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Clinical Research Article

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New Cutoffs for the Biochemical Diagnosis of Adrenal Insufficiency after ACTH Stimulation using Specific Cortisol Assays

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Abbreviations: ACTH, adrenocorticotropic hormone; AI, adrenal insufficiency; CST, synthetic $ACTH_{[1–24]}$ (cosyntropin) stimulation testing; HPA, hypothalamic-pituitary-adrenal; LC-MS/MS, liquid chromatography–tandem mass spectrometry; ROC, receiver operator characteristic curve.

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Abstract

Context: The normal cortisol response 30 or 60 minutes after cosyntropin ($ACTH_{[1–24]}$) is considered to be ≥18 μg/dL (500 nmol/L). This threshold is based on older serum cortisol assays. Specific monoclonal antibody immunoassays or LC-MS/MS may have lower thresholds for a normal response.

Objective: To calculate serum cortisol cutoff values for adrenocorticotropic hormone (ACTH) stimulation testing with newer specific cortisol assays.

Methods: Retrospective analysis of ACTH stimulation tests performed in ambulatory and hospitalized patients suspected of adrenal insufficiency (AI). Serum samples were assayed for cortisol in parallel using Elecsys I and Elecsys II immunoassays, and when volume was available, by Access immunoassay and LC-MS/MS.

Results: A total of 110 patients were evaluated. Using 18 μg/dL as the cortisol cutoff after ACTH stimulation, 14.5%, 29%, 22.4%, and 32% of patients had a biochemical diagnosis of AI using the Elecsys I, Elecsys II, Access, and LC-MS/MS assays, respectively. Deming regressions of serum cortisol were used to calculate new cortisol cutoffs based on the Elecsys I cutoff of 18 μg/dL. For 30-minute values, new cutoffs were 14.6 μg/dL for Elecsys II, 14.8 μg/dL for Access, and 14.5 μg/dL for LC-MS/MS. Baseline cortisol <2 μg/dL was predictive of subnormal stimulated cortisol values.

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Conclusion: To reduce false positive ACTH stimulation testing, we recommend a new serum cortisol cutoff of 14 to 15 μg/dL depending on the assay used (instead of the historical value of 18 μg/dL with older polyclonal antibody assays). Clinicians should be aware of the new cutoffs for the assays available to them when evaluating patients for AI.

Key Words: ACTH stimulation testing, cosyntropin, monoclonal cortisol immunoassay, LC-MS/MS

The accurate and swift diagnosis of adrenal insufficiency (AI) is imperative given the potential for life-threatening consequences if missed [1, 2]. Conversely, inappropriate assignment of AI to individuals has the potential for unnecessary glucocorticoid therapy [3]. Therefore, confirmation of the diagnosis of AI mandates precise biochemical testing, often requiring the assessment of adrenocorticotropic hormone (ACTH)-stimulated adrenal function to evaluate the integrity of hypothalamic-pituitary-adrenal (HPA) axis function.

ACTH (synthetic $ACTH_{[1–24]}$; cosyntropin; synacthen) stimulation testing (CST) is the most commonly performed dynamic test to assess the adequacy of adrenal function in patients with suspected secondary adrenal insufficiency [4]. CST assesses the maximum adrenocortical secretory response to a supraphysiologic dose of ACTH. Patients with primary AI always have an elevated plasma ACTH, so CST is typically not needed for confirmation of the diagnosis [5, 6]. Although insulin-induced hypoglycemia has previously been considered the gold standard test for decreased HPA axis function, it is very challenging to perform properly, labor intensive, and risky [7]. Accordingly, it has been abandoned in most clinical settings. The CST cortisol cutoff threshold for the diagnosis of AI 30 or 60 minutes after ACTH administration has evolved over the years, but it has become entrenched at 18 μg/dL (500 nmol/L) despite improved specificity of newer cortisol assays [5, 8-10]. Historically, immunoassays using polyclonal antibodies have been used to establish post-cosyntropin cortisol cutoff concentrations as high as 20 μg/dL [11, 12]. These assays had cross-reactivity with other serum steroids [13-15]. Newer-generation assays with greater specificity for cortisol have been developed and have already replaced polyclonal antibody assays in many institutions [13, 15, 16].

Basal morning serum cortisol concentrations are also used to either increase the suspicion for, or rule-out the diagnosis of AI [11, 12, 17-20]. Furthermore, basal morning serum cortisol concentrations ranging from 11 to 19 μg/dL have been cited as a criterion to rule-out AI. Conversely, it has been argued that very low cortisol values (ie, <3-6 μg/dL) may establish biochemical AI and thus obviate the need for dynamic CST.

The Elecsys Cortisol generation II (Roche Diagnostics, City, IN) and Beckman Access Cortisol (Beckman Coulter, City, CA) immunoassays utilize monoclonal antibodies to identify cortisol [16, 21]. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is a non-antibody, structural assay highly specific for cortisol [22-25]. Serum cortisol concentrations are approximately 20% lower with the newer assays compared with the older assays in some studies [8, 13, 15, 26, 27]. However, other studies have actually suggested that LC-MS/MS yielded a higher peak cortisol cutoff after ACTH stimulation than cortisol measured by immunoassay [28, 29]. Considering these discrepancies in the literature, ACTH-stimulated cortisol threshold values using new, more-specific cortisol assays are needed to accurately diagnose AI and minimize overtreatment.

The purpose of the current study was to evaluate basal serum cortisol and the serum cortisol response to synthetic $ACTH_{[1–24]}$ stimulation in patients suspected of having AI and compare more-specific cortisol assays (2 monoclonal antibody assays and LC-MS/MS) with a polyclonal antibody cortisol assay in order to calculate new cutoffs for the cortisol response to CST.

Materials and Methods

The study was approved by the Medical College of Wisconsin/Froedtert Hospital Institutional Review Board.

Study Design

This study used residual serum samples (n = 110) from patients undergoing CST (ambulatory and hospitalized) ordered by any clinician as part of clinical care from January 8, 2017 to April 30, 2017. This time frame was dictated by the limited availability of reagents for the Elecsys Cortisol generation I (Roche Diagnostics, IN) assay which was being phased out by the vendor during the study. Samples were identified using laboratory order codes for CST.

CST was performed as follows: cosyntropin (250 mcg $ACTH_{[1–24]}$) was administered intravenously. Blood samples for cortisol were obtained in serum separator tubes (SST) before injection (0 minutes) and at 30 minutes. If a clinician also ordered a blood sample for cortisol at 60 minutes, that was analyzed. Basal ACTH values in EDTA plasma were also obtained in some instances at the ordering provider's discretion. All samples (n = 110 for the

0- and 30-minute samples; n = 89 for the 60-minute samples) were run in parallel on the Elecsys I and II cortisol immunoassays. When specimens were available, de-identified samples were analyzed at the Mayo Clinical Laboratory using the Beckman Access Cortisol (Beckman Coulter, CA) immunoassay (n = 79 for the 0-minute sample, n = 78 for 30-minute sample, and n = 66 for 60-minute sample) and an LC-MS/MS assay (n = 79 for the 0-minute sample, n = 79 for the 30-minute sample, and n = 66 for the 60-minute sample).

Clinical data were obtained through chart review and entered into a password-protected Excel spreadsheet as follows: date, sampling times, cortisol, basal ACTH, age, sex, medical history related to potential adrenal insufficiency, relevant medications (eg, opioids, glucocorticoids), and inpatient vs outpatient setting.

Assays

The Elecsys Cortisol (referred to here as generation I) and Cortisol II electrochemiluminescence competitive immunoassays were run on the Cobas E170 (Roche Diagnostics, Indianapolis, IN) [14, 16]. The generation I cortisol assay used a polyclonal antibody and was standardized against the Enzymun test, which was standardized against isotope dilution mass spectrometry (ID-MS). It has significant cross-reactivity for 21-deoxycortisol (1 µg/mL), 45.8%; 6-β-hydroxycortisol (1 µg/mL), 158%; allotetrahydrocortisol (0.1 µg/mL), 165%; prednisolone (0.1 µg/mL), 171%; and 6-α-methylprednisolone, 389%. Intraassay variability was 1.6% to 2.4% and interassay variability was 1.9% to 2.8% for concentrations ranging from 1.4 to 60.2 µg/dL. The Cortisol II assay uses a monoclonal antibody and is standardized against a reference material, the IRMM/IFCC 451 Panel (ID-GC/MS). The assay is more specific for cortisol than its predecessor and reduced all steroid cross-reactivities to under 10%, except for prednisolone (12% at 0.1 µg/mL). Intraassay variability was 1.0% to 1.7% for concentrations ranging from 4.7 to 31.4 µg/dL. Interassay variability was 2.2% to 2.8% for concentrations ranging from 4.5 to 12.4 µg/dL

The Access Cortisol assay is a competitive binding immunoenzymatic assay run on the Beckman Coulter UniCel DxI 800 (Beckman Coulter, Brea, CA) [21]. Relevant cross-reactivities for other steroids include 11-deoxycortisol (100 µg/dL), 17.8% and prednisolone (20 µg/dL), 23.9%. Intraassay variability was 3.4% to 4.7% and interassay variability was 4.1% to 5.7% for concentrations ranging from 4.4 to 35.3 µg/dL. The serum LC-MS/MS assay method has been described previously [30]. Intraassay variability was 4.8% to 7.5% for concentrations ranging from 1.1 to 20.7 µg/dL. Interassay variability was 8.7% to 9.8% for concentrations ranging from 2.2 to 21.0 µg/dL. Plasma ACTH was measured using the Roche Cobas platform immunometric assay [31, 32].

Statistical Analysis

All statistical analyses were performed used Sigmaplot 12.5 (Systat Software Inc., San Jose, CA). Deming regressions were used to calculate the slope and intercept (with 95% CI) of the relationship between Elecsys II, Access, or LC-MS/MS vs Elecsys I cortisol assay and to then calculate the new cutoffs based on Elecsys I cutoffs. Bland-Altman plot bias data were analyzed by one-factor analysis of variance with all pairwise comparisons (Duncan multiple range test). Receiver operator characteristic curve (ROC) analysis was performed using standard algorithms with the Elecsys I data and its 18 µg/dL cutoff to assign the designation of biochemical adrenal insufficiency by maximizing both sensitivity and specificity [10, 11]. In patients with complete baseline and 30- and 60-minute post-cosyntropin data within each assay method, two-factor analysis of variance repeated on one factor (time) with all pairwise comparisons (Duncan's multiple range test) was used to compare results against Elecsys I and within time. Mann-Whitney Rank Sum Test was used to compare baseline serum cortisol results separated by whether the serum cortisol response to ACTH stimulation was normal vs subnormal. Chi-square was used to evaluate proportional data.

Results

Figure 1 shows Deming regression of serum cortisol by Roche Elecsys II, Beckman Access, and LC-MS/MS compared with Elecsys I. Shown are baseline serum cortisol (left column) and 30- and 60-minute post-cosyntropin samples for results with a baseline Elecsys I cortisol <18 µg/dL (500 nmol/L; middle and right columns). There were excellent Deming correlation coefficients between cortisol assay methods. Notice that the N values were less for Access and LC-MS/MS because of insufficient serum samples. Also, there were fewer 60- vs 30-minute samples because not all clinicians requested the 60-minute sample.

The more-specific cortisol assays (Elecsys II, Access, and LC-MS/MS) resulted in lower cortisol concentrations and lower cutoffs as the Deming slopes were all <1.00 (0.61-0.78). Elecsys II and Access cortisol were similar. The slope of LC-MS/MS vs Elecsys I regression was lower than Elecsys II and Access cortisol leading to lower cutoffs. Table 1 shows a series of baseline cutoffs (to rule-out AI) calculated from commonly used baseline Elecsys I cutoffs using Deming regression data shown in Fig. 1.

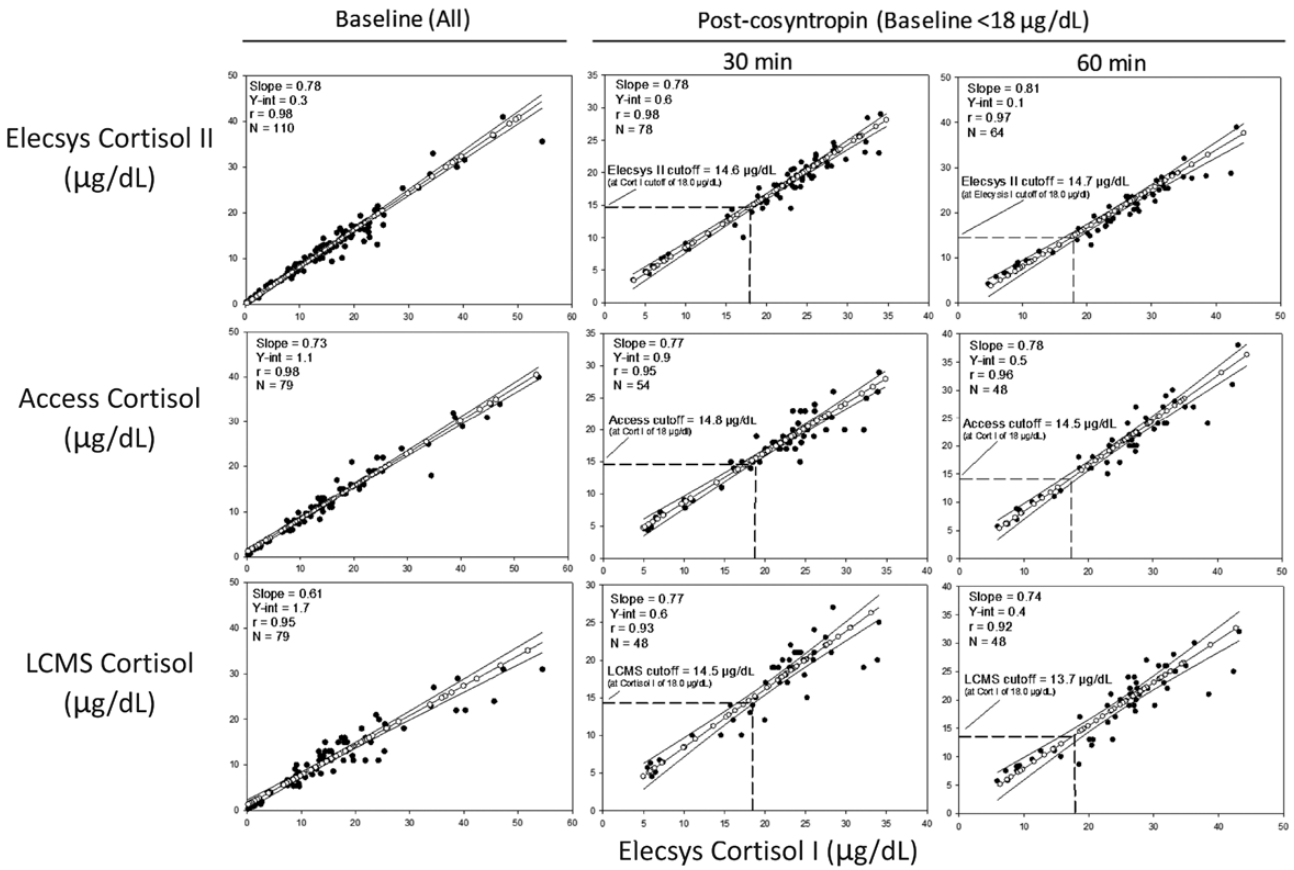

Figure 1. Deming regressions of serum cortisol by Roche Elecsys II, Beckman Access, and LC-MS/MS, compared with Elecsys I. Cortisol cutoff values for the 30- and 60-minute samples after ACTH (cosyntropin) stimulation were calculated from patients with baseline cortisol <18 µg/dL (500 nmol/L) on the Elecsys I assay. Confidence limit lines are 95%.

Table 1. Common Baseline Serum Cortisol Cutoffs Used to Rule-Out Adrenal Insufficiency (8–16 µg/dL)

Elecsys I	*Elecsys II*	*Access*	*LC-MS/MS*
8.0	6.6	6.9	6.6
10.0	8.1	8.4	7.8
12.0	9.7	9.9	9.0
14.0	11.3	11.3	10.2
16.0	12.8	12.8	11.5
N	110	79	79
Slope	0.78	0.73	0.61
95% CI	[0.75-0.81]	[0.70-0.76]	[0.57-0.66]
Y-int	0.33	1.1	1.7
95% CI	[–0.23-0.89]	[0.41-1.7]	[0.81-2.6]
***r* value**	0.98	0.98	0.95

Cutoffs were generated using Deming regression data shown. [95% CIs for slope and intercept are shown in brackets]. Cortisol units are in µg/dL. To convert µg/dL to nmol/L, multiply by 27.6.

Abbreviations: LC-MS/MS, liquid chromatography–tandem mass spectrometry; Y-int, y-axis intercept of the Deming regression.

Fig. 2 shows the Bland-Altman plots relative to Elecsys I (with the panels organized as in Fig. 1). The bias for baseline cortisol ranged from –2.9 to –4.3 µg/dL. The bias for 30-minute post-ACTH stimulation (with the cortisol baseline <18 µg/dL) ranged from –3.9 to –4.1 µg/dL and for 60-minute post-ACTH stimulation, –3.7 to –6.0 µg/dL. For the baseline cortisol data, the bias of LC-MS/MS was greater than for Elecsys II ($P = 0.019$). There were no differences in bias for the 30-minute post-ACTH stimulation results. For the 60-minute post-ACTH data, bias of LC-MS/MS was greater than Elecsys II ($P = 0.009$) and Access ($P < 0.001$).

To further explore the data, we performed ROC analyses with the assumption that Elecsys I and its associated cortisol cutoff of 18 µg/dL is the "gold standard" [10, 11]. The sensitivities and specificities of Elecsys II, Access, and LC-MS/MS were the same as one would expect considering the excellent correlations shown in Fig. 1. More importantly, the calculated post-ACTH ROC cortisol cutoffs were as follows: At 30 minutes post-ACTH, the ROC cutoffs were 14.4 µg/dL for Elecsys II, 15.5 µg/dL for Access, and 13.5 µg/dL for LC-MS/MS. At 60 minutes post-ACTH, the ROC cutoffs were 13.4 µg/dL for Elecsys II, 14.5 µg/dL for Access, and 11.5 µg/dL for LC-MS/MS.

Table 2 shows the new post-ACTH stimulation cortisol cutoffs for Elecsys II, Access, and LC-MS/MS interpolated

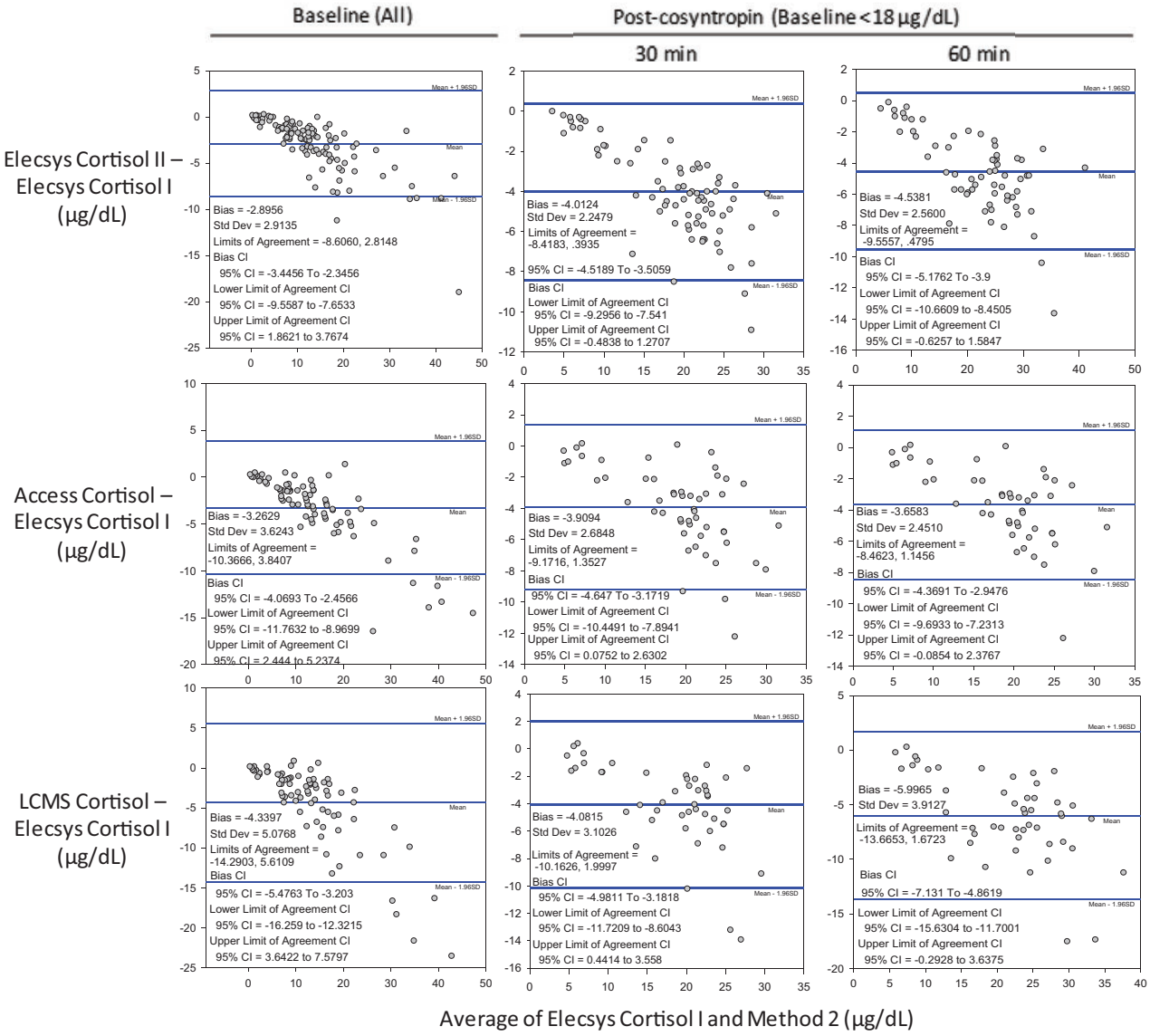

Figure 2. Bland-Altman Plots of serum cortisol by Roche Elecsys II, Beckman Access, and LC-MS/MS compared with Elecsys I. Cortisol cutoff values for the 30- and 60-minute samples after ACTH (cosyntropin) stimulation were calculated from patients with baseline cortisol <18 µg/dL (500 nmol/L) on the Elecsys I assay. N values are the same as in Fig. 1. Bland-Altman parameters are shown in each panel. For the baseline data, the bias of LC-MS/MS cortisol was greater than for Elecsys II ($P = 0.019$). There were no differences in bias for 30-minute post-ACTH (cosyntropin) results. For the 60-min post-ACTH (cosyntropin) data, bias of LC-MS/MS cortisol was greater than Elecsys II ($P = 0.009$) and Access ($P < 0.001$).

from the Deming regressions with Elecsys I from Fig. 1. Again, the slopes were <1 resulting in new cortisol cutoffs <18 µg/dL for the more-specific assays. Notice that Table 1 cannot be used to compare 30- vs 60-minute results because the data are for all patients, not only for those who had complete baseline, 30-minute, and 60-minute samples (repeated measure on all times points).

In order to accurately compare the 30- vs 60-minute response to ACTH stimulation, we then analyzed the data for only those patients who had complete 0 (baseline), 30-minute, and 60-minute results within each method (repeated measures) (Fig. 3). All the assays had statistically significantly lower values at every time point compared with the Elecsys I assay. More importantly, the 60-minute cortisol result for all methods was higher than the 30-minute result. Finally, the cortisol response to ACTH stimulation for the 3 more-specific assays were not different from each other.

When evaluating patients who had data for basal cortisol and at least one other time point (30- or 60-minutes post-ACTH stimulation), 16 of 110 (14.5%) Elecsys I ACTH-stimulated cortisol were subnormal if 18 µg/dL was used as the cutoff. For Elecsys II, 32 of 110 (29%) results would be considered subnormal if 18 µg/dL was used as the cutoff ($P = 0.014$ compared with Elecsys I). Using the LC-MS/MS assay, 25 of 78 (32%; $P = 0.007$ compared

Table 2. New Cosyntropin-Stimulated 30- and 60-Minute Cortisol Cutoffs for Elecsys II, Access, and LC-MS/MS Around Commonly Used Values (17-20 μg/dL) for the Assessment of Adrenal Insufficiency

	30-min post-cosyntropin (250 μg)			*60-min post-cosyntropin (250 μg)*		
Elecsys I	*Elecsys II*	*Access*	*LC-MS/MS*	*Elecsys II*	*Access*	*LC-MS/MS*
17.0	13.9	14.0	13.7	13.9	13.8	13.0
18.0[a]	14.6	14.8	14.5	14.7	14.5	13.7
19.0	15.4	15.5	15.2	15.5	15.3	14.5
20.0	16.2	16.3	16.0	16.3	16.1	15.2
N	78	54	48	64	48	48
Slope	0.78	0.77	0.77	0.81	0.78	0.74
95% CI	[0.74-0.82]	[0.70-0.84]	[0.68-0.86]	[0.76-0.86]	[0.71-0.85]	[0.64-0.83]
Y-int	0.6	0.9	0.6	0.1	0.5	0.4
95% CI	[−0.3 to 1.5]	[−0.7 to 2.4]	[−1.4 to 2.6]	[−1.1 to 1.3]	[−1.4 to 2.4]	[−2.1 to 2.9]
***r* value**	0.98	0.95	0.93	0.97	0.96	0.92

Cutoffs were generated using Deming regression data shown. [95% CIs for slope and intercept are shown in brackets]. Cortisol units are in μg/dL. To convert μg/dL to nmol/L, multiple by 27.6. The data are based on all patients with a baseline serum cortisol <18 μg/dL. The N values are different for 30 vs 60 minutes because these were not all paired results. Therefore, one cannot compare 30 vs 60 minutes in this Table.

Abbreviations: LC-MS/MS, liquid chromatography–tandem mass spectrometry; Y-int, y-axis intercept.

[a]18.0 μg/dL is the widely used cutoff.

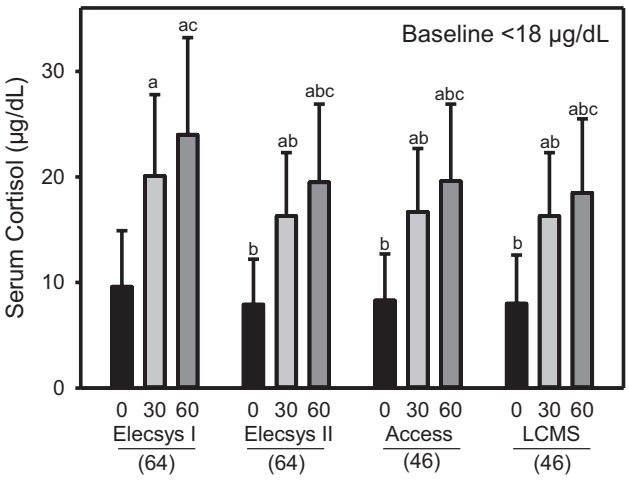

Figure 3. Comparison of baseline, 30-minute, and 60-minute cortisol values after ACTH (cosyntropin) stimulation in only those patients with data for all 3 time points within each assay method (paired repeated measures). **a,** different from 0 minutes within method. **b,** different from Elecsys I within same time point. **c,** different from 30 minutes within method. Numbers in parenthesis are N values.

with Elecsys I) would be considered subnormal if 18 μg/dL was used as the cutoff. Although not significantly different from the Elecsys I assay, 17 of 76 (22.4%; $P = 0.239$ compared with Elecsys I) would be considered subnormal using the Access assay.

To better understand if the new calculated cutoff values would provide similar biochemical diagnoses of AI (compared with the historical cutoff of 18 μg/dL in the Elecsys I assay), we identified discrepancies between assays (Table 3). Eight of the 110 patients (7.3%) had abnormal CST results (both the 30- and 60-minute results were below the cutoff) on at least one assay, but normal results using the new cutoffs on another assay. Five were abnormal in 1 of the 4 assays, and 3 were abnormal in 2 of the 4 assays. Most of the abnormal values were close to the cortisol cutoff. On chart review, 3 of these 8 patients were clinically thought to have secondary AI. LC-MS/MS would have identified all 3 of these patients as having a biochemical diagnosis of AI using the newly derived cortisol cutoff, while 2 of 3 would have been identified with Elecsys II and none with Elecsys I or Access.

Nine of the patients had abnormal 30-minute cortisol results, but normal 60-minute cortisol results on at least one assay (using the new cutoffs) (Table 3). On chart review, only 1 of these 9 patients were clinically thought to have secondary AI (case 85). Three of the patients fell into both categories (discordant results between assays and 30-minute vs 60-minute discordance). Most of the CST were performed before 9 AM (all but 3). Assay discordance could not be explained by obviously identifiable features of the clinical history including reason for CST, age, sex, time of day, inpatient (± critical illness) vs outpatient status, estrogen use, or ACTH level (Chi-square).

We further explored the data from Table 3 in only those samples with discordant post-CST results at 30 vs 60 minutes in the 8 patients in whom the clinical diagnosis of secondary AI was excluded (based on chart review). We did this analysis for the Elecsys II samples, as that is the monoclonal antibody assay currently in use for which we had complete data. In those 8 patients, 2 had a cortisol response at 30 minutes above the new cutoff whereas 7 had a cortisol response at 60 minutes above the new cutoff ($P = 0.041$).

Table 3. Discordant Cosyntropin Stimulation Test (CST) Results Between Assays and Discordant 30- vs 60-Minure Results Within Assays Using the New Cortisol Cutoffs

			Elecsys I			Elecsys II			Access			LC-MS/MS									
Case	Discordant CST result	30 Abn, 60 Nml	0	30	60	0	30	60	0	30	60	0	30	60	Age	Sex	CST time	Inpt (I) Outpt (O)	Basal ACTH	Clinical diagnosis AI	Pertinent Clinical History
2	X	X	9.6	***16.1***	18.5	7.2	***11.9***	***13.9***	9.8	***14***	18	5.3	***12***	***8.6***	68	M	12:37	I	NA	No	Syncope; on fludrocortisone for autonomic insufficiency; treated with GC 1 week prior
3	X		8.9	20	23.7	6.9	15.3	18	7.8	17	21	5.4	***12***	***13***	58	M	8:00	O	28.6	No	6 mm pituitary lesion; intraarticular GC injections 1–2 months prior
13		X	13.1	***12.9***	24.1	10.7	***10.4***	18.7			20			17	86	F	13:05	I	NA	No	Hyponatremia; SIADH and excessive PO intake; oral budesonide
14	X		12.2	23		9.8	***14.5***								65	M	8:19	O	35.8	No	Hypoglycemia
20	X		24.2	25.9		13	***14.3***		19	19		***11***	***12***		85	M	9:01	I	NA	Yes	Weakness, vomiting, weight loss; long-term GC tapered off just before stim test; chronic opioids
23	X		22.8	32.6	39.9	14.6	21.9	26.8					***12***		77	F	6:13	I	13.1	No	Unilateral adrenalectomy; bilateral adrenal nodules with cortisol excess
39		X	25.3	27.3	31.8	17.3	19.5	21.7	19	20	22	13	***14***	17	74	M	7:02	I	33	No	New brain metastasis and weakness; s/p unilateral adrenalectomy
42		X	13.8	19.5	21.7	10.6	***14.5***	16							52	F	8:57	O	26.1	No	Unilateral adrenal nodule with cortisol excess s/p unilateral adrenalectomy
47	X	X	15.4	18.2	20.5	11.7	***13.9***	14.8	12	***14***	16	11	***13***	***12***	55	F	6:13	I	NA	No	Unilateral adrenalectomy; bilateral adrenal nodules with cortisol excess
59		X	10.2	***15.2***	18.1	8.9	***13.3***	15.1							28	M	9:01	O	38.8	No	Hypopituitarism: GH deficiency; hypogonadism, hypothyroidism; no GC
69		X	9.1	***15.8***	18.7	8.4	***14.3***	16.4	7.8	15	16	10	***14***	17	47	F	13:20	O	49.9	No	S/p unilateral adrenalectomy for pheochromocytoma
85	X	X	17.7	***17.1***	20.7	10.1	***10***	***12.8***	15	15	18	11	***10***	***13***	73	M	6:00	I	6.2	Yes	Subtotal gastrectomy c/b small bowel necrosis; critically ill on opioids and vasopressors; low albumin
89	X		16	19.3	20.1	12.4	15.4	15.4	12	15	16	12		***13***	50	M	5:44	I	NA	Yes	Acute liver injury and pancreatitis; critically ill on opioids and vasopressors
99		X	9.7	18.5	23	7.9	15	17.1	7.3	15	15	7.7	***14***	16	24	F	7:55	O	15.1	No	7 mm pituitary adenoma

CST time refers to time of day the test was performed. Cortisol units (columns 4-15) are µg/dL. To convert µg/dL to nmol/L, multiply by 27.6. ACTH units are pg/mL. To convert to pmol/L, multiply by 0.2202. For inpatients, reason for hospitalization is listed first in Pertinent Clinical History. None of the female patients were on estrogen. Patients were not critically ill unless specified.

Abbreviations: Abn, abnormal; AI, adrenal insufficiency; c/b, complicated by; GC, glucocorticoids; Inpt, inpatient; NA, not available; Nml, normal; Outpt, outpatient; s/p, status-post.

Figure 4 shows the data from patients with baseline cortisol <8 µg/dL separated by whether they had a normal or subnormal response to CST. The baseline cortisol was statistically lower in the patients with a subnormal ACTH-stimulated cortisol response, but there was still considerable overlap with the baseline cortisol value in the patients with a normal response. Elecsys I baseline cortisol had to be very low (less than ~2 µg/dL) to reliably predict an abnormal post-ACTH stimulated cortisol. These differences were not accounted for by age, sex, inpatient or outpatient status, or baseline plasma ACTH (Chi-square).

Discussion

This study demonstrated that newer, more-specific cortisol assays have a lower threshold for the normal cortisol response to CST in patients with suspected AI. Although there was excellent correlation between these new assays and an older polyclonal antibody cortisol assay, the results were 22% to 39% lower than the less-specific Elecsys I assay. Accordingly, the new cortisol response to CST should be 14 to 15 µg/dL rather than 18 µg/dL. Not unexpectedly, there were differences in the 30- and 60-minute post-CST cortisol levels; however, these differences rarely caused diagnostic confusion and there was excellent correlation among all the specific assays at every time point.

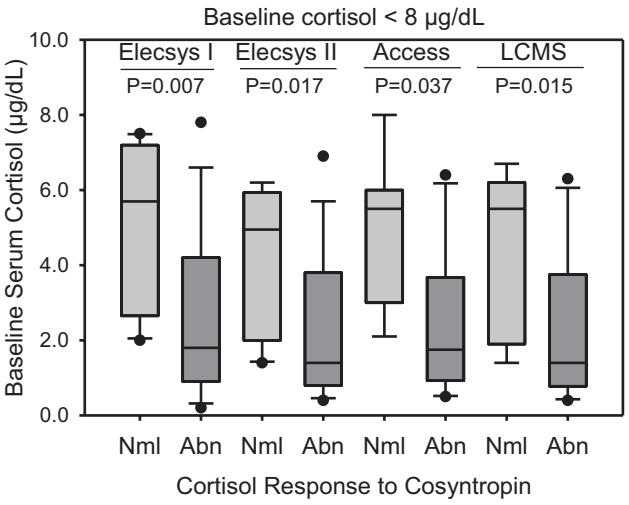

Figure 4. Box-whisker plots of comparison of patients with baseline cortisol values <8 µg/dL on Elecsys I separated by whether they had a normal or subnormal response to ACTH (cosyntropin) stimulation. New cortisol cutoff values were used to determine the adequacy of response (cortisol value above the cutoff at the 30- or 60-minute time points). *P* values refer to differences between normal and subnormal response within each method. Abbreviations: Nml, normal; Abn, abnormal. N values for Nml and Abn are 8 and 13, respectively, for Elecsys I and Elecsys II. N values for Nml and Abn are 6-7 and 9-10, respectively, for Access and LCMS (LC-MS/MS).

The CST is the most commonly used dynamic test to probe for a decrease in HPA axis function. The advantage of CST is that it can be performed at any time of the day and elicits the maximal cortisol production from the adrenal glands in a short time. Both high dose (250 mcg) and low dose (1 mcg) CST have each been advocated, but meta-analysis and consensus statements have concluded that these doses yield similar diagnostic accuracy [4]. This is not surprising since the high dose and low dose cosyntropin generate plasma ACTH concentrations of 22 000 to 100 000 pg/mL and 2000 pg/mL, respectively, at 2 minutes after injection [33, 34]. The concentrations of plasma ACTH greatly exceed the maximum endogenous physiologic stimulus to the adrenal cortex [4]. In fact, plasma ACTH >100 pg/mL generates a near maximum cortisol response [4]. Since patients with primary AI always have elevated plasma ACTH, CST will stimulate very small increments in cortisol if at all. Accordingly, the *increase* in cortisol stimulated by cosyntropin in these patients is not a reliable criterion for the adequacy of adrenal function. Historically, a cortisol level at 30 or 60 minutes of >18 µg/dL has been considered normal [5, 9, 10, 35, 36].

Newer, more-specific cortisol assays with lower cross-reactivity to other endogenous steroids have replaced older, less-specific assays [8, 13, 15, 26, 27]. Cortisol concentrations are approximately 20% lower in these more-specific assays. We have shown that the threshold cortisol response to CST of 14 to 15 µg/dL in a large group of patients with suspected secondary adrenal insufficiency is comparable to the 18 µg/dL cutoff previously used. Grassi et al also found lower cortisol cutoff values of 12.7 and 13.3 µg/dL using Elecsys II and LC-MS/MS assays, respectively [37]. Cutoff values 30 minutes after ACTH stimulation have been proposed to be 14.9 µg/dL for LC-MS/MS and 16 µg/dL for Elecsys II based on the 2.5th centile in healthy controls [26]. In another study with healthy subjects, a new lower reference limit 30 minutes after ACTH stimulation has been suggested to be ~16.6 with the Access assay [27]. If the historically accepted cutoff of 18 µg/dL after ACTH stimulation were used for the newer, more-specific cortisol assays, approximately twice as many patients in our study would have been given the biochemical diagnosis of AI. This means many more patients may have been unnecessarily treated with glucocorticoids, putting them at risk of adverse complications.

We did not perform other studies of HPA function, such as insulin-induced hypoglycemia or metyrapone stimulation. These studies are cumbersome, difficult to execute in an inpatient setting, and therefore rarely used in clinical practice. Regardless, the cortisol response to insulin-induced hypoglycemia would be lower than 18 µg/dL with specific cortisol assays [13].

As expected, the 60-minute post-ACTH cortisol was higher than the 30-minute cortisol on each of the cortisol assays evaluated [26, 28, 38]. Most laboratories and guidelines do not distinguish between these different time points when recommending a cutoff value to exclude AI and some electronic medical records and laboratory information systems even fail to provide a normal cortisol threshold after ACTH stimulation. Nine of the 110 patients undergoing CST had normal 60-minute cortisol concentrations (when using new cutoff values), but subnormal 30-minute cortisol concentrations in at least one assay. Of note, only one of these 9 biochemically abnormal patients was assigned the clinical diagnosis of AI based on chart review. These data would support the notion that 30-minute cortisol values after high dose CST provide more sensitivity, but less specificity than 60-minute cortisol values [39]. However, it was not certain whether any of these patients had AI, since none had follow-up testing or other testing of HPA axis function. Since some of these patients were hospitalized, it is possible that low levels of cortisol binding proteins may have accounted for some of the impaired cortisol responses [40]. This has implications for clinical practice. In some institutions, it is standard to only obtain a post-ACTH stimulation sample at 30 minutes. This means that approximately 7% (8/110) of patients may have been assigned the biochemical diagnosis of AI when the diagnosis could have been excluded if a 60-minute sample had been obtained. Alternatively, it is possible that these patients may have been misassigned by the 60-minute result and that glucocorticoid support during stress may have been prudent.

As noted in Table 3, 8 of the 110 patients had a cortisol value after CST on one assay method that was discordant with one of the other assay methods. In most of these instances, the cortisol value was very close to the cutoff value. Discordant results may be accounted for in part by normal assay variability (up to 6% to 10% depending on the assay method). As with any endocrine test, no absolute cortisol cutoff for the ACTH stimulation test is perfect for the diagnosis of AI [4, 7, 11, 12]. It also serves as a reminder that results should not be interpreted in isolation and that careful interpretation in the context of clinical history by an endocrinologist is still essential to establish the diagnosis of AI.

Varying cutoffs for basal cortisol levels have been suggested to predict AI [11, 17-19, 41]. In this study, there was a difference in low baseline cortisol values (defined as a cortisol of <8 µg/dL) in patients identified as having a subnormal cortisol response after CST (using the new cortisol cutoff values) vs those who had a normal response. However, there was considerable overlap in patients with cortisol values between 2 and 8 µg/dL. It is likely that basal cortisol values are not a reliable predictor of the cortisol response to ACTH unless they are <2 µg/dL. This is similar to results by Kalaria et al, who found that a cortisol <2.8 µg/dL was required to have 100% sensitivity for failed CST [20]. Age, male vs female, inpatient vs outpatient setting, and baseline ACTH value did not account for patients with low basal cortisol who had normal cortisol responses to ACTH stimulation.

Limitations of this study include lack of an independent endocrine test to determine if a patient had AI. Many of our patients did not have long-term follow-up available to confirm the diagnosis of chronic AI and the need for sustained glucocorticoid support. Patients in this study were from diverse clinical settings (eg, inpatient vs outpatient, relatively healthy vs critically ill, older vs younger). The study also illustrates the limitations of interpreting CST without an assessment of the pre-test probability of AI and the many clinical circumstances that might affect the results such as serum protein levels, concurrent medications that impact HPA axis function, and the proper timely execution of CST itself. Another limitation of this study was our inability to study a cohort of healthy subjects. Despite that, previous studies in healthy subjects using more-specific cortisol assays including LC-MS/MS have found a lower peak cortisol response to ACTH stimulation that was very similar to the lower cortisol threshold reported in our study [10, 26].

In summary, our study calculated new cortisol cutoff values for highly specific cortisol assays utilizing LC-MS/MS or a monoclonal antibody. To reduce the number of false positive results, we recommend a new cortisol cutoff of 14 to 15 µg/dL depending on the assay used (instead of the generic, historical value of 18 µg/dL derived from polyclonal antibody assays). It is important for clinicians to be aware of the new, assay-specific cutoff for the method available in their institution when evaluating patients for AI. Laboratories should consider providing these values when CST is performed. Baseline cortisol values <2 µg/dL were consistently associated with stimulated cortisol values below threshold. Finally, discordant results between different assays underscores the importance of clinical judgment from an experienced physician when determining the diagnosis of AI.

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Additional Information

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