: 39%–75%), for 17OHS was 43% (95% CI: 27%–61%), for the morning corticotropin was 51% (95% CI: 37%–65%), for the midnight serum cortisol was 95% (95% CI: 74%–100%), and for HDDST was 92% (95% CI: 73%–99%).

**Imaging Studies**

The NPV for MRIs of the pituitary gland was 43% (95% CI: 30%–58%), whereas for the CT scans of the adrenal glands, it was 92% (95% CI: 84%–96%).

**Diagnostic Utility of Each Test (ROC Calculations)**

Area under the ROC curve calculations showed that the midnight cortisol had a high ROC area under the curve (0.998) and was the most accurate test in diagnosing CS; in addition, the diagnostic power of the HDDST was superior to that of the oCRH stimulation in distinguishing between pituitary-dependent CD and adrenal-dependent CS (Fig 6).

**Additional Testing for Children With Adrenal Disease**

Indications for obtaining the Liddle’s test were nonsuppression of cortisol levels in serum during the HDDST and/or negative imaging studies and/or suspected adrenal disease (Fig 7). As proved by their postoperative diagnoses, 20 children with primary adrenal disease and 12 children with CD underwent the Liddle’s test during subsequent visits to our clinical center.

None of the children with primary adrenal disease suppressed beyond ~90% their UFC levels on day 6 when compared with baseline values. Baseline 24-hour excretion for UFC during the Liddle’s test for children with micronodular adrenal disease was 298 ± 80 µg/m² per 24 hours or 822 ± 221 nmol/day (range: 68.5–1373 µg/m² per 24 hours or 188–3788 nmol/day). On day 6, UFC values increased and the mean values were 752 µg/m² per 24 hours or 2075 nmol/day (range: 211–3441 µg/m² per 24 hours or 582–9494 nmol/day). Among children with other causes of primary adrenal disease (unilateral adenoma and micronodular disease), baseline UFC values during the Liddle’s test were 355 ± 125 µg/m² per 24 hours or 979 ± 345 nmol/day (range: 148–636 µg/m² per 24 hours or 408–1755 nmol/day); on day 6, UFC values for these children were 425 ± 124 µg/m² per 24 hours or 1173 ± 342 nmol/day (range: 211–700 µg/m² per 24 hours or 582–1931 nmol/day). Only children with bilateral micronodular adrenal disease showed an increase in UFC on day 6 >100% from baseline values, in agreement with previously published studies.21 Also, consistent with previously published studies, an increase of 50% or more in UFC levels on day 6 of the Liddle’s test identified 69% of the patients with micronodular adrenal disease (11 of 16; 95% CI: 42%–88%) and excluded all patients with micronodular adrenocortical disease and/or unilateral adrenal adenoma. One child with micronodular adrenal disease with UFCs in the upper limit of normal had a baseline of 68.5 µg/m² per 24 hours (188 nmol/day); in this patient, UFC increased to 246 µg/m² per 24 hours (679 nmol/day) on day 6 of the Liddle’s test. In another patient with CT of the adrenal glands consistent with micronodular disease and a histopathologic diagnosis of primary pigmented
nodular adrenocortical disease, there was no increase in urine cortisol level, and, in fact, UFC suppressed from the baseline values on day 6 of the Liddle’s test to $74\%$. In 12 children who had CD and underwent the Liddle’s test, the mean 24-hour excretion for UFC was 195 $\mu$g/m$^2$ per 24 hours or 538 $\pm$ 155 nmol/day (range: 78–763 $\mu$g/m$^2$ per 24 hours or 215–2105 nmol/day) at baseline; they all suppressed to a mean UFC value on day 6 of 42 $\pm$ 11 $\mu$g/m$^2$ per 24 hours or 116 $\pm$ 30 nmol/day (range: 5–120 $\mu$g/m$^2$ per 24 hours or 14–331 nmol/day), which represents a suppression range of $36\%$ to $97\%$ from baseline values. This is consistent with the published data from Liddle’s test results in adults with CD: $67\%$ (8 of 12) of children with CD had a decrease of UFC excretion of $90\%$ or more from the mean basal value on the last day of the HDDST.

Long-term Follow-up
One child who had primary adrenal disease and had a positive MRI of the pituitary gland (see “Imaging Studies”) developed a growth hormone–secreting adenoma 7 years later. This patient received a diagnosis of Carney complex.

The median follow-up for all children who did not have CS (control group in this study) has been 4 years. None of these children has developed any significant disease; almost all of them, however, remain overweight or even obese but have progressed normally to puberty and continued to grow (data not shown). Additional testing during the follow-up time in these 20 children included imaging studies and biochemical testing such as measurements of cortisol levels in serum and urine (data not shown). All of these studies remain normal in these children.

DISCUSSION
The purpose of this study was to evaluate the tests that most accurately diagnose CS in a pediatric population referred for evaluation for this disorder. Once CS is suspected on the basis of clinical manifestations, the diagnostic evaluation involves the administration of multiple tests in a logical sequence. In theory, these tests should have high diagnostic accuracy and be easily available and at a low cost (Table 2). Before this study, all tests (and their criteria for interpretation) used for the diagnosis of CS in children were derived from studies on adults and in fact sometimes significantly older groups of patients.

Overproduction of cortisol is the biochemical hallmark of CS regardless of its cause. Testing for increased urinary glucocorticoid excretion therefore is a standard screening procedure, and our study confirms its usefulness. However, increased UFC excretion may not be a consistent finding, and serial measurements of UFC excretion are necessary to rule out CS. This study also indicates that 17OHS excretion in the first stages of the diagnostic workup for CS offers no added value in children.

Abnormalities of diurnal variation in plasma cortisol secretion have been exploited diagnostically for the confirmation of CS in several studies, since first reported by
Doe et al in 1960. Unfortunately, studies on adult patients differ significantly in the criteria and the methods used; it has been reported that a single midnight plasma cortisol measurement indicates the presence of CS when values are as low as 1.8 μg/dL (50 nmol/L) or as high as 7.5 μg/dL (207 nmol/L). In our study a cutoff point for midnight cortisol concentration of 4.4 μg/dL (121 nmol/L) identified 104 of the 105 children with CS with a specificity of 100%. In fact, all children with CS in our study had midnight cortisol levels ≥3.2 μg/dL (88 nmol/L), but at least 2 of the referred normal children also had a midnight cortisol value higher than that number. It should be noted that our study did not follow the strict guidelines regarding sleep monitoring followed by others during the diurnal cortisol variation test, which may explain why our cutoff value is higher than the suggested 1.8 μg/dL (50 nmol/L). In addition, most of our patients who were evaluated for CS were overweight, and obesity is a pseudo-Cushing state in both children and adults. However, the difference of the midnight cortisol value of 4.4 μg/dL (121 nmol/L) vs the 7.5 μg/dL (207 nmol/L) obtained under exactly the same conditions and an identical hospital setting may reflect the differences between adult and pediatric hypothalamic-pituitary-adrenal axis regulation.

A high urinary cortisol averaged over 3 days and a midnight cortisol value >4.4 μg/dL (121 nmol/L) confirmed the diagnosis of CS in all children but did not indicate the source. Although low morning corticotropin levels suggest adrenal disease, neither corticotropin values nor imaging studies were accurate in determining the cause, as studies in adults have also shown.

### TABLE 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Value</th>
<th>Sensitivity, % (n/N)</th>
<th>Specificity, % (n/N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midnight cortisola</td>
<td>Newell-Price et al (1995)</td>
<td>1.8 μg/dL (50 nmol/L)</td>
<td>100 (105/105)</td>
</tr>
<tr>
<td></td>
<td>This study</td>
<td>4.4 μg/dL (121 nmol/L)</td>
<td>99 (104/105)</td>
</tr>
<tr>
<td></td>
<td>Papanicolaou et al (1998)</td>
<td>7.5 μg/dL (207 nmol/L)</td>
<td>97 (102/105)</td>
</tr>
<tr>
<td>Corticotropina</td>
<td>Newell-Price et al (2006)</td>
<td>&lt;5 pg/ml (1.1 pmol/L)</td>
<td>68 (17/25)</td>
</tr>
<tr>
<td></td>
<td>This study</td>
<td>29 pg/ml (6 pmol/L)</td>
<td>70 (56/80)</td>
</tr>
<tr>
<td>CRH testb</td>
<td>Nieman et al (1993)</td>
<td>Cortisol increase 20%</td>
<td>74 (59/80)</td>
</tr>
<tr>
<td>HDDSTc</td>
<td>This study</td>
<td>20%</td>
<td>97.5 (77/79)</td>
</tr>
<tr>
<td></td>
<td>Tyrell et al (1986)</td>
<td>50%</td>
<td>77 (61/79)</td>
</tr>
<tr>
<td></td>
<td>Dichek et al (1994)</td>
<td>68%</td>
<td>75 (59/79)</td>
</tr>
</tbody>
</table>

* Different assays and methods to collect samples may have contributed to yield different results.
* Corticotropin level was not available for 1 patient with CD.
* HDDST results were not available for 1 patient with CD. Two children with adrenal disease did not undergo HDDST but underwent Liddle’s test.

![Figure 7](image_url)

Mean change in 24-hour excretion of UFC during the Liddle’s test. Filled bars represent baseline UFC excretion values, the mean of 2 days of 24-hour urine collection (day 1 and day 2 of the Liddle’s test). Open bars represent UFC on day 6 of the Liddle’s test. For children with CD (n = 12), 24-hour UFC excretion decreased on day 6 of the Liddle’s test. For children with micronodular adrenal disease (primary pigmented nodular adrenocortical lesions) (n = 16), 24-hour UFC excretion increased significantly on day 6 of the Liddle’s test. Children with other causes of adrenal disease (unilateral adenomas and micronodular adrenal disease) (n = 4) had a modest increase in their 24-hour UFC excretion on day 6 of the Liddle’s test. Error bars represent the SE. * P < 0.05; baseline 24-hour UFC excretion compared with UFC on day 6.
the other developed a growth hormone–secreting adenoma. This child received a diagnosis of a hereditary form of CS, Carney complex. Tomography of the adrenal glands, like corticotropin hormone levels and MRI of the pituitary glands, was not a sensitive test in determining the source of CS: in our study, some of the children with primary adrenal disease had negative adrenal scans. Normal or small adrenal glands can be seen in patients with primary adrenal disease as a result of pigmented nodular adrenocortical hyperplasia.2,20

For the differential diagnosis of pituitary adenomas versus other causes of CS, it has been reported that the oCRH stimulation test has a sensitivity of 85% to 91% and specificity of 88% to 100% for a stimulated cortisol value of 14% to 22% over baseline and a sensitivity of 93% and specificity of 100% for a stimulated corticotropin value of 35% over baseline.18,28 Other studies have reported that an increase of corticotropin hormone by 50% carries a sensitivity of 85% to 86% and specificity of 90% to 100%.29–31 In this study, the oCRH stimulation test was performed as described in previous studies in the same institution.18 In our series, this test did not identify all patients with CD, and some children with adrenal tumors had normal responses to oCRH. Why did children with adrenal disease responded after oCRH stimulation with an increase of cortisol and corticotropin secretion? These children usually have intermittent hypercortisolemia, and their hypothalamic-pituitary-adrenal axis may not be completely suppressed23; in addition, differences in the physiology of the axis between children and adults may account for the relative lack of complete suppression. We did not have any children with ectopic CS in this series, and the specificity of the test could not be calculated; however, the results of the sensitivity of the oCRH test in our series were comparable to those obtained in studies of adult patients with CS in our institution (Table 2).18

HDDST, which was originally developed by Liddle32 to distinguish pituitary from adrenal tumors that cause CS and modified by Dichek et al.19 was very accurate in differentiating pediatric CD from other cortisol-producing tumors in our patients. There are several versions of the HDDST, including the standard 2-day-long oral high-dosage (2 mg every 6 hours for 8 doses), the 8-mg overnight oral,19 and the intravenous 4- to 7-mg tests.33 Plasma and/or urinary cortisol levels are evaluated before, during, or after dexamethasone administration. In earlier studies, a criterion of a 50% decrease in plasma cortisol after overnight administration of dexamethasone had a sensitivity of 77% to 92% and a specificity of 57% to 100%.3–7 According to the study on which we based our testing,19 a 68% suppression of morning plasma cortisol after the oral administration of 8 mg of dexamethasone at 11:00 pm resulted in a sensitivity of 71% and a specificity of 100% for distinguishing CD from all other causes of CS. That study by Dichek et al19 was performed almost exclusively in adult patients. In this study, the same test, in the same institution, but in a pediatric population of patients with CS, showed that a 20% cortisol suppression from baseline had a sensitivity and specificity of 97.5% and 100%, respectively. As expected from these data, the area under the ROC curve showed that the diagnostic power of the HDDST was superior to that of the oCRH stimulation in distinguishing between pituitary-dependent CD and adrenal-dependent CS, although the 20% suppression cut-off may change in future studies because of the increased proportion of children with micronodular adrenocortical hyperplasia in these series.18

In addition, a subset of children in our investigation underwent the “classic” Liddle’s test (Fig 7); this test has been shown to be useful in patients who have suspected adrenal disease in whom adrenal imaging studies and other tests might be of limited value23 and aims to identify patients who will show “paradoxic” stimulation of their cortisol secretion, something that cannot be seen with the short HDDST. This test can then identify patients with primary pigmented nodular adrenocortical disease from those who have other forms of primary adrenocortical lesions.23 Indeed, in this series, most of the children with primary pigmented nodular adrenal disease showed an increase in UFC levels on day 6 of the test. None of the children with adrenal disease had decrease in UFC of −90% from baseline on day 6 of the test. Because this test requires 6 days of urine collection and the administration of an oral medication for 4 days every 6 hours, it should only be reserved for patients who have adrenal-dependent CD and meet the criteria outlined by Stratakis et al.21

The strength of the present study lies in the number of children examined and that all procedures were performed during an inpatient admission to a controlled environment. In addition, all children were examined because of clinical suspicion of CS and were evaluated by standardized tests that are currently in clinical practice. The limitations of our study were as a follows: (1) We did not perform salivary cortisols as suggested elsewhere34; salivary cortisols are convenient (may be obtained on an outpatient basis), spare children from venipuncture, and have a reported sensitivity and specificity of 93% to 100% and 95.2% to 100%, respectively.14 However, the assays are not well standardized and they are not available everywhere. (2) We did not perform a 1-mg dexamethasone test; this test has not been studied well in children and has not been part of the admission testing in our center. (3) A retrospective study such as ours from a large referral center that specializes in certain forms of CS may be biased in terms of both the diagnostic breakdown of our patients and the consistency of the control group. However, the majority of the children in this study had CS as a result of pituitary tumors (CD).
consistent with the literature data. In addition, the study was designed specifically to address the testing that will differentiate patients with CS from those with a state that would lead any clinician to think of this diagnosis. Because the results obtained in this particular study apply to children in whom there is clinical suspicion of CS, the NPV and PPV of each test are relatively unbiased.

CONCLUSIONS
In this series of pediatric-only patients with CS, we found that a single cortisol value at midnight followed by overnight HDDST was the most rapid and accurate way for confirmation and diagnostic differentiation, respectively, of hypercortisolemia caused by pituitary or adrenal tumors. These data suggest that the biochemical workup of pediatric patients with suspected CS can be condensed in less than a week’s time, including 3 days of UFC collection, diurnal testing, morning corticotropin, and the HDDST. Diurnal testing does require an inpatient overnight admission, and this limits its use as a routine screening test. However, this test might be extremely helpful in patients who pose a diagnostic dilemma. In addition, this study provides useful guidelines for the use and interpretation of biochemical data, imaging studies, and other testing (eg, Liddle’s test) that are used in the evaluation of pediatric CS.

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