Published in final edited form as:

Endocr Dev. 2010 ; 17: 96–107. doi:10.1159/000262532.

Pitfalls in the Diagnosis of Central Adrenal Insufficiency in Children

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Abstract

The diagnosis of central adrenal insufficiency relies heavily on laboratory testing of cortisol levels in the systemic circulation. The lack of cortisol assay standardization challenges the reliability of dynamic tests of the hypothalamic-pituitary adrenal axis. Although the insulin-induced hypoglycemia or metyrapone tests remain the accepted standards for evaluating central adrenal insufficiency in children their associated risks and inconvenience make them unattractive for routine use. Corticotropin testing is an effective first step to evaluate for chronic central adrenal insufficiency for children older than 2 years who are ambulatory, have normal sleep-wake cycle and normal serum protein levels. The low-dose (1 µg) corticotropin test may be superior to standard-dose (250 mcg) for patients with suspected hypothalamic-pituitary disease.

Chronic central adrenal insufficiency (CAI) is characterized by the impaired synthesis and release of adrenocorticotrophic hormone (ACTH) from the pituitary gland, impaired release or effect of corticotropin-releasing factor (CRH) from the hypothalamus, due to disease or injury to the hypothalamus-pituitary area or from prolonged exogenous glucocorticoid administration that exceeds physiological doses. The consequence of ACTH or CRH deficiency is reduced synthesis and release of cortisol. One of the cardinal differences distinguishing CAI from primary adrenal insufficiency is that CAI does not significantly affect the mineralocorticoid axis, as mineralocorticoids are primarily regulated by the renin-angiotensin system, and only partially by ACTH.

The reference tests for establishing the integrity of the hypothalamic-pituitary-adrenal (HPA) axis require assessing the response to either a strong stimulus (e.g. insulin-induced hypoglycemia) or an interruption of the negative feedback from cortisol (overnight metyrapone test). However, these reference tests have major drawbacks. The insulin tolerance test is contraindicated in children with a history of seizures and requires continuous physician supervision to monitor for serious adrenergic or neurological symptoms [1]. The overnight metyrapone test carries a risk of adrenal crisis, and errors can occur from other drugs affecting metyrapone clearance [2]. Thus, there is a great clinical demand for alternative tests that are quicker, cheaper, and safer.

The rationale for using the corticotropin stimulation test is the assumption that in chronic endogenous corticotropin deficiency, acute responsiveness of the adrenal zona fasciculata is diminished and fails to mount an adequate cortisol response [3]. We examined the published literature for evidence on two tests – using either a standard (250 µg) or low-dose (1 µg)

corticotropin analog – and summarized the results in a quantitative meta-analysis [4] in the ‘gold-standard’ diagnosis of central adrenal insufficiency, defined by results of the insulin tolerance test or overnight metyrapone test. In this paper, we examine the results for studies evaluating pediatric patients.

Methods

Data Collection

We searched the PubMed (www.PubMed.gov) database from 1966 to 2006 for articles with the key words ‘adrenal insufficiency’ and ‘diagnosis’ and limited the search to human studies published in English. We selected studies with at least 10 subjects with suspected CAI and required that the disease be verified with either the insulin tolerance test or the metyrapone test. We then contacted the principal investigator of each relevant study to request their patient-level data on the following variables: results of the integrated HPA axis reference test, baseline cortisol value, and cortisol values after the standard-dose and low-dose corticotropin tests.

To be eligible for inclusion, subjects had to be suspected of CAI from disease or injury to the pituitary or hypothalamus or from prolonged exogenous glucocorticoid administration in supraphysiological doses. Patients had to be affected by hypothalamic-pituitary disease for at least 4 weeks in order to exclude acute hypothalamic or pituitary disorders. A normal sleep-wake cycle was required (or assumed, if no information) as cortisol secretion has a diurnal cycle. We did not include data from studies performed in the critical care setting.

In actual clinical practice, testing is performed when there is some suspicion for CAI. We therefore investigated the performance of the tests in at-risk patients in order to avoid the problem of spectrum bias [5], which occurs when tests are evaluated among patients who are different from the ones who will be tested in practice. Thus, we excluded those studies that included control subjects who were described as normal healthy children (‘healthy volunteers’ in whom there was no suspicion of pituitary disease based on signs, symptoms, or imaging).

The main reason for HPA axis testing was growth hormone deficiency or multiple pituitary hormone deficiencies, although some children were investigated after the treatment of brain tumors, leukemia, and other central nervous system malignancies [6].

Reference Tests

The diagnosis of CAI was based on an abnormal response to one of the two reference standards for evaluating the integrity of the HPA axis: insulin tolerance test or overnight metyrapone test. We relied on individual study investigators to correctly dichotomize the reference test results into CAI present or absent.

For the overnight metyrapone test in children, a single oral dose of metyrapone was administered at 24.00 h [6] or at 23.00 h [7]. Metyrapone was given orally in a dose of 1 g/m² for children weighing 30 kg or less, 2–3 g/m² for 30–60 kg, and 3 g/m² for those weighing above 60 kg [6], which approximates doses of 30–40 mg/kg used in another study [7]. Blood glucose was monitored hourly during the night and serum cortisol and 11-deoxycortisol were measured on the following morning at 08:00 h. Response to metyrapone was defined as low if serum 11-deoxycortisol was $< 7 \mu\text{g/dl}$ (200 nmol/l) with serum cortisol $< 5 \mu\text{g/dl}$ (140 nmol/l).

For the insulin tolerance test [8], 0.1 U/kg of human regular insulin was injected and serum cortisol and glucose were measured at baseline and at 30, 60, 90 and 120 min after insulin

administration. The test was positive if the cortisol was $<20 \mu\text{g/dl}$ (550 nmol/l) when the glucose was below 40 mg/dl (2.2 mmol/l) with glucopenic symptoms.

Cortisol Assay

Cortisol assays are not standardized and vary across hospitals and studies [9–11]. The cortisol assay methods used in various studies included commercially available radioimmunoassays or immunometric methods.

Standard-Dose Corticotropin Stimulation Test

One of the two available synthetic corticotropin analogs – cosyntropin (Cortrosyn, Amphastar Pharmaceuticals, Inc.) or tetracosactrin (Synacthen, Novartis Pharma, Switzerland) – was administered intravenously at a dose of $250 \mu\text{g}$, and serum cortisol levels were obtained at baseline and at least once after injection (most commonly at 30 or 60 min).

A standard-dose corticotropin stimulation test involves a dose of $250 \mu\text{g}$ (0.25 mg) of either cosyntropin or tetracosactrin, which are equivalent to 25 USP units of corticotropin. For brevity, we use the term ‘corticotropin’ for both analogs, while acknowledging that Synacthen and Cortrosyn are synthetic corticotropin analogs, different from the native ACTH molecule.

Low-Dose Corticotropin Stimulation Test

The low-dose test was performed in the morning with patients fasting. One of the two synthetic corticotropin analogs (cosyntropin or tetracosactrin) was administered intravenously after being prepared using the method of Dickstein et al. [12]. The dose for children varies by study. Maghnie et al. [8] used a $1\text{-}\mu\text{g}$ dose, Gonc et al. [7] used $0.5 \mu\text{g/m}^2$, and Rose et al. [6] used $1 \mu\text{g/m}^2$ body surface area. Serum cortisol was measured at baseline and at 30 min postinjection, except for the study by Rose et al. [6], where it was measured at 20 min.

Basal Cortisol

All studies measured serum cortisol between 08.00 h and 10.00 h after an overnight fast (basal cortisol).

Statistical Analysis

We conducted data analysis using Stata statistical software, version 10.0 (StataCorp LP, College Station, Tex., USA).

To compare the performance of LDCT and SDCT, we used receiver-operator-characteristic (ROC) curve analysis. From each study’s data, we calculated the area under the ROC curve (AUC) with 95% CIs (fig. 1). We categorized cortisol response into 3 intervals (high, indeterminate and low likelihood of CAI), which were defined by two thresholds. The first threshold was the cortisol value below which there was a high likelihood of CAI (likelihood ratio [LR] >9 ; rule-in threshold). The second threshold was the cortisol value above which there was a low likelihood of CAI (LR <0.15 ; rule-out threshold). Cortisol values between these thresholds (LR 0.15–9) defined the interval with indeterminate likelihood of CAI.

Likelihood ratios were calculated as the ratio of two probabilities: the probability of the test result among patients with CAI, divided by the probability of the same test result among patients without CAI.

Results

We were able to analyze pediatric patient-level data from 3 published studies, with 2 studies having paired data comparing results of the low-dose and standard-dose corticotropin stimulation tests on the same patients (table 1). The prevalence of CAI in the study samples ranged from 27% to 58%, with a mean of 33% (table 1). The age of the children varied from 1 to 18 years [6], 5 to 20 years [7], and 4 to 20 years [8].

Cortisol Testing

Cortisol testing methods varied from individual radioimmunoassay kits [6, 8] to immunometric test kits [7]. The lack of a standard cortisol assay method [9–11] explains some of the variability in diagnostic cortisol thresholds reported across studies.

Basal Cortisol

According to pediatric data from 3 studies (211 children), the lowest basal cortisol threshold was 3 µg/dl (88 nmol/l) to diagnose CAI and the highest basal threshold was 7 µg/dl (15 µg/dl (415 nmol/l) to exclude CAI. For comparison, in a meta-analysis of 12 studies of adults (635 subjects), a basal cortisol less than 5 µg/dl (138 nmol/l) best predicted CAI, while values greater than 13 µg/dl (365 nmol/l) best predicted a normal HPA axis.

Standard-Dose Corticotropin-Stimulated Cortisol Test

After standard-dose corticotropin stimulation, there was variability across studies in the optimal timing for measuring cortisol response; however, in no study was there a statistically significant difference in diagnostic discrimination at 30 min, 60 min, or at peak response.

A 30-min cortisol value of less than 16 µg/dl (440 nmol/l) was highly predictive of CAI. Values greater than 39 µg/dl (1,076 nmol/l) virtually exclude CAI in children (ruling out CAI). (For comparison, adults with a stimulated cortisol greater than 30 µg/dl (833 nmol/l) rules out CAI.)

Intermediate values – 16–39 µg/dl – should be considered diagnostically indeterminate in children. The area under the ROC curve for these test results is depicted in figure 1.

Low-Dose Corticotropin-Stimulated Cortisol Test

After low-dose corticotropin stimulation, 30-min cortisol measurements in 2 studies had superior test characteristics compared to measurements at other times [7, 8]. In our analyses of low-dose corticotropin stimulation, we used 30-min cortisol values, or, if not available, then the 20-min value [6].

A 20- to 30-min cortisol value of less than 16 µg/dl (440 nmol/l) was highly predictive of CAI. Values greater than 22 µg/dl (600 nmol/l) virtually exclude CAI in children (ruling out CAI). The area under the ROC curve using these diagnostic thresholds was 0.99 (95% CI 0.98–1.00). Given the a small number of pediatric studies and to achieve higher fidelity diagnosing or excluding CAI, the ‘rule in’ threshold value was selected as the lowest cortisol threshold to predict CAI (whereas weighted mean was 17 µg/dl or 470 nmol/l), the ‘rule out’ threshold value was selected as the highest cortisol threshold to exclude CAI (whereas weighted mean was 20 µg/dl or 550 nmol/l), and were reasonably close to match LDCT thresholds in adults. Using weighted mean thresholds did not significantly change the results.

For comparison, our meta-analysis of 11 studies using the low-dose stimulation test (589 subjects, primarily adults) found that values less than 16 µg/dl (440 nmol/l) best predicted CAI, while values greater than 22 µg/dl (600 nmol/l) were best for ruling out CAI. The area under the ROC curve using these diagnostic thresholds was 0.94 (95% CI: 0.90–0.94).

Comparison of Low-Dose and Standard-Dose Stimulation Tests

In the 2 studies with paired 30-min cortisol data for both tests (53 children) and one study with unpaired data (158 children), the low-dose test had a larger area under the ROC curve compared to the standard-dose test (see fig. 1 for area under the ROC curve). In the two studies that used either 1 µg or 1 µg/m² of the corticotropin analog, the low-dose test was statistically superior to the standard-dose test in area under the ROC curve.

Optimal Testing Strategy Algorithm in Children

We applied the previously published testing algorithm [4] to the data on children (fig. 2). The basal cortisol and LDCT thresholds described in figure 2 were based on the mean cortisol values weighted by study sample size (last row of table 1). The thresholds for the two sequential tests defined 5 subgroups: (1) low basal cortisol; (2) high basal cortisol; (3) indeterminate basal cortisol and low stimulated cortisol; (4) indeterminate cortisol and indeterminate stimulated cortisol, and (5) indeterminate basal cortisol and high stimulated cortisol. We calculated the expected probability of CAI, with 95% CIs, within each of the 5 subgroups.

We found that overall LDCT thresholds for evaluation of CAI performed well in children. However, basal cortisol thresholds, particularly the one to rule in CAI (<5 µg/dl) was not reliable.

Discussion

Our analysis suggests that the low-dose corticotropin stimulation test is superior to the standard-dose test in diagnosing central adrenal insufficiency in children, similar to findings in adults. Because all study subjects were ambulatory and presumably had normal sleep-wake cycles, these findings may not generalize to hospital settings or patients with acute illnesses. Nevertheless, there may be clinical settings where standard-dose testing is more appropriate to diagnose CAI, especially if the quality of the low-dose testing protocol cannot be assured.

The 3-step approach for evaluating patients with possible hypothalamic-pituitary adrenal insufficiency [4] may be used in children (fig. 2). However, in the first step of measuring a morning basal cortisol, one may consider using lower threshold (3 µg/dl or 88 nmol/l) to achieve higher fidelity in the diagnosis of adrenal insufficiency. The second step, a low-dose corticotropin stimulation test, performs well in children diagnosing or excluding adrenal insufficiency. If this test is indeterminate and there are no contraindications to integrated HPA axis testing, we suggest the third step of an insulin hypoglycemia test or metyrapone test. Although this three-step approach will accurately diagnose the majority of children, because it is not perfect, there will still be an important role for clinical judgment, especially regarding use of glucocorticoid supplementation during extreme stress. For convenience, in appropriate clinical circumstances, the first and second steps (basal and LDCT or SDCT) can be done at the same clinical visit to reduce the number of visits for testing.

Due to the lack of cortisol assay standardization and other reasons for measurement variability, the error in measuring cortisol can be up to 6 µg/dl (165 nmol/l), thus caution is advised when making clinical decisions based on cortisol values close to threshold values. In addition to high variability in the cortisol diagnostic thresholds, especially one to exclude

CAI, a low likelihood of CAI does not exclude the possibility of future CAI, especially after progression of hypothalamic-pituitary disease or radiation therapy. Therefore, longitudinal assessments may be necessary.

The low-dose corticotropin stimulation test has not been validated in patients with acute illnesses, abnormal sleep-wake cycles, or acute hypothalamic-pituitary disorders (e.g. within 1 month of pituitary surgery). In addition, all studies of the low-dose test that we were able to analyze were conducted in the morning with the patients fasting. Afternoon cortisol values tend to be lower by 1–1.5 $\mu\text{g/dl}$ (28–58 nmol/l) [12, 13], and the effect of eating or drinking is uncertain. We also have no information on how the low-dose corticotropin stimulation test would perform among patients with low serum protein levels, as cortisol in the circulation is highly protein bound.

There are several technical details to performing a low-dose test that must be rigorously addressed to avoid false-positive test results (falsely low 30-min stimulated cortisol value). Currently, there are two acceptable corticotropin analogs that can be used – cosyntropin (Cortrosyn, Amphastar Pharmaceuticals) or tetracosactrin (Synacthen, Novartis Pharma) – supplied in vials containing 250 μg of powder. Preparing the 1- μg dose requires a several-step process of first reconstituting with 250 ml of normal saline and then using a 1-ml aliquot (1 μg) for intravenous injection. There are additional steps for minimizing adherence of the medication to plastic tubing [14]. In addition, the timing of cortisol sampling after low-dose corticotropin administration is very important (we recommend collecting the blood sample 20–30 min after corticotropin analog administration), as later sampling may result in a false-positive result [15]. Thus, low-dose testing should be performed only by personnel knowledgeable of the multiple steps required for preparation and administration. If the quality of administering a 1- μg dose is suspect, then we recommend using the standard dose of 250 μg (reconstituted with 1 ml of sterile diluent) and measuring serum cortisol 30 min after intravenous injection. A result less than 16 $\mu\text{g/dl}$ (440 nmol/l) – which is the same threshold used for low-dose testing – strongly suggests hypothalamic-pituitary AI. However, with standard-dose testing, the 30-min cortisol value must be greater than 30 $\mu\text{g/dl}$ (833 nmol/l) to be reasonably confident in ruling out adrenal insufficiency.

In the two studies that used 1- μg corticotropin analog testing (Maghnie and Rose studies in fig. 1), the low-dose tests perform better than standard-dose test – the area under the ROC curve is better for the low-dose test. Although we do not have a head-to-head comparison of the 1- μg or 1- $\mu\text{g/m}^2$ and 0.5- $\mu\text{g/m}^2$ corticotropin testing, the latter dose appears to be inferior (the discriminatory capacity of 0.5 $\mu\text{g/m}^2$ LDCT is similar to SDCT). Therefore, the 1- μg corticotropin analog testing is preferable. None of the studies were designed to validate a dose adjustment for body surface for the 1- μg corticotropin stimulation test. Similar to adults, children with a mature hypothalamic-pituitary axis (typically older than 3 years) [16] may achieve the maximal total daily ACTH production rates which can reach 250 μg . Therefore, using corticotropin analog in a 1- μg dose without body surface adjustment is logical and feasible [8], given the precision due to technical difficulties diluting and administering available corticotropin formulations in doses even lower than 1 μg .

It is worth mentioning that low-dose corticotropin analog testing in children younger than age 3 years has not been well studied.

Research suggests [17, 18] that dehydroepiandrosterone sulphate (DHEA-S) blood levels might also help with assessing the hypothalamic-pituitary-adrenal axis, particularly when the results of the low-dose stimulation test are close to either of the two threshold values.

We found no studies testing the performance of the low-dose corticotropin analog test against a reference standard (insulin hypoglycemia test or metyrapone test) in the diagnosis

of glucocorticoid-induced adrenal insufficiency. The diagnosis of asthmatic children using inhaled synthetic glucocorticoids is challenging for several reasons. First, the basal cortisol levels in children have a relatively low discriminatory capacity diagnosing central adrenal insufficiency, and synthetic glucocorticoid absorbed into the systemic circulation may affect basal cortisol levels (certain synthetic glucocorticoids may partially cross-react in cortisol assay). Second, the performance of the low-dose corticotropin analog test in patients has not been well validated against a reference standard. The washout period of the synthetic glucocorticoids and their metabolites (3–5 elimination half-times) may require prolonged discontinuation of inhaled glucocorticoid in order to obtain valid results of insulin hypoglycemia or overnight metyrapone test. Finally, the diagnostic thresholds used in the low-dose corticotropin test may prove to be different (dependent on the degree of cortisol suppression and on partial cross-reactivity of synthetic glucocorticoid in cortisol assay) in patients who have synthetic glucocorticoids in their circulation.

Establishing the diagnosis of hypothalamic-pituitary adrenal insufficiency, the accepted reference standard is an abnormal insulin tolerance test or metyrapone test. Both tests, however, can be unreliable. The average intra-subject variability in peak cortisol response to insulin-induced hypoglycemia is 8 to 12 percent [19], but in males with hypopituitarism it can vary by 42 percent [20]. Healthy control subjects have been known to ‘fail’ this test. Neither of these reference tests has been validated by assessing predictive accuracy, that is, the ability to predict adrenal crisis.

A limitation of our analysis is that we were unable to include data of one study that had published paired results of LDCT and SDCT, because we were unable to obtain the patient-level data [21]; however, spectrum bias in this study limits the value of its use for the purpose of our analysis, as the children were selected for Weintrob et al. [21] study either due to unequivocal adrenal insufficiency or unequivocally normal HPA axis.

In summary, the performance of the three step algorithm, including low-dose corticotropin stimulation test for diagnosis of central adrenal insufficiency is valid in children older than age 3 years. The low-dose corticotropin stimulation test appears to be superior to the high-dose test for evaluating hypothalamic-pituitary adrenal insufficiency. However, it must be used by personnel, knowledgeable of the multiple steps required for preparation and administration.

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Fig. 1. ROC curves for LDCT (20- to 30-min cortisol levels; light lines) and SDCT (30-min cortisol levels; darker lines) diagnosing HPA insufficiency.

Fig. 2. Performance of adult optimal testing strategy for evaluating children with possible CAI. * A normal DHEA-S blood level makes CAI highly unlikely [17] and therefore supports the finding of a normal basal cortisol level. ** At <3 µg/dl (88 nmol/l) threshold probability CAI 100% (95% CI 99–100).

Table 1

Study characteristics

Study	Basal cortisol (early morning)			Standard-dose stimulated cortisol (30 min/peak)			Low-dose stimulated cortisol (20-30 min)		
	n	HPAI [†]	no HPAI [‡]	n	HPAI [†]	no HPAI [‡]	n	HPAI [†]	no HPAI [‡]
<i>Studies with paired data</i>									
Gonc et al. [7]	11/29	6	9	11/29	16	36	11/29	16	19
Maghnie et al. [8]	14/24	9	15	13/23	20	33	14/24	20	22
<i>Study with unpaired data</i>									
Rose et al. [6] //	42/158	3	13	14/38	16	39	28/120	17	20
Adult mean [§] (95% CI)	33% 210/635	5[§] (4.7–5.3)	13[§] (12.9–13.6)	40% 140/346	16[§] (15.2–16.4)	30[§] (29.9–32.3)	33% 193/586	16[§] (15.2–16.0)	22[§] (20.9–21.9)

HPAI = Hypothalamic-pituitary adrenal insufficiency.

Suggested pediatric and adult thresholds are in bold.

* Serum cortisol in µg/dl, to convert to nmol/l multiply by 27.56.

† Threshold below which cortisol values had a LR >9 (rule-in threshold).

‡ Threshold above which cortisol values had a LR <0.15 (rule-out threshold).

§ Mean, weighted by size of the studies.

// SDCT and LDCT were performed on different subsets of patients (no paired data).